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43327—24

ERRATA AND AUTHORS' EMENDATIONS.

Page 106, line 10, should read "western yellow pine (*P. ponderosa* Lawson, var. *scopulorum* Eng.), Douglas fir (*Pseudotsuga taxifolia* [Lambert] Britt.), lodgepole pine (*P. contorta*, Loud., = *P. murrayana* Balf.), and Engelmann spruce (*P. engelmanni* [Parry] Eng.), instead of "western yellow pine, Douglas fir, lodgepole pine, and Engelmann spruce."

Page 106, line 13, should read "Lake States pines (White pine, *P. strobus* Linn., Norway or red pine, *P. resinosa* Sol., and Jack pine, *P. divaricata* Gordon) and" instead of "Lake States pines and."

Page 195, footnote 5, third line from bottom, insert after the words "sour orange" the following: "*Citrus Aurantium L.*; pomelo."

Page 251, line 11, should read "bactericidal" instead of "bacteriacidal."

Page 253, lines 15 to 16 from bottom, should read "*Andropogon scoparius*" instead of "*Andropogon scopiarus*."

Page 442, line 5, should read "they produced" instead of "they provided."

Page 897, Table I, head "Fruits inoculated" should stand over columns 2, 3, 4, 5, Head "Fruits infected" should stand over columns 6 to 13.

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JOURNAL OF AGRICULTURAL RESEARCH

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TEMPERATURE RELATIONS OF ELEVEN SPECIES OF RHIZOPUS [1]

J. L. WEIMER and L. L. HARTER, *Pathologists, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Harter, Weimer, and Lauritzen (*12*) [2] have recently shown that the typical softrot of sweet potatoes may be produced by nine different species of the genus Rhizopus. It has been demonstrated likewise that these fungi can produce a similar decay of a large number of fruits and vegetables when the host and parasite are brought together under suitable conditions (*11*). The importance of temperature as one of the conditions essential for infection by these fungi was pointed out, it having been found necessary in all cases to expose the hosts to temperatures within the range most suitable for the growth of the fungus in order to obtain infection. These fungi were placed roughly into high, low, and intermediate temperature groups. More recent studies have been made [3] in which the temperature limits for growth of these fungi upon the sweet potato have been determined. The behavior of these fungi upon the living host is of special interest, since it is under these conditions that they become of economic importance. However, in studies of this nature two living organisms are involved, each of which may respond quite differently to various conditions of the environment. At high temperatures the physiological activities of the host are accelerated, while the reverse is true at low temperatures. The growth of the fungus will likewise be stimulated or retarded, depending upon the temperature employed; consequently any living host as a medium for testing the response of the species of Rhizopus to temperature would not be uniform at different temperatures and would therefore be unsatisfactory. It was the object of these investigations to determine the influence of temperature on the development of 11 species of Rhizopus when grown upon an artificial culture medium of uniform composition.

The studies, the results of which are presented below, include the effect of temperature first on the germination of the spores, second on the growth of the mycelium, and third on the fruiting of the fungi.

SPECIES STUDIED

The species of Rhizopus studied were the same as those used in former investigations: Namely, *nigricans* Ehrnb., *reflexus* Bainier, *chinensis* Saito,

[1] Accepted for publication May 29, 1922.

[2] Reference is made by number (italic) to "Literature cited," p. 39-40.

[3] By J. I. Lauritzen, of the Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture. The results have not yet been published.

tritici Saito, *artocarpi* Racib., *delemar* (Boid.) Wehmer and Hanzawa, *maydis* Bruderl, *nodosus* Namysl, *oryzae* Went and Pr. Geerligs, *microsporus* v. Tieg., and *arrhizus* Fischer. As previously explained (*12*) all the original cultures except those of *nigricans* and *arrhizus* were obtained from Mr. E. D. Eddy. Each organism was pure-lined by isolation from a single spore. A comparison with the original descriptions of each species showed no appreciable disagreement.

METHODS

In order to keep the stock cultures in a vigorous state of growth they were renewed about once each month by transferring to sweet potato agar in small Erlenmeyer flasks and incubated at a temperature of from 22° to 26° C. Cultures of from 5 to 10 days old were used in the experiments. Preliminary investigations showed that Rhizopus spores germinate more readily in some good nutrient medium such as sweet potato decoction than in water, hence the former medium made as previously described (*10*) was used.

In order to have available a solution of uniform composition for all the spore germination tests, a large volume of sweet potato decoction was prepared and stored in small flasks stoppered with cotton and covered with several thicknesses of oiled paper to retard evaporation. Each flask contained about 120 cc. of the decoction, which was about the amount required for the study of the spore germination of each organism. By storing the solution in small flasks the necessity for resterilizing the stock solution each time some of it was removed and thereby possibly changing its chemical composition was avoided. The loss by evaporation was restored by bringing the solution up to its original volume by the addition of distilled water. A loop of a spore suspension made in this medium was placed upon a clean cover slip, which was then sealed with vaseline in an inverted position to a glass ring cemented to a slide with a mixture of beeswax and vaseline. In the bottom of a cell so constructed was placed a small quantity of the same solution. The cells used were comparatively large (13 mm. high by 16 mm. in diameter) and provided sufficient air for the germinating spores.

In view of the fact that some investigators have found the hanging-drop method of studying spore germination unsatisfactory, the writers made several preliminary comparative tests of different methods. These showed that so far as these species of Rhizopus are concerned as reliable results could be obtained by the hanging-drop method as by any of the others, as, for example, in small volumes of solution in test tubes. Furthermore, since repeated observations must necessarily be made to determine accurately the time of germination the hanging-drop method lent itself more readily to manipulation than the test-tube method in which a fresh mount must be made for each observation. The hanging-drop cultures were placed in the incubators as soon as possible after they were prepared. The incubators were manufactured by Paul Altmann, were electrically controlled, and ranged in temperature from 1.5° to 50° C. Usually the temperatures in the individual chambers remained almost constant, varying for the most part less than a degree except in the lower ones, where there was sometimes a variation of 2° or 2.5° C. Pans of water were kept in the bottom of the chambers to keep the air moist. At least four and more often eight hanging drops were used at each temperature.

The rate of growth of these fungi at various temperatures was studied by growing them in Petri dishes containing 10 cc. of a 2 per cent Irish potato agar without additional sugar. A small drop of a suspension of spores in water was placed in the center of each plate with a 2-mm. platinum loop. The plates were placed in the incubators as soon as they were prepared, and the rate of growth was determined by measuring twice daily the diameter of the mycelial felt formed. Five Petri dishes were placed at each temperature. When the results were not entirely satisfactory the experiment was repeated.

The effect of temperature on sporangia formation was determined by growing the fungi at different temperatures in 100-cc. Erlenmeyer flasks on 30 cc. of 2 per cent Irish potato agar and observing the temperatures at which the sporangia were produced and the time required for their formation.

INFLUENCE OF TEMPERATURE ON SPORE GERMINATION

The effect of temperature upon spore germination has been studied by a number of investigators and by several different methods. The percentage of germination at different temperatures has been used as a measure of the influence of temperature upon spore germination by Melhus (*14*), Doran (*4*), Reed and Crabill (*18*), and others. Naturally the method used in any particular investigation must depend upon the type of data desired. Preliminary tests showed that practically 100 per cent of the spores of *Rhizopus* spp. germinated except at extreme temperatures; hence, the percentage of germination could be used as a measure of the effect of temperature only by taking into consideration the time factor. The number of spores which germinate and cause infection in the case of decay-producing fungi of this type is not of as much importance as it is in the case of organisms producing other types of diseases, such as leafspots. In the former case a host may be completely destroyed as a result of a single infection, while in the latter the total amount of damage done often depends upon the number of individual infections. In view of these facts the writers decided to use the time required for germination to begin as the measure of the influence of temperature upon this phenomenon. Anderson (*2*), Melhus (*14*), Ames (*1*), Rands (*16*), Ravaz and Verge (*17*), Shapovalov (*19*), and others have used this same criterion in their investigations. However, in no case is it made clear when these writers considered germination to have taken place. It is possible to use as a criterion the time when the maximum percentage of the spores have germinated or when the spores which germinate first have produced germ tubes. With Rhizopus a few spores in each drop produce a germ tube first, followed very soon by others, the number gradually increasing so that in a short time nearly 100 per cent of the spores have germinated. The period intervening between the time when the germ tubes appear on the spores which are the first to germinate and the time when all the spores have germinated varies with the temperature, being shortest at the optimum and gradually increasing as the upper and lower temperature limits are approached. As a criterion of germination the writers decided to use the time necessary for the spores which germinate first to produce a germ tube equal in length to the diameter of the spores. Using the figures thus obtained, curves were plotted which show the variation in time due to the difference in temperature. The time necessary for the germ tubes to reach some specific

number of microns in length might have been employed; but the measure selected was easier to determine, since the spore was always present with the germ tube for quick comparison, while considerable time would have been required to bring the spore in line with the micrometer scale for measurement. In these studies time was a very important factor, since germination often occurred in three or four incubators at nearly the same time, often not over from 5 to 15 minutes apart. Some care had to be exercised, especially at the less favorable temperatures, as often one or a very few spores would start to germinate considerably in advance of the remainder. However, by examining the hanging drop cultures frequently it was possible to determine with a fair degree of accuracy the point sought. Slides were examined near the incubators and were kept out usually less than a minute. Only at the higher temperatures was there any appreciable fluctuation as a result of opening the doors, and in such cases the normal temperature was quickly restored.

The results obtained from the study of the germination of the spores of 10 of the species is shown by the curves in figure 1. The germination of *Rhizopus maydis* spores was not studied for the reason that under the conditions under which the cultures were grown spores were not produced in sufficient quantity to carry out the experiment.

In the curves in figure 1 the time in hours necessary for the germ tubes to reach the length of the diameter of the spores was plotted on the abscissa, while the temperature in degrees centigrade was plotted on the ordinate. The maximum temperature for germination as indicated by the table is that temperature at which no germination took place. The temperature at which germination will just take place is very difficult to determine, since at 1° or 2° C. below the maximum it frequently starts and then stops before the germ tube reaches the diameter of the spore. For example, *Rhizopus artocarpi* spores germinated readily at 32.3° while at 33.4° only about one-half of 1 per cent of the spores sent out germ tubes, which finally reached a length equal to twice the diameter of the spore and then stopped. At 34.5° the spores became somewhat swollen, which is a condition always preceding the extrusion of the germ tube. After 48 hours the spores had been killed at the two higher temperatures, as indicated by the fact that they failed to germinate when placed at a temperature favorable for germination. Similar results were obtained with other species. No attempt was therefore made to establish a definite maximum, but it may be said that in general it is somewhere from 1° to 2° lower than the temperature plotted as being the inhibiting temperature. In every case where there was no germination within 24 to 48 hours the spores were found to have been killed. The temperatures just above the maximum which inhibited germination of the spores of the different species are as follows: *artocarpi*, 34.5°; *tritici*, *delemar*, *oryzae*, *nodosus*, and *arrhizus*, 45.5°; *chinensis*, 52°; *reflexus*, 38°; *nigricans* and *microsporus*, 34°. The lower temperature limits for germination for only two of the species, *nigricans* and *microsporus*, are shown by the curves, since in all other cases the time for germination at the minimum temperature was more than 100 hours. The spores of *chinensis*, *oryzae*, and *delemar* had not germinated during 30 days' exposure at 8.5°, 7°, and 7°; but germination did take place in that length of time at temperatures of 10°, 9°, and 8.7°, respectively. On the other hand, the spores of the remaining species, *reflexus*, *arrhizus*, *tritici*, *nodosus*, and *artocarpi*, germinated at 1.5°, the lowest temperature tried, in 5, 15, 22, 14, and 6 days, respectively. The percentage of spore germination at

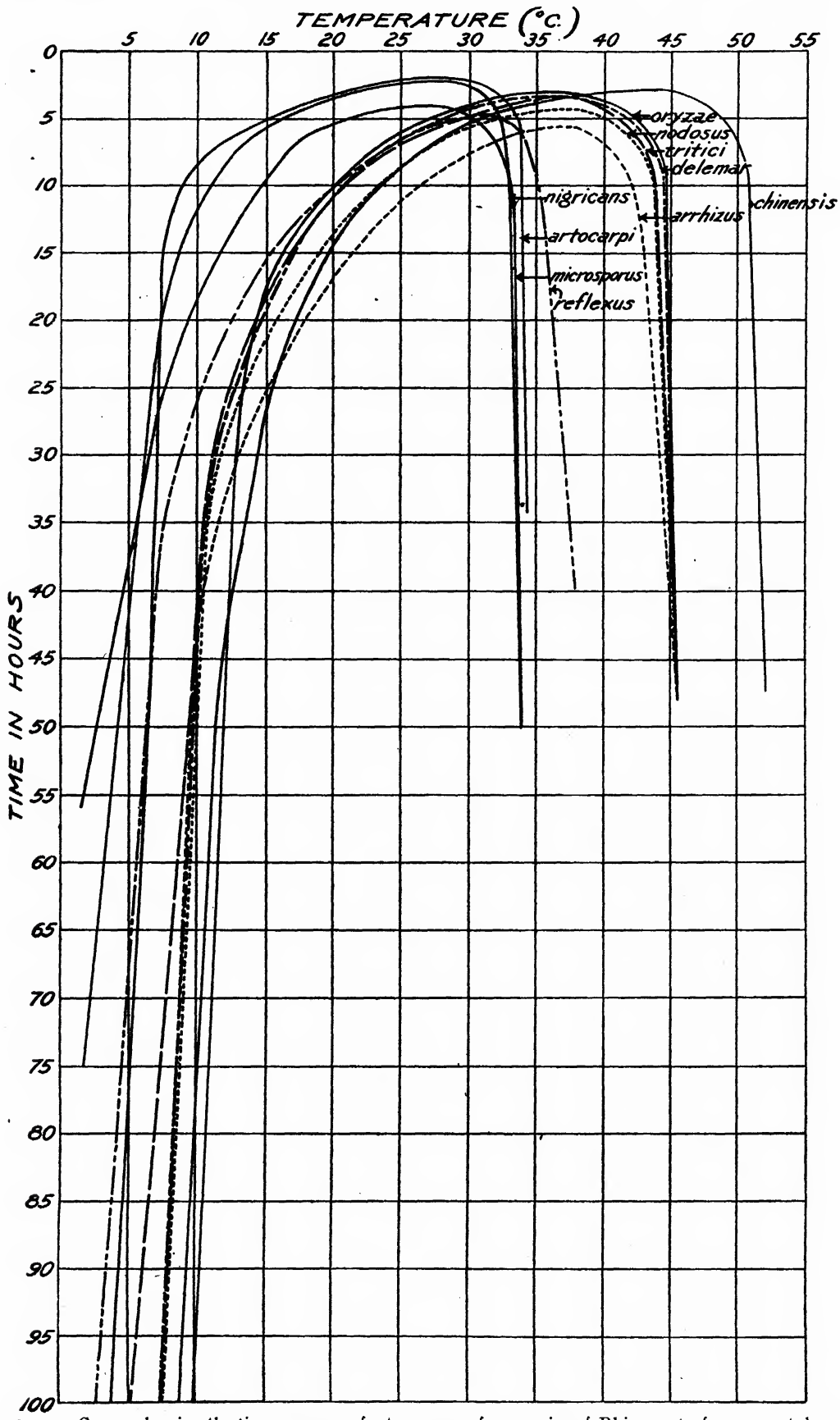

FIG. 1.—Curves showing the time necessary for the spores of 10 species of Rhizopus to form germ tubes equal in length to the diameter of the spore at different temperatures.

this temperature was often small, and only in the case of *reflexus* did any appreciable growth take place within the time limit of these experiments (30 days).

The optimum temperatures as obtained by this method are as follows: *artocarpi*, 26° to 29° C.; *nigricans* and *microsporus*, 26° to 28°; *tritici*, *delemar*, *nodosus*, *oryzae*, and *arrhizus*, 36° to 38°; *reflexus* 30° to 32°; *chinensis*, 43° to 45° C. Any attempt to determine a closer optimum seemed impracticable, since the rate of germination at these temperatures is so nearly the same. For the convenience of the reader and for the greater ease of comparing the effect of temperature on germination, growth, and fruiting these data are set forth in Table I and will be referred to later.

The curves (fig. 1) show that the species studied fall into three groups according to their response to temperature. *Chinensis* stands out conspicuously as a species with a high optimum, although there is a considerable range through which good growth will take place. In fact, this species has a wider temperature range, about 30° C., through which the spores germinate readily, than any of the others studied, which indicates that it is less sensitive to heat. *Artocarpi* probably stands at the other extreme, although its spores germinate well within a range of 20° (10° to 30°). The drop on both sides of the curve is very abrupt. Owing to its sensitivity to heat considerable difficulty was at first experienced in keeping this fungus alive. This species was the most erratic in its behavior, requiring more study to determine its temperature relations than any of the other species.

Along with *artocarpi* as species with comparatively low optimums and maximums may be placed *reflexus*, *nigricans*, and *microsporus*. The results seem to indicate that the two latter species have the lowest minimum, since their spores germinated much more quickly at the lowest temperature tried.

The remaining five species are very similar in regard to their maximum and optimum temperatures, differing only in the time necessary for germination, which varies at the optimum from 3 hours (*oryzae*) to 5½ hours (*arrhizus*). As pointed out above, however, these species also differ in their lower temperature limits in that the spores of *oryzae* and *delemar* failed to germinate at 7° C. while those of the other three germinated at 1.5°.

The temperature relations of several species of Rhizopus have been studied by Hanzawa (*9*) and Lendner (*13*, *p. 111–127*). The former separated *nigricans* from the other species studied by him by the fact that it was the only one which did not grow at 37° C., and the latter separated it from *oryzae* because it did not grow on potato at 39°. Hanzawa states that *nigricans* would not grow at blood temperature (35° to 37°). He also found that *chinensis* spores did not germinate at 6° and that those of *delemar* would not germinate below 12° or above 42°. Hagem (*8*), working with *Mucor* (*Rhizopus*) *nodosus* (Namyslowski), found that its spores did not germinate at 43° to 44° C. Ames (*1*) found that the spores of *R. nigricans* failed to germinate at 1° or at 42° but germinated at 3° to 4° and 41°, the optimum being from 38° to 41°, where germination took place in 5½ hours. Stevens and Wilcox (*20*) found that this fungus could mature a few sporangia on ripe strawberries at 36° to 37°. The difference in temperature limits given by different investigators for this fungus is difficult to understand. This disagreement in results might be due to several causes, such as the use of different strains, to

incorrect identification or to mixed cultures, to the influence of the culture media, etc. A comparative study of different strains of *nigricans* which the writers have under way may throw some light upon this subject. The variation in temperature between different parts of an artificially heated incubator is a factor which can not be overlooked. The upper strata of air in an incubator whose temperature is measured by means of a thermometer inserted through an opening at the top has been found to be in some cases from 1 to several degrees warmer than that at the center or bottom. In chambers heated by a water jacket the writers have recorded a difference of 5° between the temperature of the air at the top and bottom of a chamber, a distance of only 16 inches. In the present investigations the thermometers were compared with a standardized thermometer and then the bulbs were lowered to the immediate vicinity of the cultures in order to obviate as nearly as possible this source of error.

Dunn (*5*) found that the plus and minus strains of *nigricans* with which she worked differed somewhat in their temperature relations. The minus strain seemed to be more vigorous, since it had a slightly higher optimum than the plus and grew at both a slightly higher and a lower temperature. The optimum for growth by the minus strain lay between 25° and 28° C., which agrees closely with that obtained by the writers—approximately 25°. The maximum temperature for the growth of the minus strain was about 31°, which also agrees closely with that obtained for the strain studied by the writers. Dunn found a considerable difference in the optimums for different strains of Rhizopus from strawberries. The optimum temperature for one strain was about 36°, while that for other strains was approximately 27° or 28°. However, she expresses doubt as to whether she was working with strains or species, and since several species of Rhizopus are capable of decaying strawberries (*11*) she may have worked with a species which thrived best at a higher temperature rather than with a strain.

INFLUENCE OF TEMPERATURE ON GROWTH

Having determined the effect of temperature on germination, its influence on the continued growth of the germ tubes and resulting mycelium was next studied. This was done by measuring the daily increment of growth on agar in Petri dishes. The results are shown graphically in figures 2 to 12, in which the base line shows the temperature in degrees centigrade and the perpendicular shows the diameter of the mycelial disks at stated intervals of time.

An examination of these figures shows that they possess some common characteristics. Each series, for example, originates at scattered points to the left (the lowest temperatures tried at which growth did not take place within the given time), then rises in the direction of the optimum, and finally falls to a point at the right (the temperature at which no growth took place). From these figures it is evident that some growth took place at the maximum temperatures during the first 24 hours, while at the minimum temperatures growth did not begin until sometime later. The optimum temperature in all cases remains the same within the time shown in these graphs.

The lowest temperature at which growth of *maydis* and *arrhizus* was observed was 7.4° C. after 7 and 12 days, respectively. Hanzawa (*9*) found that *arrhizus* could make some growth at 6°. The writers did not

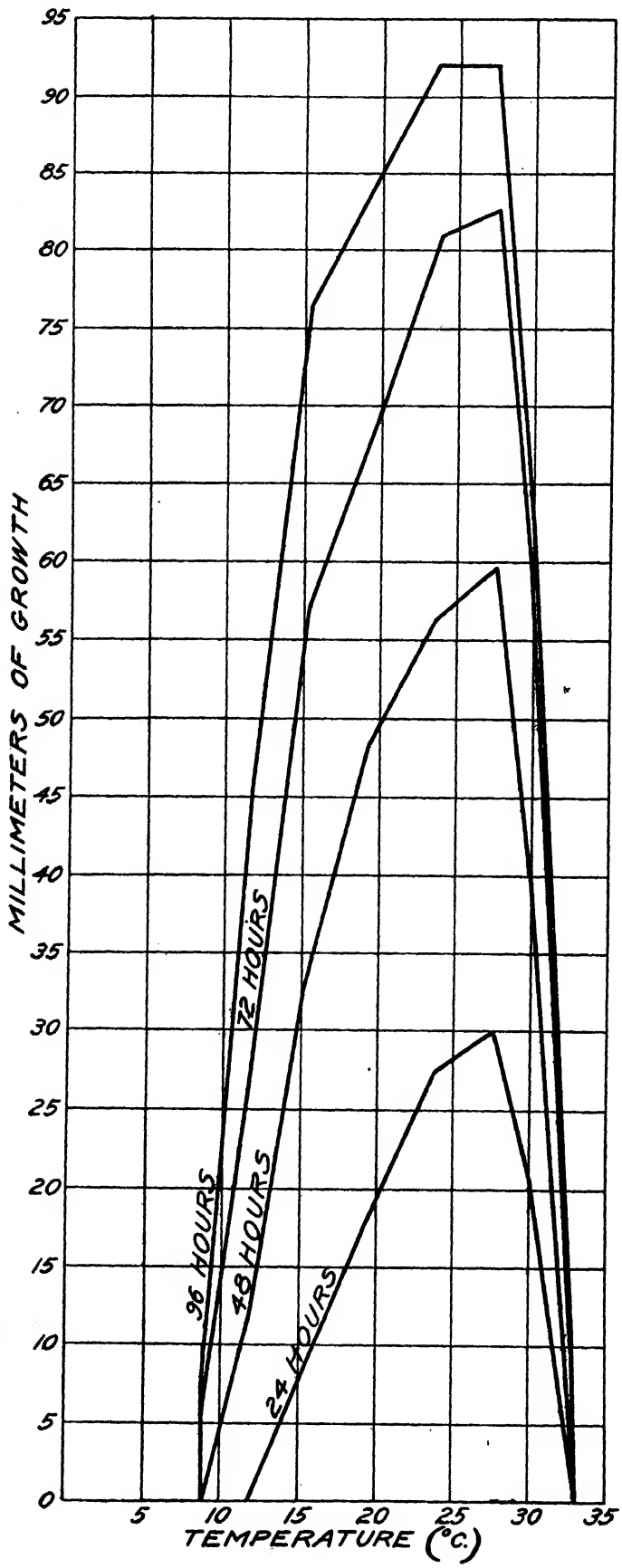

FIG. 2.—Graph showing the rate of growth of *Rhizopus artocarpi* at different temperatures.

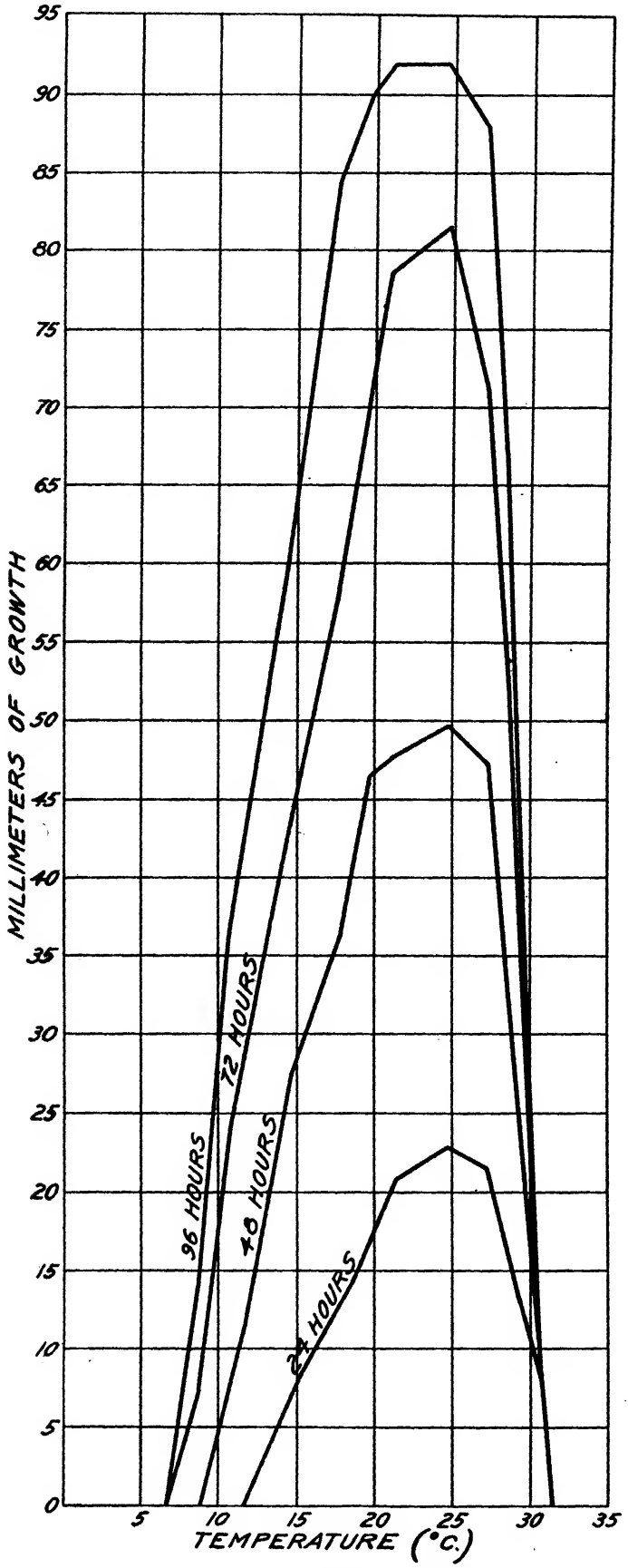

FIG. 3.—Graph showing the rate of growth of *Rhizopus nigricans* at different temperatures.

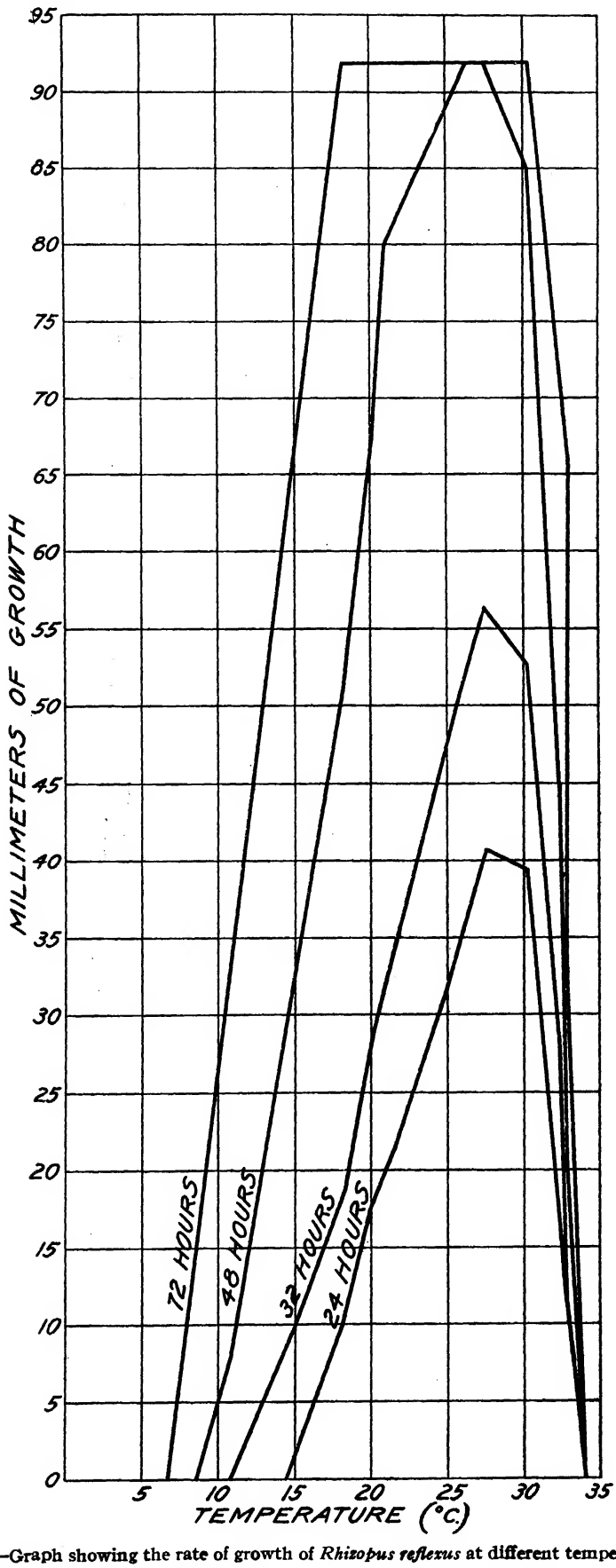

FIG. 4.—Graph showing the rate of growth of *Rhizopus reflexus* at different temperatures.

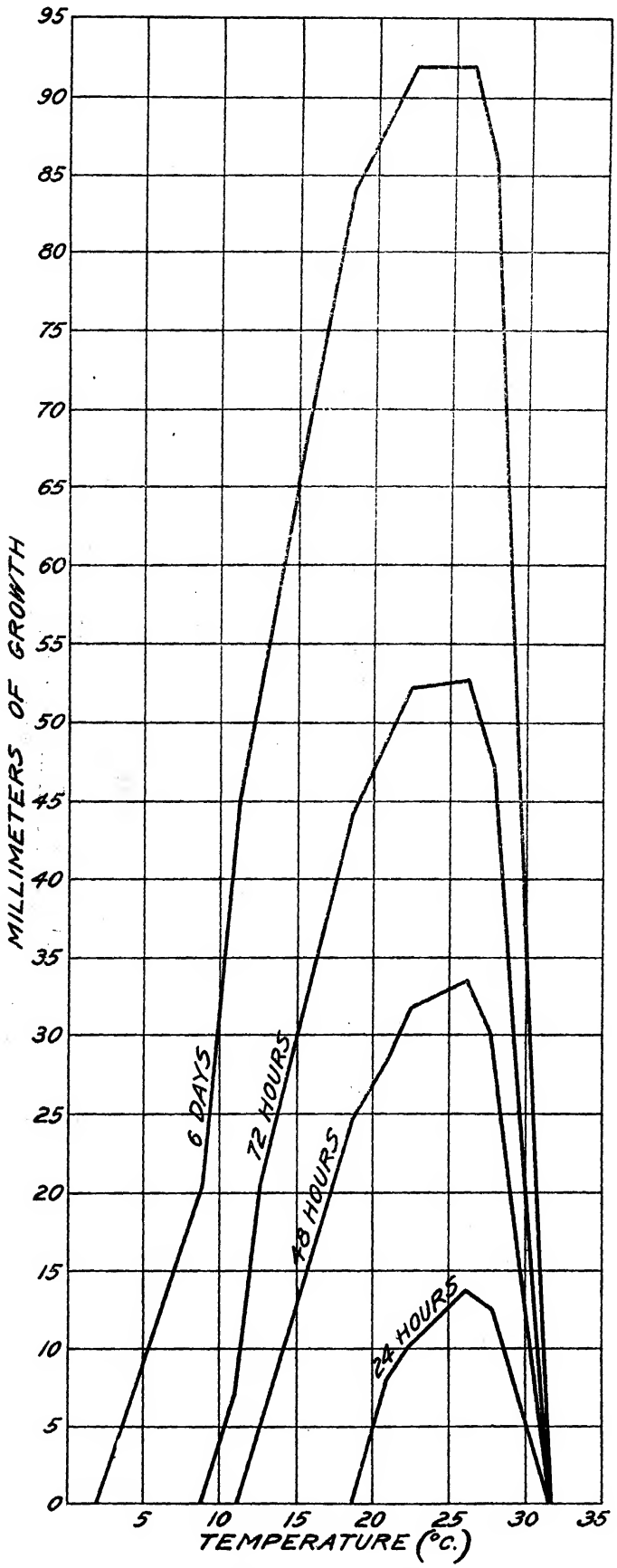

FIG. 5.—Graph showing the rate of growth of *Rhizopus microsporus* at different temperatures.

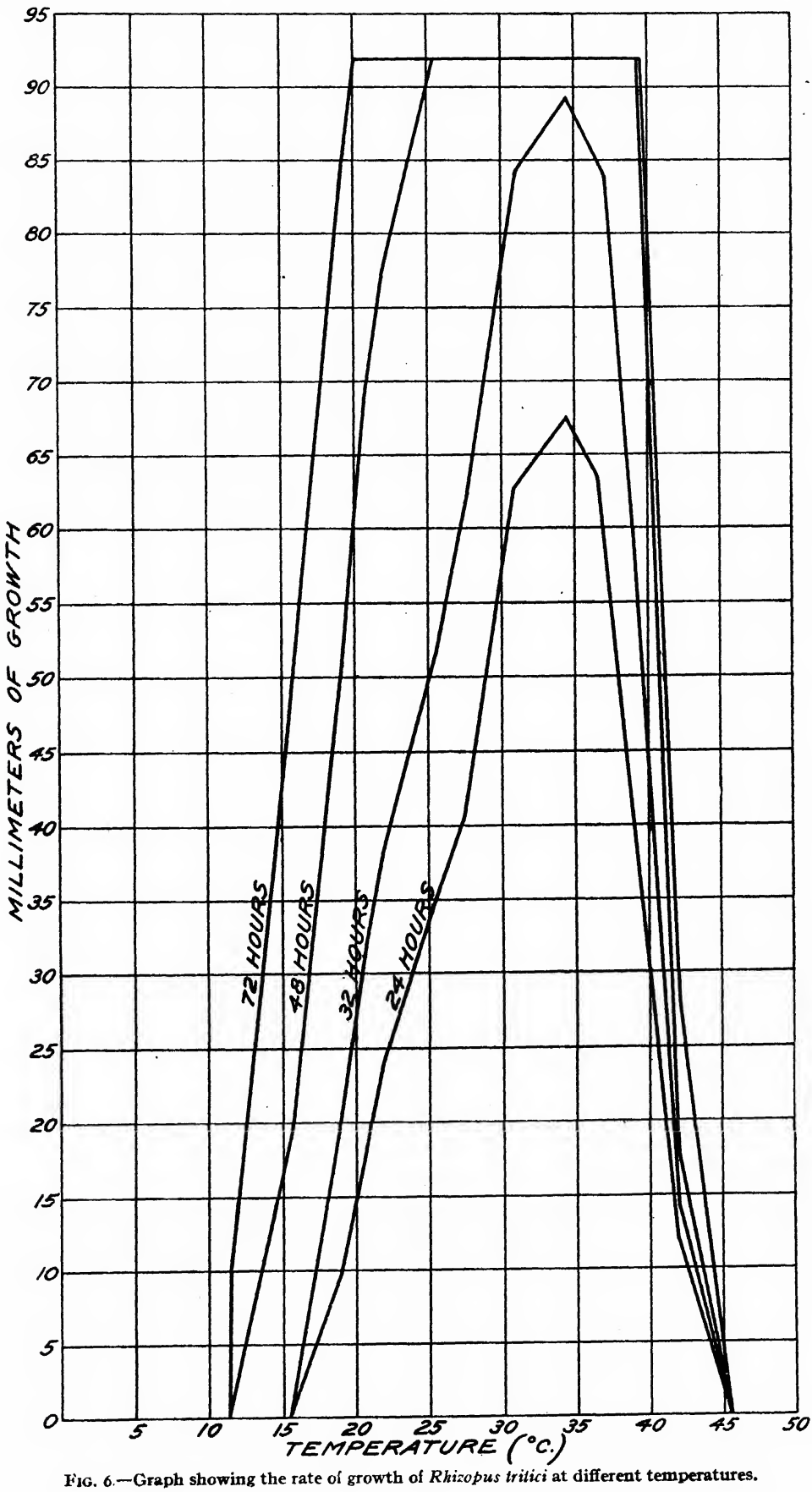

FIG. 6.—Graph showing the rate of growth of *Rhizopus trilici* at different temperatures.

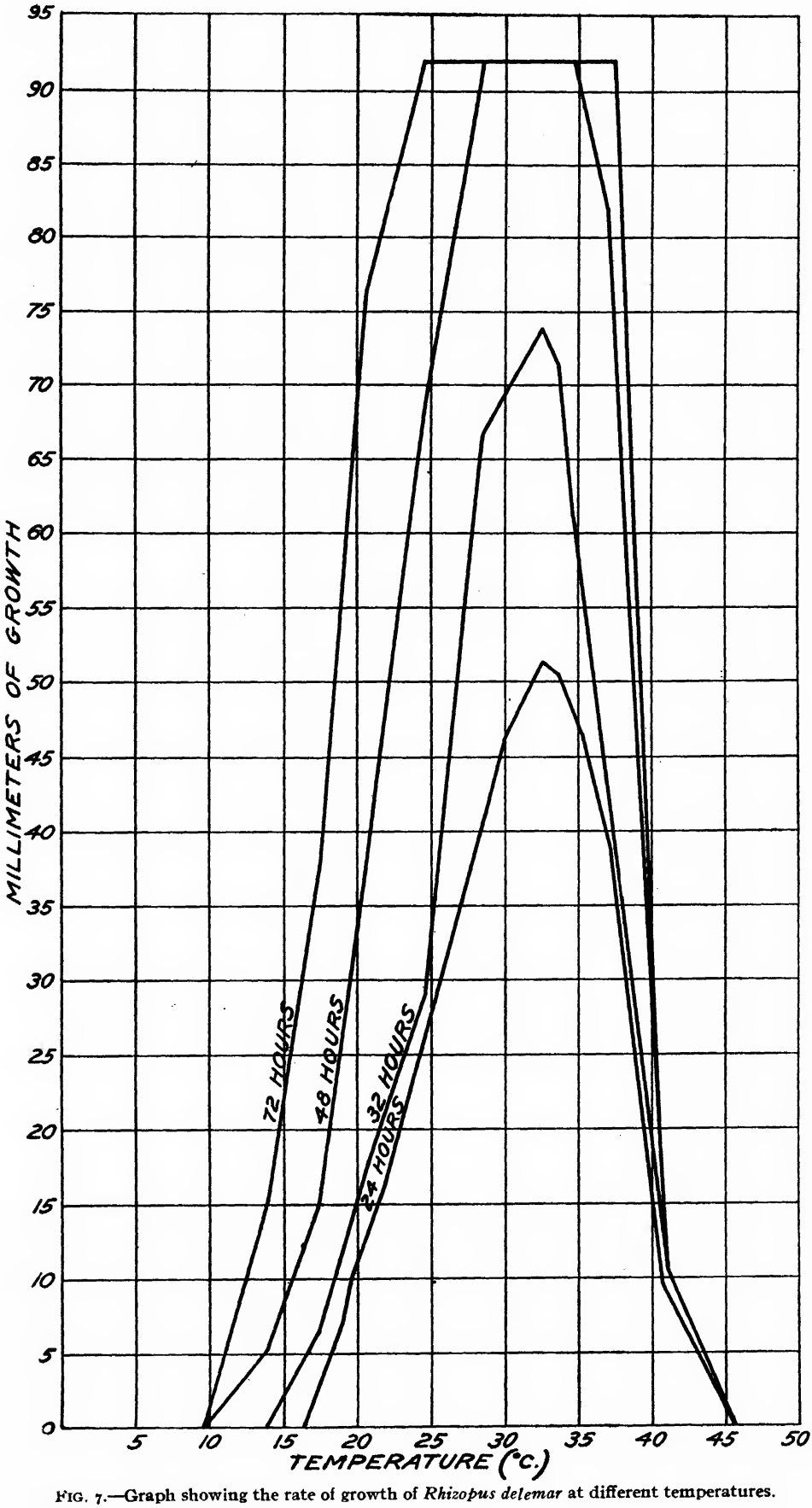

FIG. 7.—Graph showing the rate of growth of *Rhizopus delemar* at different temperatures.

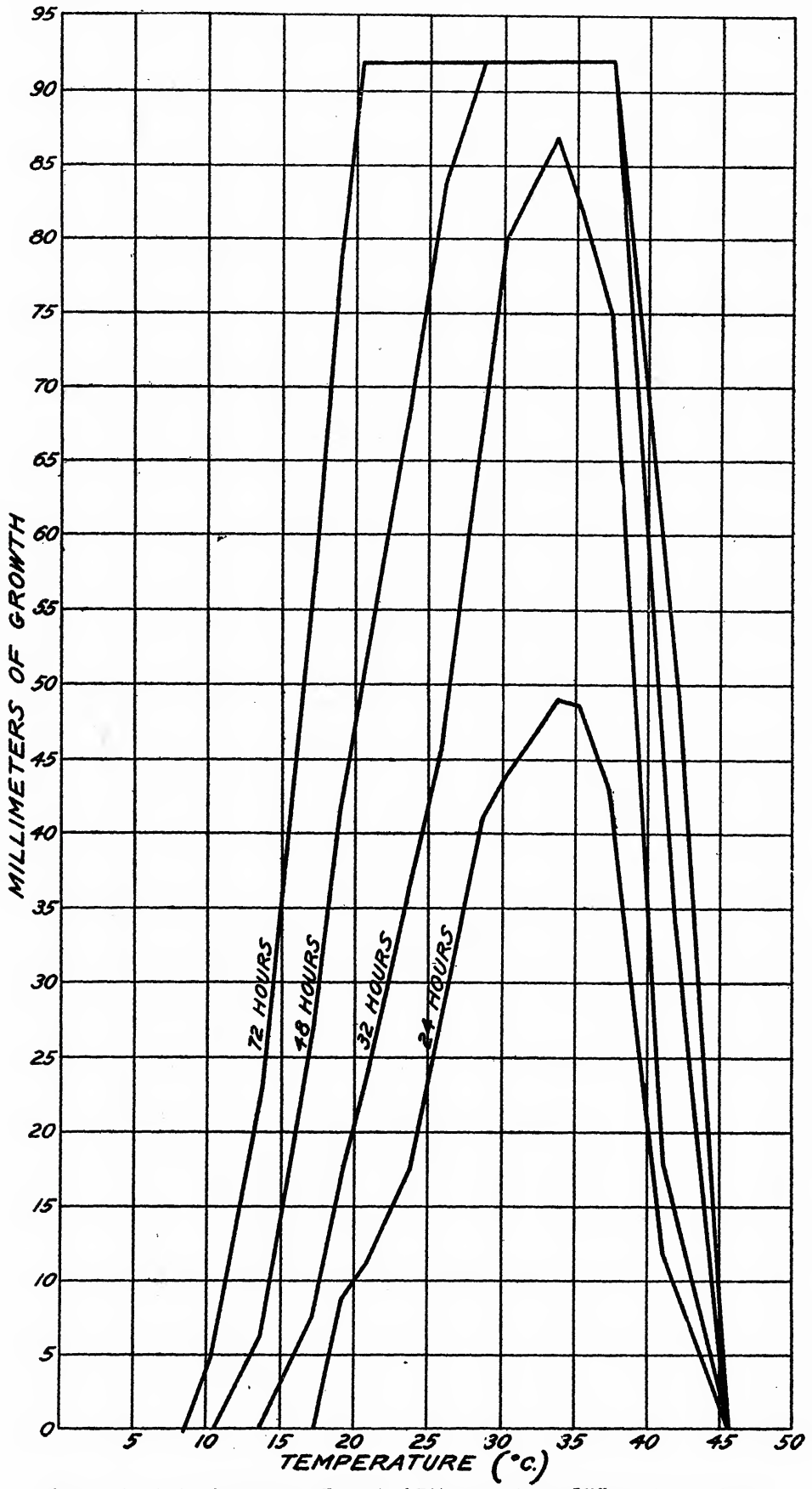

FIG. 8.—Graph showing the rate of growth of *Rhizopus nodosus* at different temperatures.

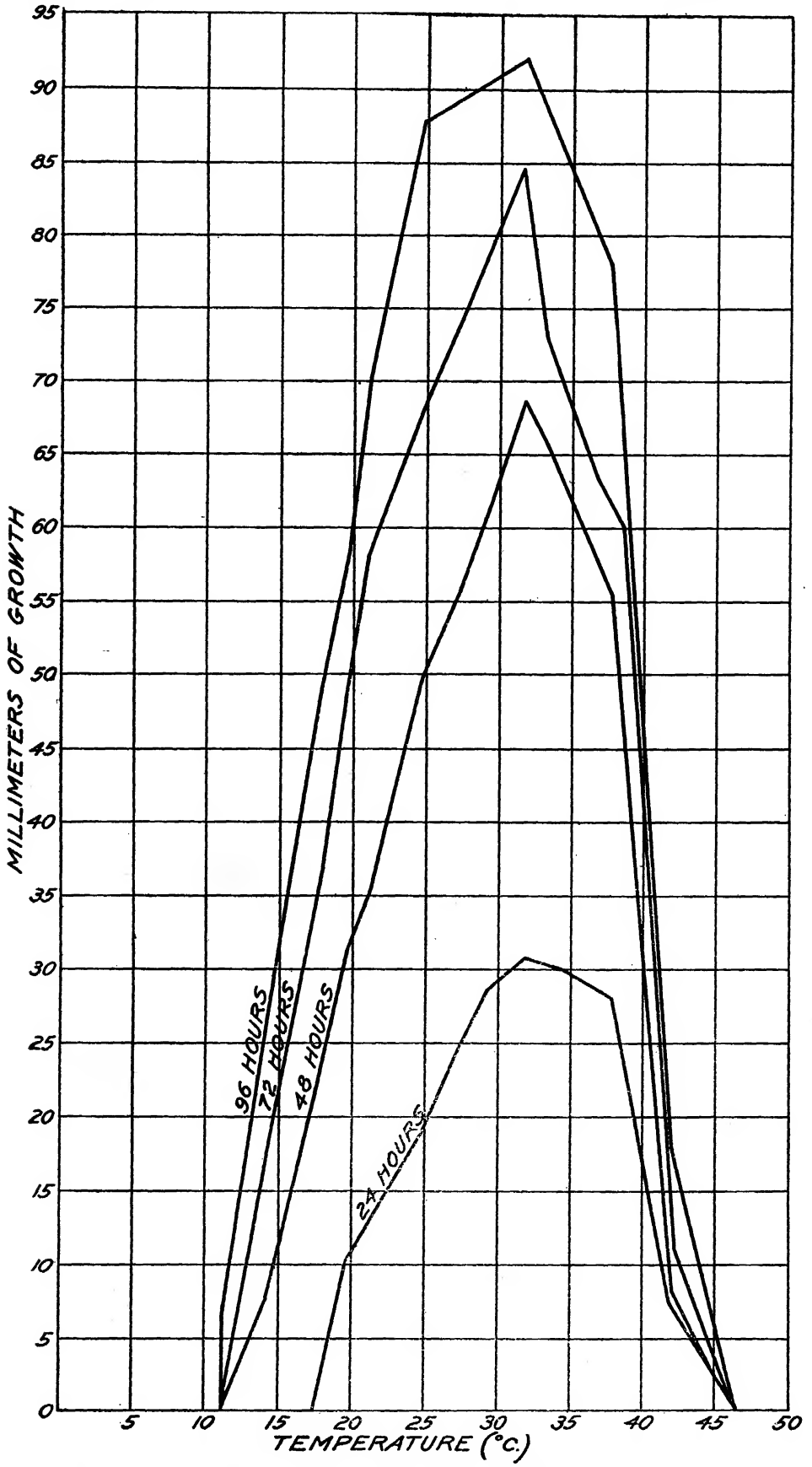

FIG. 9.—Graph showing the rate of growth of *Rhizopus oryzae* at different temperatures.

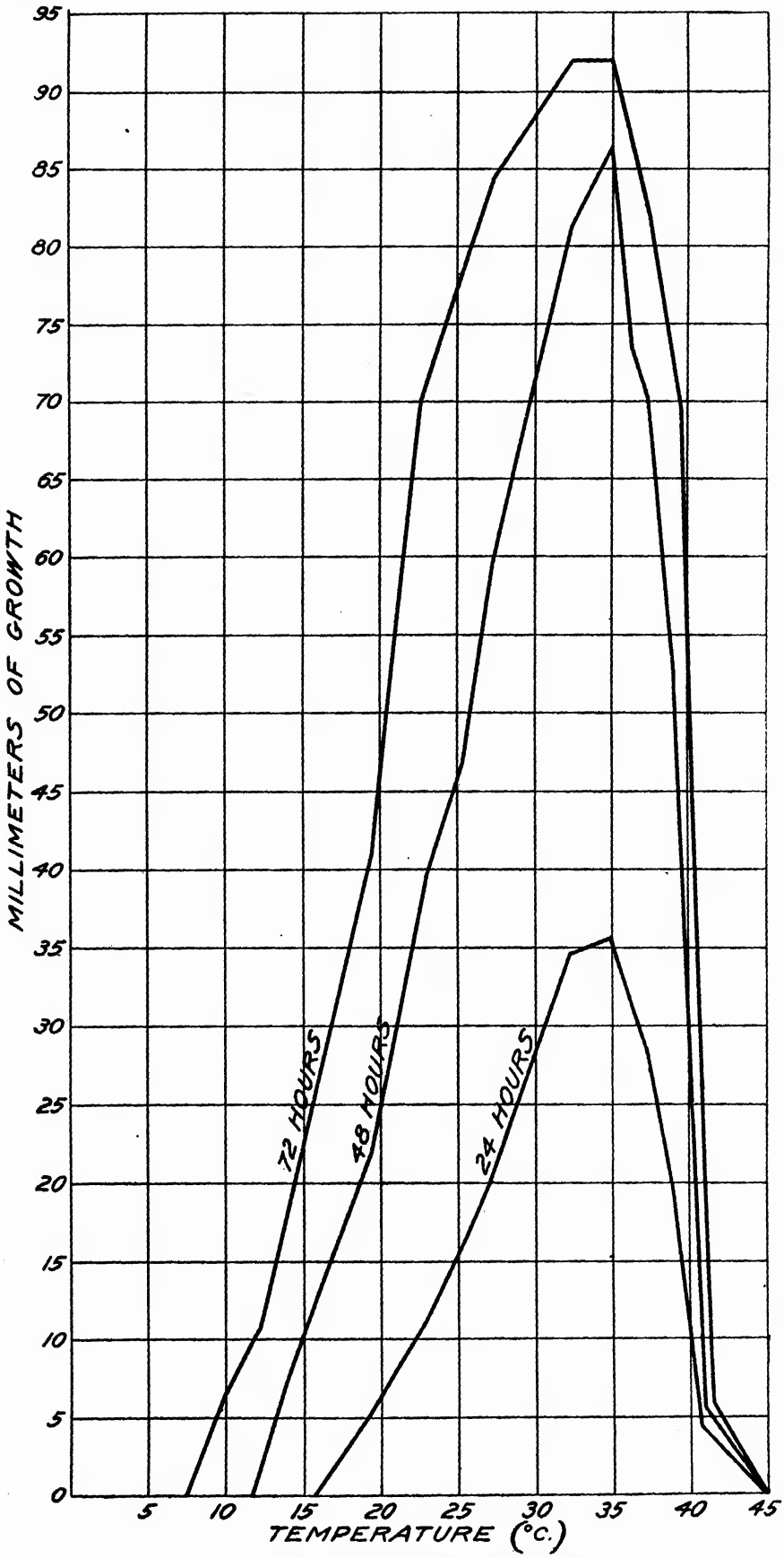

FIG. 1.—Graph showing the rate of growth of *Rhizopus arrhizus* at different temperatures.

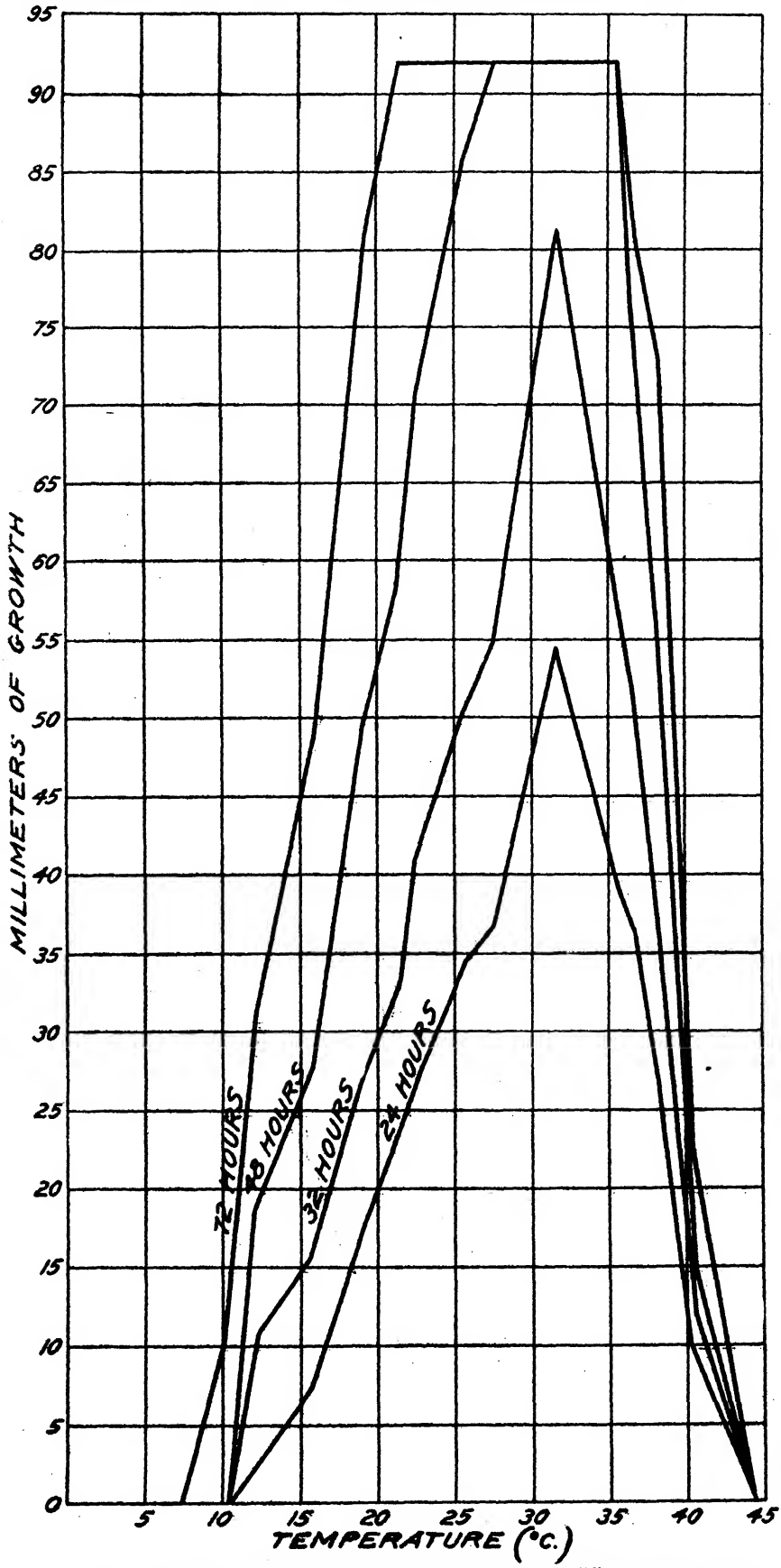

FIG. 11.—Graph showing the rate of growth of *Rhizopus maydis* at different temperatures.

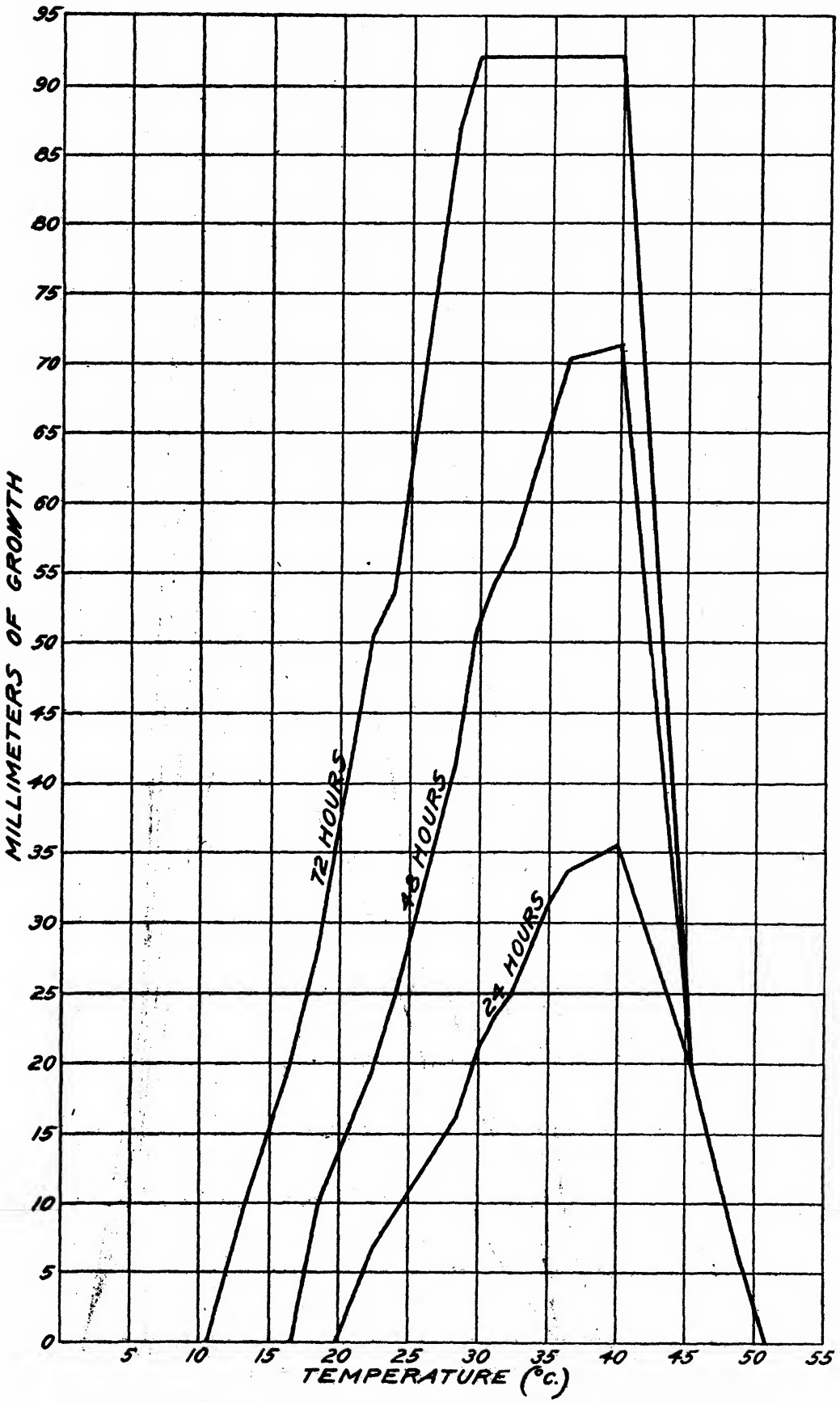

FIG. 12.—Graph showing the rate of growth of *Rhizopus chinensis* at different temperatures.

try a temperature between 7.4° and 1.5°, but it is quite probable that growth will take place below 7.4° if sufficient time is allowed, since the spores germinated at 1.5°. Growth by *tritici*, *nigricans*, and *nodosus* at 6.5° began after 12, 8, and 10 days, respectively. These fungi also made some growth at 6° to 8° in Hanzawa's experiments. *Microsporus* and *reflexus* are the lowest temperature forms so far as growth is concerned. They began to make an appreciable growth at 1.5, the lowest temperature tried, after 10 days. *Artocarpi*, a low-temperature form as indicated by its optimum, made some growth at 9° but none at 7°, while *chinensis*, which has a high optimum, made some growth at 10.4° but none at 7.5°; in other words, growth was inhibited at practically as high a temperature in the low- as in the high-temperature form. At 11° and 9.8° *oryzae* and *delemar*, two forms very closely related both morphologically and physiologically, made some growth in 4 and 16 days, respectively. However, at 7.5° growth was entirely inhibited. *Oryzae* was not subjected to any temperature between 11° and 7.5°, so it is possible that some growth might have taken place at a temperature somewhat below 11°. Hanzawa found that this species made some growth at 6°, while the upper and lower limits for *delemar* were found to be 12° and 42°, respectively. It may be said, then, that the minimum temperature for growth varies with the time for the first 5 to 15 days, depending upon the species. After that time the true minimum—that is, the lowest temperature at which growth will take place regardless of the time—is reached. Thus growth decreases as the temperature is lowered until a point is reached where it is entirely inhibited. However, cultures which failed to grow after 30 days at the inhibitive temperature or lower develop rapidly when transferred to a more favorable temperature, showing that the protoplasm of the spore has suffered no harmful effects. These results are in general accord with those of other workers with other organisms.

It was pointed out above that there was visible growth at the maximum temperature by the end of the first 24 hours in each species. It would appear from the graphs that the maximum temperature in all cases is fixed—that is, that it does not change during the course of the experiments as does the minimum. However, this is not the case. *Delemar*, for example, made a growth about 9 mm. in diameter at 41° C. in the first 24 hours, which reached 10.5 mm. during the following 24-hour period and then ceased altogether, the mycelium failing to grow when placed at a favorable temperature. In this case the maximum for the first 48 hours was about 41°, after which even at 38° the growth was considerably retarded although not inhibited within the time limit of this experiment. Shifting of the maximum was noted with the other species. No doubt if temperatures sufficiently close were tried over a considerable length of time and by a method sufficiently delicate to determine accurately very small increments of growth a so-called shifting of the maximum would be found in all cases. As the temperature rises above the optimum a retarding soon followed by an inhibiting effect is noted. A comparatively short exposure to the inhibiting temperatures results in the death of the organism, although it is possible that a temperature slightly below the maximum established might be found which would inhibit growth for a considerable length of time and yet would not kill the fungus. Fawcett (*6*) has found a similar shifting of the maximum. Shifts from 36° to 31°, 38° to 35°, 46° to 35° were noted in *Pythiacystis citrophthora* Smith and Smith, *Phytophthora terrestria* Sherbakoff, and *Diplodia natalensis* Evans, respectively. All of these fungi

showed a lowering of the apparent optimum of from 3.5° to 6°. *Phomopsis citri* Fawcett, however, did not show similar changes. No such change in the optimum for growth of the species of Rhizopus studied by the writers was noted. It is possible that if the fungi had been grown for longer periods of time some such shifting of the optimum would have

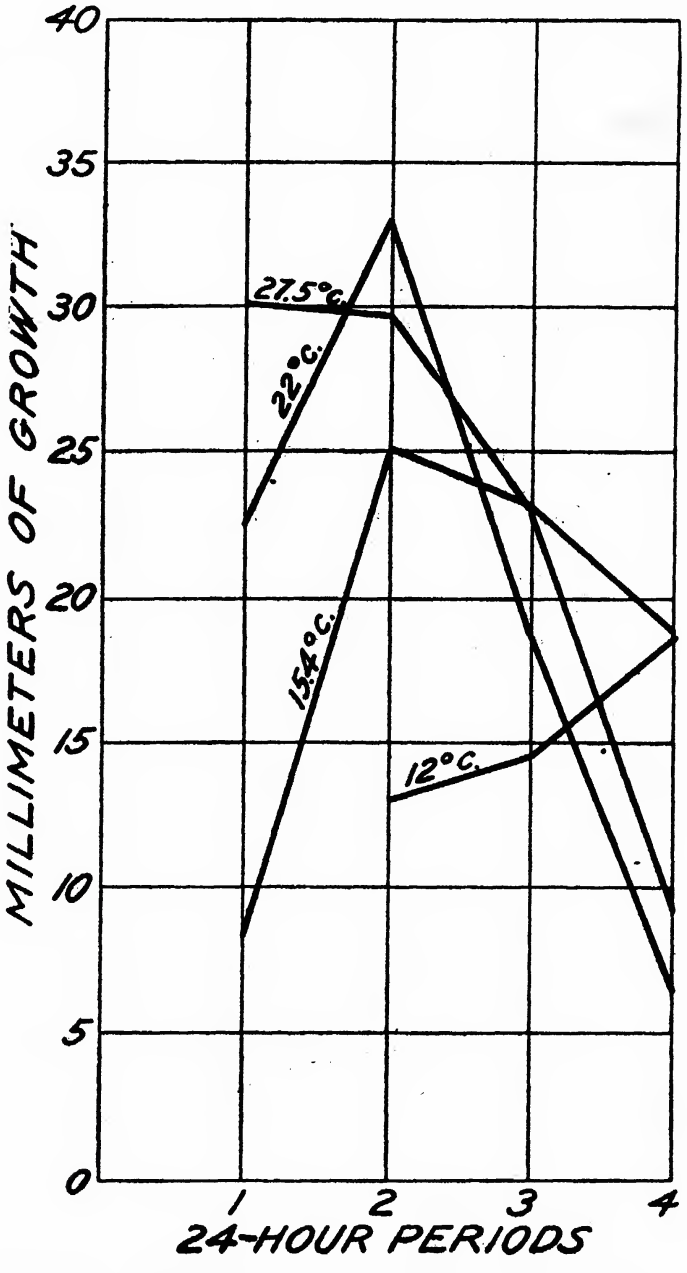

FIG. 13.—Graph showing the increase of growth of *Rhizopus artocarpi* for consecutive 24-hour periods.

become apparent. Hanzawa (*9*) found that the cardinal temperatures for some of these fungi were as follows: *Delemar*, *nigricans*, *arrhizus*, and *nodosus* have maximums of 42°, 35° to 37°, 42°, and 43° to 44°, respectively; *oryzae*, *tritici*, and *chinensis* all made a good growth at 38° to 42°; while the optimums for *delemar*, *oryzae*, and *tritici* were 30°, 30° to 40°, and 30° to 35°, respectively. Hagem (*8*), on the other hand, found that

nodosus, *nigricans*, and *arrhizus* had for their upper limits 43° to 44°, 33.5°, and 42°, respectively; Lendner (*13*) gives 30° to 35° as the optimum for *tritici* and 30° to 40° as that for *chinensis;* while Bruderlein (*3*) states that *maydis* makes its optimum growth at 39°. This is considerably higher than that obtained by the writers. No explanation for this apparent difference can be given.

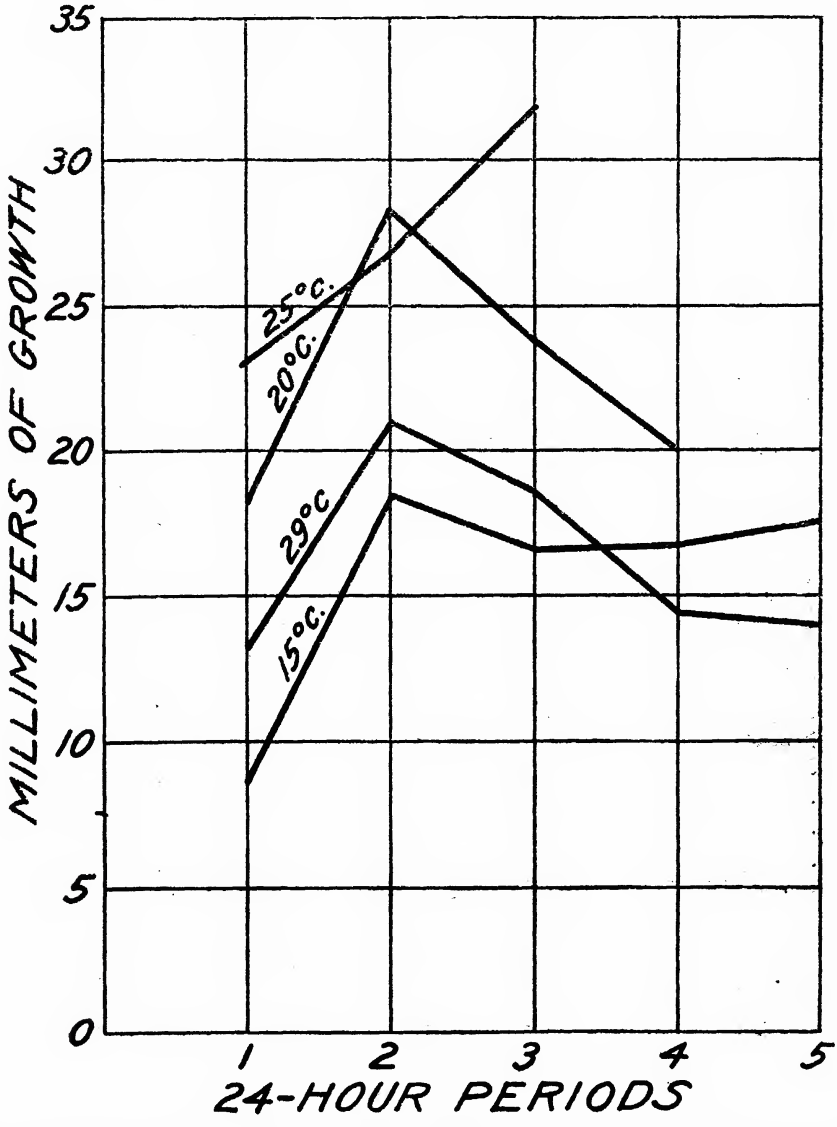

FIG. 14.—Graph showing the increase of growth of *Rhizopus nigricans* for consecutive 24-hour periods.

Graphs (fig. 13 to 23) were constructed to show the variation in the growth rate at different temperatures for successive 24-hour periods. The ordinates represent diameter increase in millimeters while the abscissas represent successive 24-hour periods after exposure to a given temperature. An inspection of these graphs shows that for most of the fungi the maximum rate of growth was attained during the second 24-hour period, the exceptions being *delemar* and *chinensis*, which reached their maximums one day later. The data for *nodosus*, although not alto-

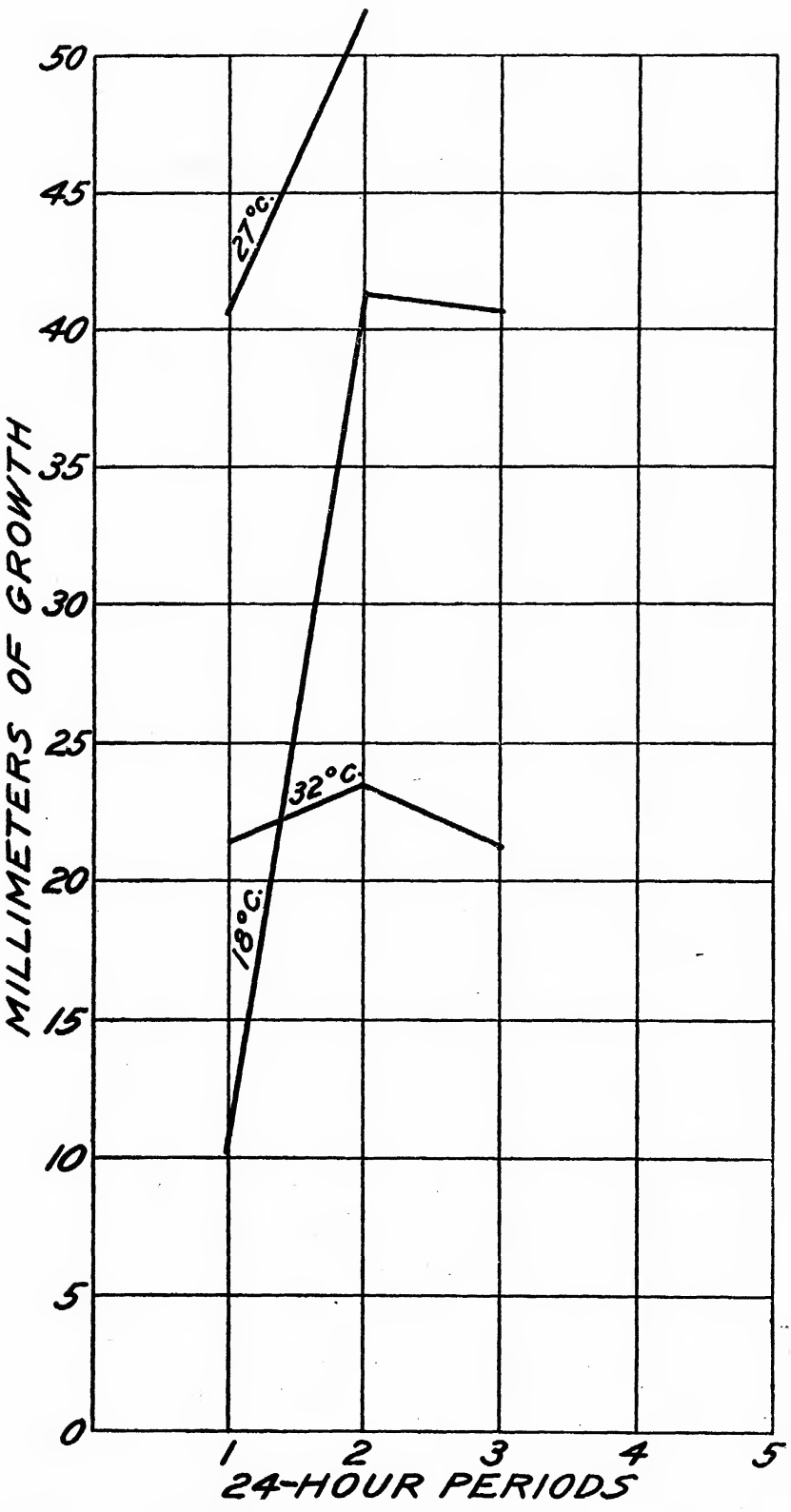

FIG. 15.—Graph showing the increase of growth of *Rhizopus reflexus* for consecutive 24-hour periods.

gether consistent, seem to indicate that it makes its most rapid growth some time after the second day. The growth rate varied with the temperature in most cases. In *arrhizus*, *tritici*, *maydis*, and *artocarpi* the obvious discrepancies are at the low temperatures, where the curve continues to rise after the second day. Certain of the fungi—*oryzae*, *artocarpi*, *arrhizus*, and *nigricans*—show a rather rapid decline in the rate of growth after the maximum is attained, while others, such as *microsporus*, *delemar*, and *reflexus*, show a more gradual decline. In *chinensis*, so far as the data show, there was no reduction in the rate of growth up to the time the plates were covered.

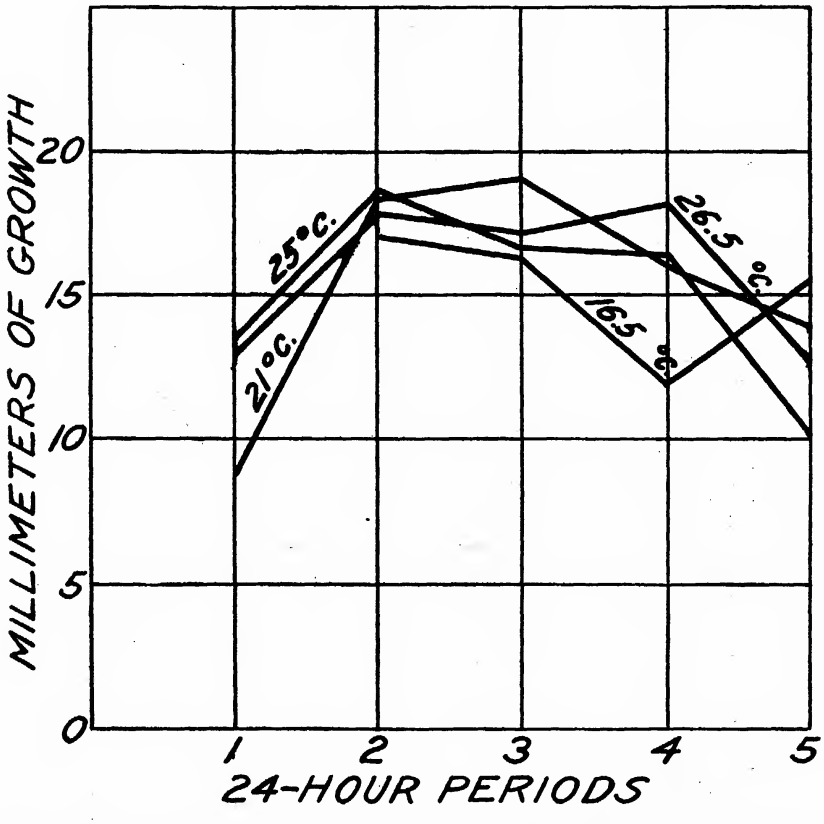

FIG. 16.—Graph showing the increase of growth of *Rhizopus microsporus* for consecutive 24-hour periods.

Fawcett (*6*) found that the fungi investigated by him made their greatest increase in growth during the first two days at the lower temperatures and continued to increase at a more gradual rate thereafter. Over a small range of the higher temperatures the rate first increased and then remained more or less constant till the end of the experiment, while at the highest temperatures a continuous decrease was noted from the first observational time. In general these results agree with those of the writers. Only in *oryzae*, *artocarpi*, and *tritici* at the highest temperatures (38°, 27.5°, and 42° C., respectively) do the graphs show a decrease, beginning with the first 24 hours. Nevertheless the fact that the maximum shifts, as explained above, indicates that such a condition would have been found to exist had the proper temperatures—for example, temperatures very near the maximum—been used.

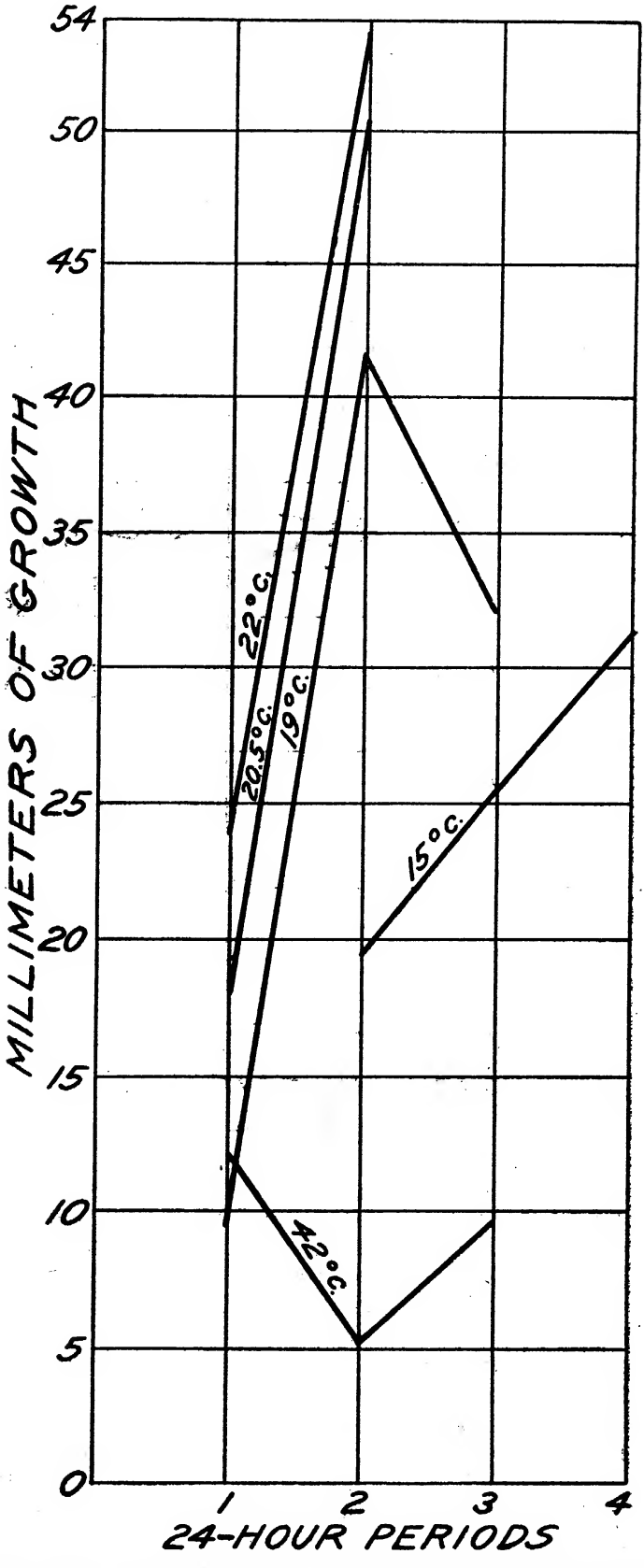

FIG. 17.—Graph showing the increase of growth of *Rhizopus tritici* for consecutive 24-hour periods.

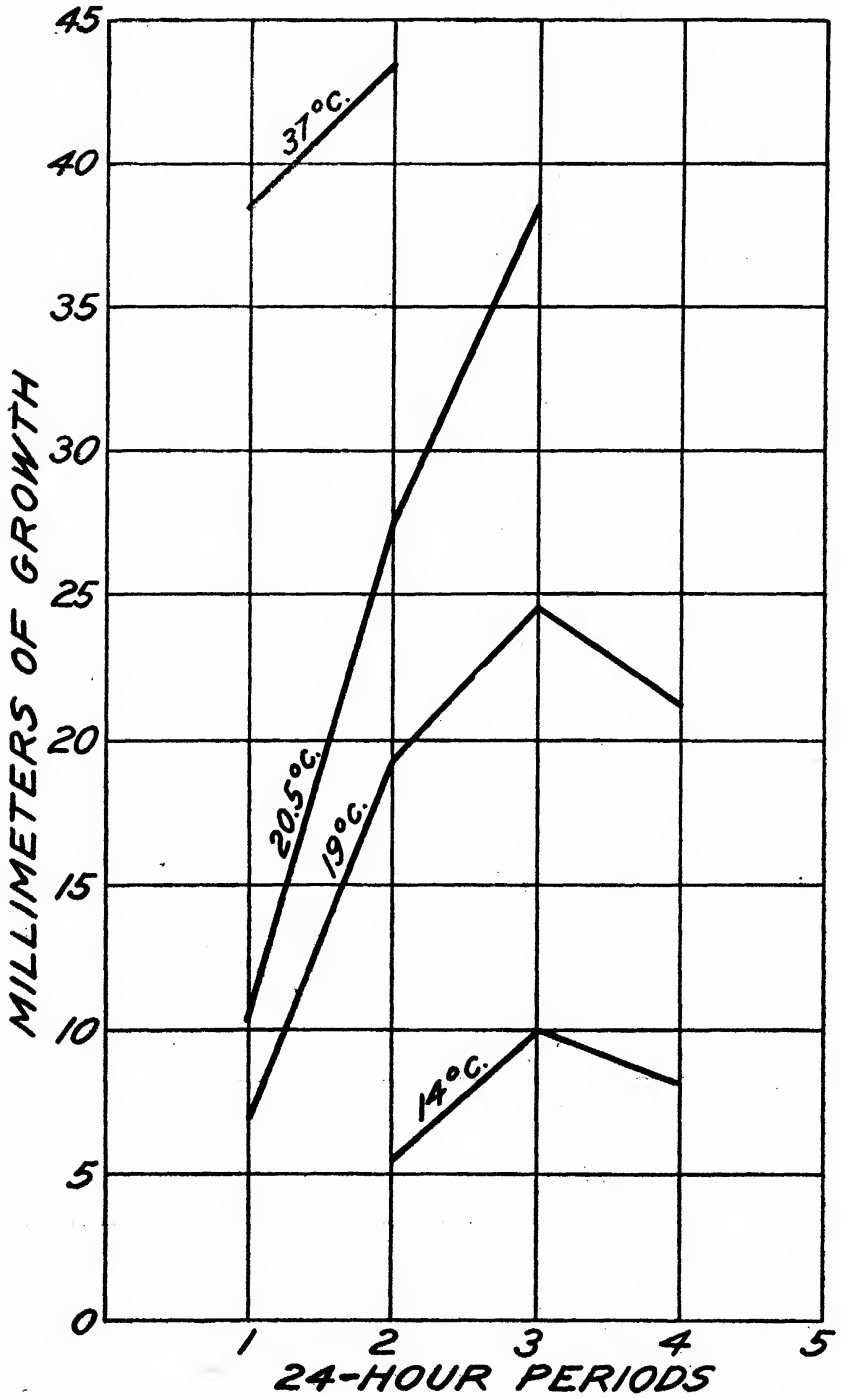

FIG. 18.—Graph showing the increase of growth of *Rhizopus delemar* for consecutive 24-hour periods.

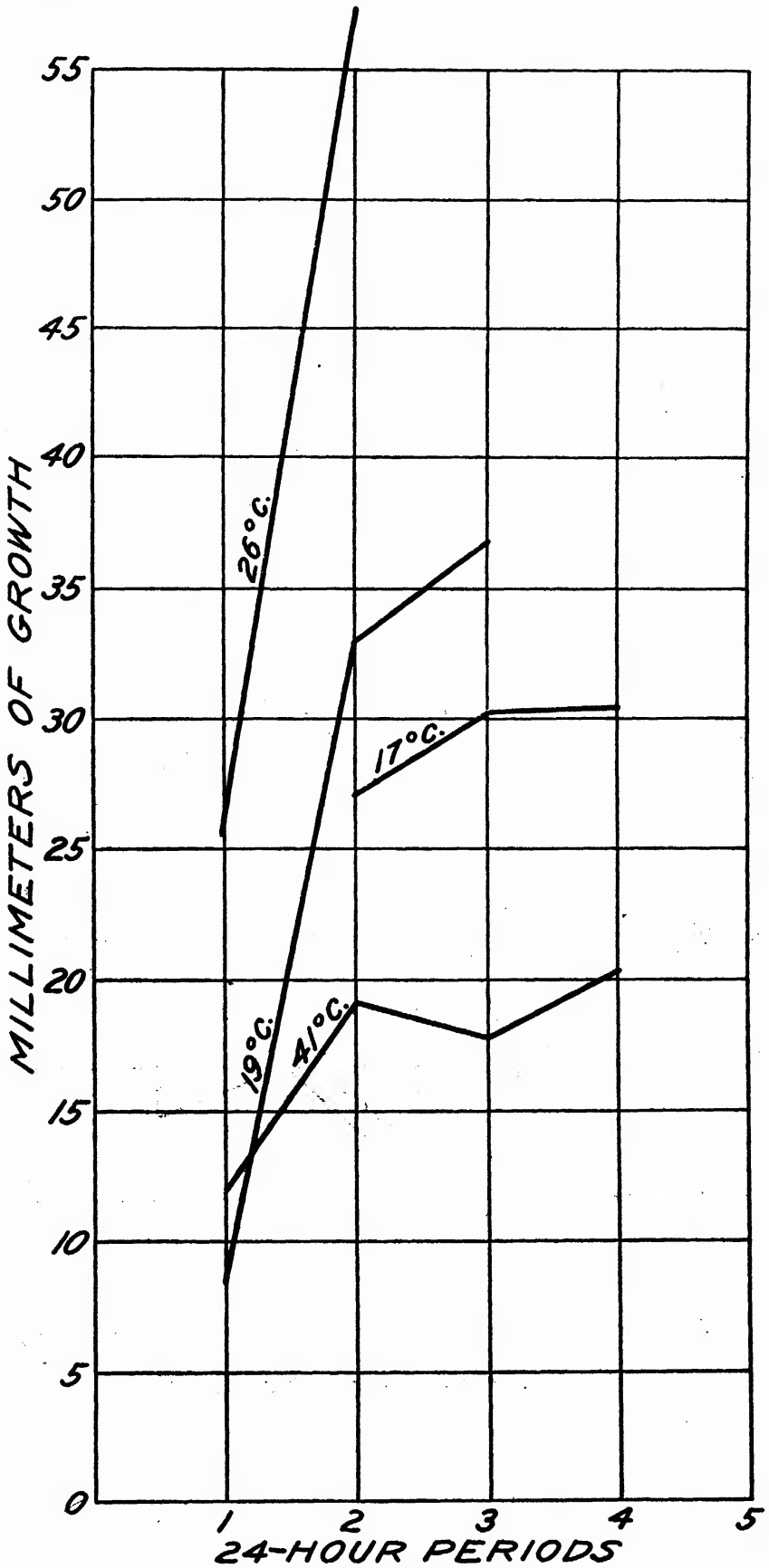

FIG. 19.—Graph showing the increase of growth of *Rhizopus nodosus* for consecutive 24-hour periods.

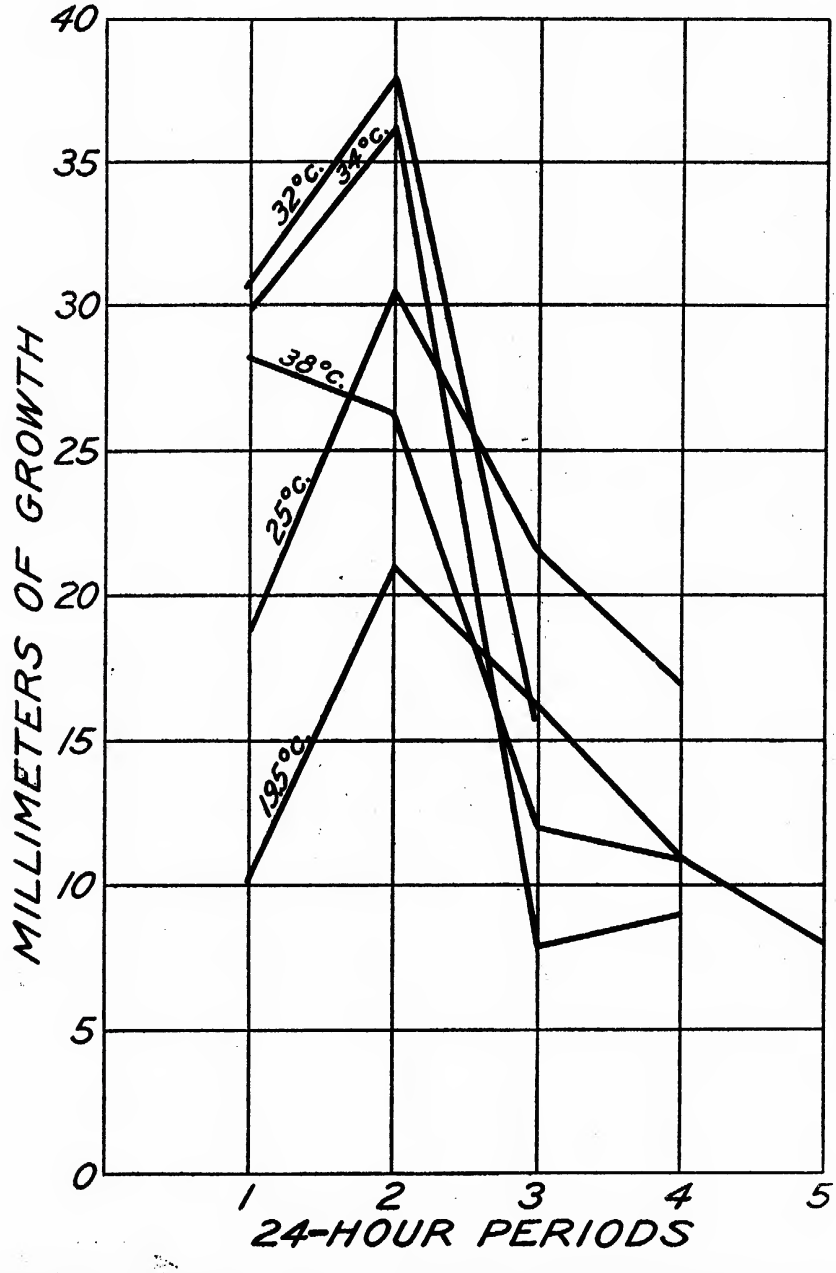

FIG. 20.—Graph showing the increase of growth of *Rhizopus oryzae* for consecutive 24-hour periods.

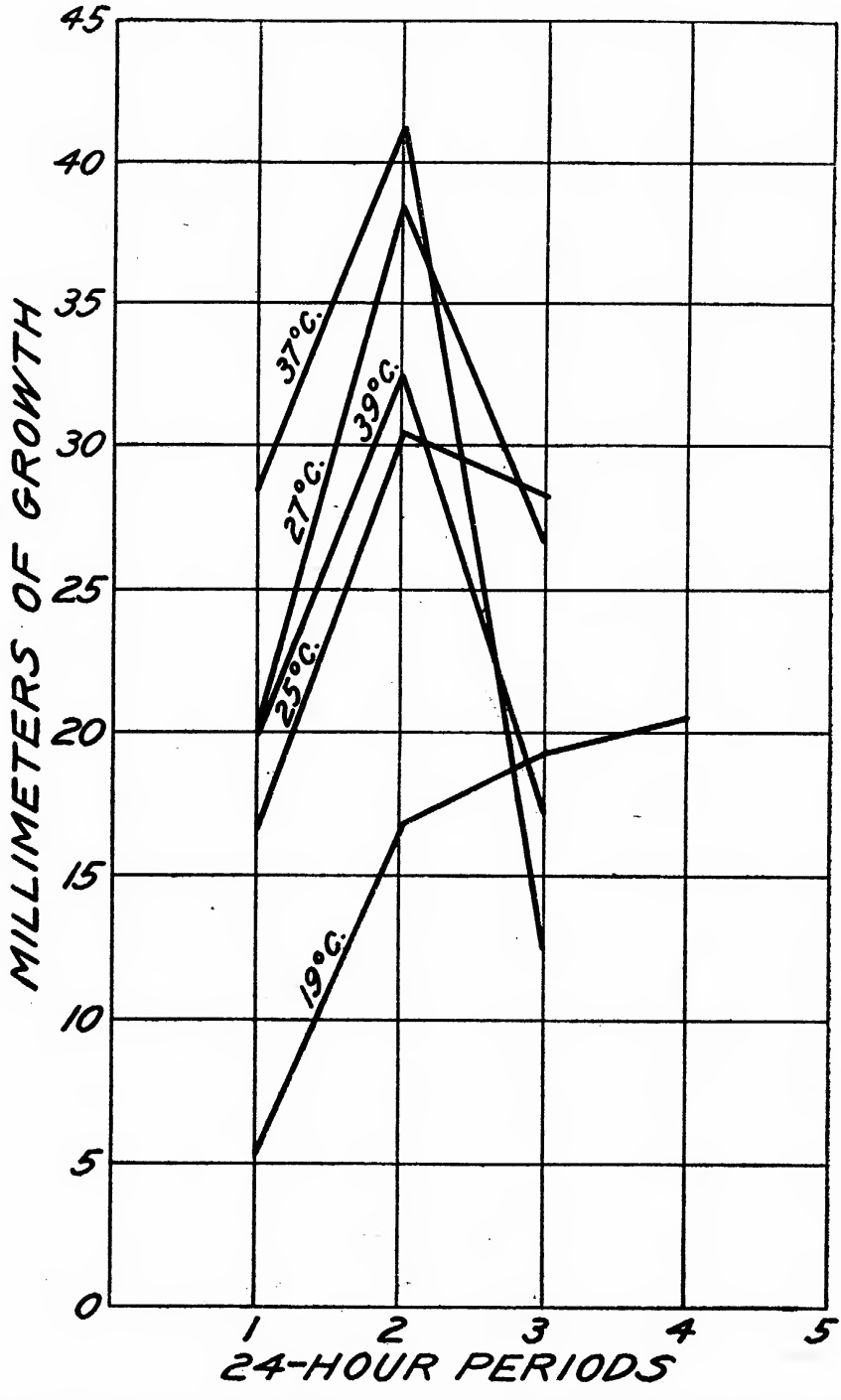

FIG. 21.—Graph showing the increase of growth of *Rhizopus arrhizus* for consecutive 24-hour periods.

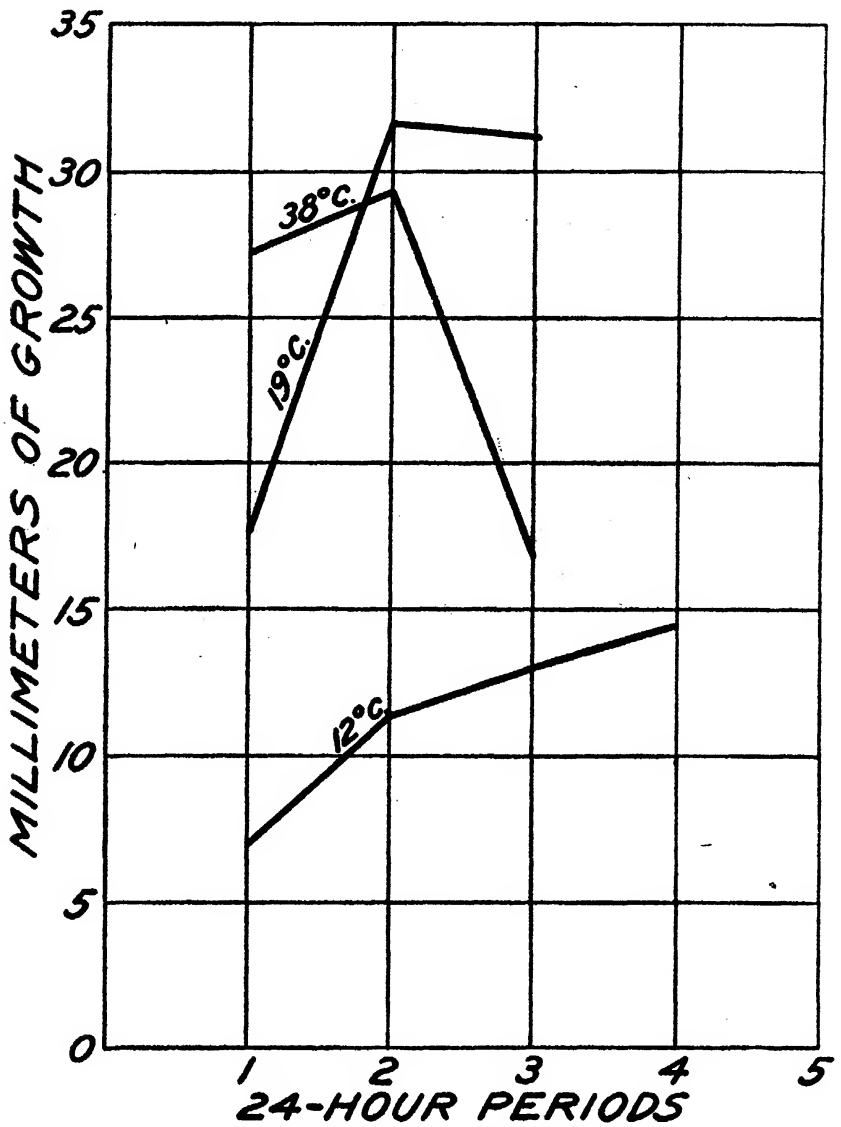

FIG. 22.—Graph showing the increase of growth of *Rhizopus maydis* for consecutive 24-hour periods.

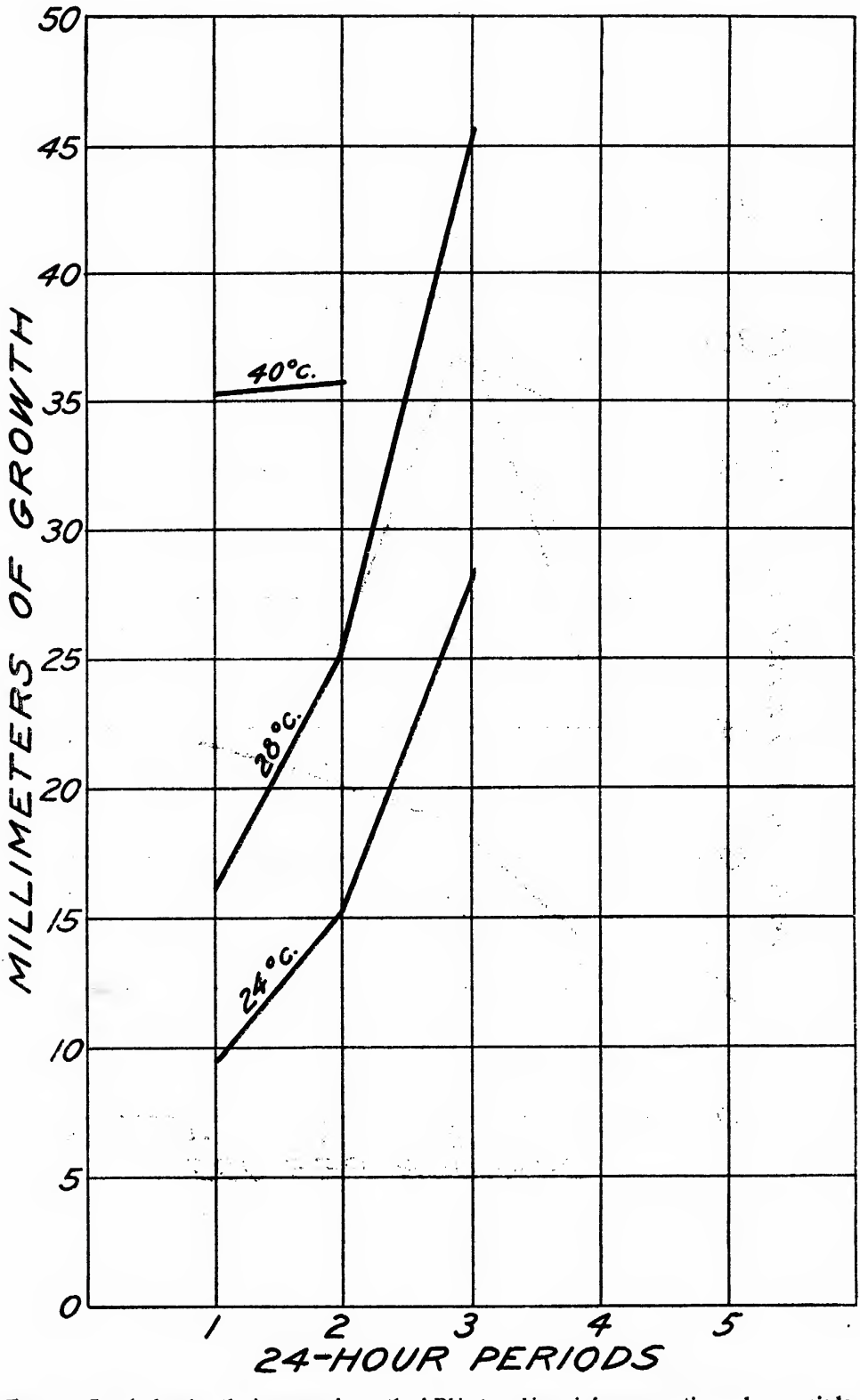

FIG. 23.—Graph showing the increase of growth of *Rhizopus chinensis* for consecutive 24-hour periods.

INFLUENCE OF TEMPERATURE ON FRUITING.

It has been repeatedly demonstrated by different investigators that the range of temperature at which fungi will sporulate is not coextensive with that at which they will grow. It has been proved that the various species of *Rhizopus* often cause considerable damage to vegetables and fruits. Although under suitable environmental conditions the causal organism can spread from host to host by means of their mycelium, their distribution is accomplished largely by means of the spores. It was therefore thought that information regarding the effect of temperature on sporangia formation, especially at the lower temperatures, might be of considerable practical value. The data accumulated during these studies are given, together with the data on the effect of temperatures on spore germination and growth, in Table I. In all cases those temperatures at which no growth took place and those at which some growth was made are listed in the columns headed "absent" and "present," respectively. The results show that fruiting of these fungi takes place over a considerable range of temperature. The optimum for some of the species is rather sharp and can be easily determined, while in other cases it extends over several degrees. *Chinensis*, *nigricans*, and *artocarpi*, which showed very sharp optimums for spore germination, fruited about equally well over a considerable range of temperatures. On the other hand, *arrhizus*, *reflexus*, and *nodosus* respond readily to temperature, both in regard to germination and fruiting.

Although *artocarpi* belongs to the low-temperature group, when its optimum is considered it stands among the highest-temperature forms with respect to its minimum for fruiting. *Nigricans* and *reflexus*, two low-temperature forms, also have a low minimum for fruiting; whereas *chinensis*, which has the highest optimum and maximum, has about the same minimum as the intermediate forms. The group consisting of *tritici*, *delemar*, *arrhizus*, *oryzae*, and *nodosus* has practically the same minimum, optimum, and maximum temperatures for both growth and fruiting. Hanzawa (*9*) found that *delemar* and *nodosus* did not fruit at 37° to 42° and 38° C., respectively. The maximum for *arrhizus* and *chinensis* was found by Hanzawa to be 36° and 38° to 42°, respectively. Of the five species just mentioned, *tritici* and *nodosus* alone formed sporangia at 8° to 10°. Hagem (*8*) gives 38° as the upper limit for *nodosus* and 36° as the maximum for *arrhizus*.

TABLE I.—*Minimum, optimum, and maximum temperature in degrees centigrade for spore germination, mycelial growth, and fruiting for 11 species of Rhizopus*

Species.	Spore germination.				
	Minimum.		Optimum.	Maximum.	
	Absent.	Present.		Present.	Absent.
artocarpi	.....	1. 5	26 to 29	33. 5	34. 5
nigricans	.....	1. 5	26 to 28	33	34
reflexus	.....	1. 5	30 to 32	36. 6	38
microsporus	.....	1. 5	26 to 28	33	34
tritici	.....	1. 5	36 to 38	44	45. 5
delemar	7	8. 7	36 to 38	44	45. 5
nodosus	.....	1. 5	36 to 38	44	45. 5
oryzae	7	9	36 to 38	44	45. 5
arrhizus	.....	1. 5	36 to 38	43. 6	45. 5
maydis	.....	.....	.....	.....	.....
chinensis	8. 5	10	43 to 45	51	52

Species.	Mycelial growth.				
	Minimum.		Optimum.	Maximum.	
	Absent.	Present.		Present.	Absent.
artocarpi	7	9	26 to 28	32	33
nigricans	1. 5	6. 5	23 to 26	30. 7	31. 5
reflexus	.....	1. 5	26 to 28	33	34
microsporus	.....	1. 5	25 to 27	30	32
tritici	1. 5	6. 5	33 to 35	42	45. 5
delemar	7. 5	9. 8	32 to 34	41	45. 5
nodosus	1. 5	6. 5	33 to 35	41. 5	45. 5
oryzae	7. 5	11	31 to 34	42	45. 5
arrhizus	1. 5	7. 4	32. 5 to 35. 5	40. 9	44. 9
maydis	1. 5	7. 4	30. 5 to 32. 5	40	44. 5
chinensis	7. 5	10. 4	37. 5 to 40. 5	49	51

Species.	Fruiting.				
	Minimum.		Optimum.	Maximum.	
	Absent.	Present.		Present.	Absent.
artocarpi	12. 5	17	22 to 27	30	32
nigricans	7	10	23 to 28	30	32
reflexus	7. 6	10. 6	26 to 28	31	32. 5
microsporus	(1)	(1)	(1)	(1)	(1)
tritici	12	16. 5	32 to 34	40	45
delemar	12	15	32 to 34	38	40
nodosus	12. 5	17	32 to 35	37	38. 5
oryzae	12	15	32 to 36	37	40
arrhizus	11	15	32 to 34	37	40
maydis	.....	.....	.....	.....	.....
chinensis	12	16	35 to 40	45	50

[1] Not tested.

CERTAIN ENVIRONMENTAL FACTORS INFLUENCING GERMINATION AND GROWTH

TEMPERATURE AT WHICH THE SPORES ARE PRODUCED

Wiesner (*22*) found that the temperature at which the mycelium of *Penicillium glaucum* Link developed influenced the time required for the germination of the spores. For example, he showed that spores which matured at 14° C. germinated more quickly at 3° than those which had developed at 3°, while the spores that were produced at 22° germinated more slowly than those produced at 14°. The influence which the growing temperature of *Rhizopus nigricans* has on the germination of its spores has been studied to some extent by the writers. Stock cultures were grown for 7 days on Irish potato agar in Erlenmeyer flasks at 16°, 24°, and 27°. Spore suspensions were prepared in portions of the same solution. The hanging drops were prepared as previously described and incubated at 26°. The spores grown at 16°, 24°, and 27° formed germ tubes as long as the spores in 2 hours, in 2 hours and 5 minutes, and in 2 hours and 25 minutes, respectively. Similar tests were made with *delemar* with the following results: Spores produced at 20.5°, 27.5°, 31.5°, and 36.5° germinated at 36.5° after 3 hours and 45 minutes, 3 hours and 50 minutes, 4 hours and 45 minutes, and 7 hours + (no germination within 7 hours but some later), respectively. Tests with some of the other species showed a similar tendency—that is, spores which were produced at the lower temperatures germinated more quickly than those from cultures grown at higher temperatures. It was thought that possibly the difference in the rate of germination of the spores produced at different temperatures was due to a difference in age. The physiological activities of the organism are admittedly more rapid at the higher temperatures, the spores therefore being produced more quickly and probably aging more rapidly. In order to determine whether the difference in the rate of germination of spores produced at different temperatures might have been due to the age of the spores in the foregoing experiments, another test was made in the following manner: Fifteen 100-cc. Erlenmeyer flasks, each containing 30 cc. of Irish potato agar, were inoculated with a loop of a suspension of *nigricans* spores. Five of these flasks were then held at each of the following temperatures: 10°, 20°, and 26°. The time required for the development of the sporangia was noted in each case, and germination tests were made from time to time. The tests were conducted at 25.5°. The spores from cultures which had been fruiting for 3, 5, 8, and 12 days at 20° and 26° germinated in 3¼ hours. One flask held at 26° became contaminated and was discarded. The final test for the spores produced at 20° was made after 20 days, and the time required for germination was the same as when the other tests had been made. The spores produced at 10° when 3, 5, and 15 days old required only 2¾ hours for germination to start. The other two cultures failed to fruit. These experiments show first that spores produced at the lowest temperature (10°) germinated 30 minutes earlier than those formed at 20° and 26°, and second that the age of the spores, at least up to 20 days, did not influence the time necessary for the beginning of germination. It is quite evident from these results that it is important to grow the stock cultures used in comparative experiments at the same temperature.

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Experiments were next conducted to determine whether the influence of temperature on the germination of the spores is reflected in the rate of mycelial growth. Irish potato agar contained in 5 Petri dishes was inoculated with a loop of a spore suspension from cultures of *tritici* and *delemar* grown at 20.5°, 27.5°, 31.5°, and 36.5° C. The plates were held at 34° to 35°. At the end of 24 and 48 hours the growth of *tritici* had reached a diameter of 50.6, 50.8, 48, and 45.8 mm. and 84, 92, 82, and 79.5 mm., respectively. The growth of *delemar* measured at the end of 24 and 48 hours 46, 42, 40.5, and 34.6 mm. and 79.5, 81.4, 77, and 78.8 mm., respectively. In general *tritici* showed a slight decline in the rate of growth when the spores were grown at the two highest temperatures. *Delemar* showed a more marked difference at the end of the first 24 hours, there being a gradual decrease from 46 to 34.6 mm.; but this was largely overcome during the next 24 hours, as shown by the fact that there was less than 1 mm. difference between the two extreme temperatures. The temperature at which the spores are produced undoubtedly influences their rate of germination and the early period of the growth of the mycelium developed therefrom. The evidence seems to point to the fact that different species react differently in this respect, some being much more sensitive to small changes in temperature than others.

CULTURE MEDIA

Tests were also made to determine to what extent, if any, different media would affect the rate of germination of *nigricans* spores. The media used were sweet potato decoction, beef bouillon, distilled water, string bean agar, Irish potato agar, and a synthetic agar. The media were placed on cover slips, spores from a single culture were sifted on, and the slips were then inverted over glass rings on slides in the usual manner and incubated at 26° C. The time necessary for germination in each case was as follows: Sweet potato decoction, 2 hours; beef bouillon, 2 hours and 25 minutes; distilled water, 3½ hours; string bean agar, 2 hours and 10 minutes; Irish potato agar, 2 hours and 20 minutes; and synthetic agar, 3 hours. Sweet potato decoction proved the best solution tried, and string bean the best agar. This experiment was duplicated on different days, using spores from a different culture with very similar results. It seems clear that Rhizopus spores require something more than water for good germination, since in distilled water a much smaller percentage of the spores germinated, and the germ tubes produced were considerably more slender than those supplied with nutrients. Tap water was found by comparative tests to be a less favorable medium for the germination of these spores than distilled water.

In order to test the effect of the substrate upon the rate of mycelial growth, plates were prepared in the usual manner, using string bean and Irish potato agars. The plates were inoculated with a platinum loop of the same suspension of *nigricans* spores in sterile distilled water. Five plates of each agar were used at each temperature. Measurements of the diameters of the mycelial disks were made in the usual way, and an average taken of the growth on each medium at the different temperatures. The results are given in Table II.

TABLE II.—*Comparative rate of growth of Rhizopus nigricans on string bean and Irish potato agars at different temperatures*

Temperature.	24 hours.		48 hours.	
	String bean agar.	Irish potato agar.	String bean agar.	Irish potato agar.
°C.	*Mm.*	*Mm.*	*Mm.*	*Mm.*
33	0	0	0	0
25.7	41.4	23.8	92	46.6
23.3	40.4	22	92	42.2
19.4	22.4	13.8	73.8	39
12.9	7.4	6.2	48	24
11.9	0	0	9	7.8
10.7	0	0	0	0

The data show that for the most part *nigricans* grows nearly twice as fast on string bean agar as on Irish potato agar. The plates of bean agar at 25.7° C. after 48 hours were completely covered, and it was evident that the diameter of the growth would have been somewhat greater had it not come in contact with the edge of the dish. The plates at 23.3° were just covered. The difference in the amount of growth on the two agars at 11.9° was very slight but yet apparent. The cardinal temperatures on the two media appear from the result of these experiments to be about the same. However, to determine this point with certainty, much closer temperatures than those used by the writers would have to be employed.

DEXTROSE

While studying the effect of different culture media upon growth it was observed that *nigricans* grew at 30° C. on a synthetic agar but failed to do so on the other media used.

It was thought that perhaps the differences in the acidity of the media might account for this shifting of the maximum temperature. Hence, an experiment was prepared in which Irish potato and beef agars made up to the same H-ion concentration as the synthetic agar, were used, together with synthetic agar as a control. The fungus, however, failed to grow on either of the modified agars at 30° C., but did grow on the synthetic agar. This experiment demonstrated that the H-ion concentration of the media was not the cause of the shifting of the maximum temperature of this fungus.

The synthetic agar differed from the other agars in sugar content as well as acidity, and for this reason tests were made to determine the effect of the addition of different quantities of dextrose to the media. Irish potato and beef agars were prepared to contain roughly 1, 5, 10, 15, and 20 per cent and carrot agar 1, 5, and 10 per cent dextrose. Ten plates were prepared in the usual way with these as well as with unmodified string beans, corn meal, and sweet potato agars. Plates of synthetic, Irish potato, beef, and carrot agars were prepared in the same manner and held as controls. All the plates were held at 30° C. for 48 hours, when the diameter of the growth was measured. This experiment was repeated several times. The average figures for all tests are presented

in Table III. In the cases where no growth is recorded the spores usually had germinated and formed short germ tubes but never formed a spot which spread appreciably beyond the area covered by the drop of spore suspension. Exposure for 48 hours to a temperature at which no growth took place was sufficient to kill the fungus, as was shown by the fact that it failed to make a further growth when held for 24 hours at a temperature suitable for its development.

TABLE III.—*Comparison of the growth made by Rhizopus nigricans at 30° C. on different agars with and without dextrose added*

Agar used.	Not modified.	With addition of dextrose.				
		1 per cent.	5 per cent.	10 per cent.	15 per cent.	20 per cent.
			Mm.	*Mm.*	*Mm.*	*Mm.*
Irish potato	No growth	No growth.	7	49	59	57
Beef	do	do	No growth.	30	52	54
Carrot	do	do	do	23	.....	.....
String bean	do	.....	.....	.....	.....	.....
Corn meal	do	.....	.....	.....	.....	.....
Sweet potato	do	.....	.....	.....	.....	.....
Synthetic	[1] 47	.....	.....	.....	.....	.....

[1] Contained 20 per cent dextrose.

The table shows that the strain of *nigricans* used (4652) did not grow at 30° C. on any of the unmodified agars tried except synthetic agar, which contained 20 per cent dextrose as originally prepared. Upon the addition of 10 per cent or more of dextrose to Irish potato, beef, or carrot agar growth did take place at this temperature. The addition of more than 15 per cent of sugar seemed to have little effect.

After it was determined that the maximum temperature for growth was raised by the addition of dextrose to the medium, experiments were conducted to learn the extent of this change. A loop of the same spore suspension was placed in the center of each of 20 Petri dishes, one-half of which contained unmodified Irish potato agar and the other half some of the same agar to which 20 per cent dextrose was added. The plates were then held at 31° C. Observations after 48 hours showed that the spores had not germinated on the unmodified agar while an average growth about 5 mm. in diameter had been made on the agar with 20 per cent dextrose added. A slight additional growth was made after 48 hours longer on the agar with sugar added. The results of these experiments seem to justify the conclusion that the maximum temperature for the growth of this organism on Irish potato agar is raised from 1° to 1.5° C. by the addition of 20 per cent dextrose to the medium.

That the presence of dextrose in the medium influences the minimum temperature was demonstrated by placing a loop of spore suspension in the center of each of 20 plates of Irish potato agar to one-half of which 20 per cent dextrose was added. The plates were then placed at a temperature of from 0.8° to 2° C. The spores had not germinated at the end of 17 days on the unmodified agar. On the other hand, germ

tubes of from three to five times as long as the diameter of the spores had formed on the agar containing 20 per cent dextrose. The differences on the two agars seem to indicate that the presence of 20 per cent dextrose in Irish potato agar lowers slightly the minimum temperature for germination.

To determine whether the optimum would be changed by the addition of dextrose, plates of each of the agars were prepared and placed at temperatures of 23, 26.5, 27.8, 28.7, and 30° C. Measurements of the diameters of the mycelial growths after two days at 23, 26.5, 27.8, 28.7, and 30° on the unmodified agar were 42, 65.6, 61, 51.8, and 0 mm., and on agar with 20 per cent dextrose added 66.4, 87.8, 92, 92, and 83.2 mm., respectively. These figures show that the optimum for growth on the unmodified agar was 26.5°, while that on the agar containing 20 per cent dextrose probably lay between 27.8 and 28.7°. The measurements of the diameters of the growths at 26.5, 27.8, and 28.7° after 24 hours were 26, 36, and 34 mm., respectively, showing that the optimum was nearer 27.8° than 28.7° but nearer the latter than 26.5°, and was raised 1.5° to 2°.

These experiments show rather conclusively that the presence of dextrose in Irish potato agar shifts the cardinal temperatures of this strain of *Rhizopus nigricans*. Thiele (*21*) also found that the addition of dextrose to glycerine and formic acid changed the maximum temperatures for the growth of one species of both Penicillium and Aspergillus. He observed that dextrose added to glycerine and to formic acid caused a shifting of the maximum temperatures for the growth of Penicillium of 5° and 4° C., respectively. The temperature maximums for the germination of Aspergillus spores and for mycelial growth were raised about 2° and 3°, respectively, by the addition of different amounts of dextrose. On the other hand the maximum for total growth was raised about 2° when growing on glycerine and lowered 3° on the higher concentrations of formic acid. He concluded that the nutritive value of a substance is correlative to a certain extent with the temperature. Similarly, Bruderlein (*3*) found the growth of *R. maydis* to be retarded on potato, almost arrested on carrot, and prohibited entirely on agar at 42°. Peltier (*15*) showed that the minimum temperature for the growth of *Psuedomonas citri* Hasse when grown in beef bouillon and on soluble starch agar is slightly higher than on cooked potato cylinders, its maximum being slightly higher in the former than on the two latter media. Likewise, Goss (*7*) found that *Fusarium trichothecioides* Wollenw. made its best growth on a synthetic solution at 25° and no growth at 5°. On the other hand, the optimum lay between 15° and 20°, and a weighable growth was made at 5° when this fungus grew on Link's potato extract.

Although it has been demonstrated by the writers and others that the cardinal temperatures of some fungi vary with the nature of the substrate, the principles underlying this phenomenon have never been explained. Thiele (*21*) attributes the shifting of the maximum temperature of the fungi with which he worked to the different nutritive value of the substrate at different temperatures. It may be in the case of the writers' experiments that the dextrose when present in considerable concentration (10 per cent or over) was more available and hence supported growth at a higher temperature than when it was present only in small amounts. It is a well-known fact that increasing the molecular concentration of a solution raises its boiling point and lowers its freezing point. It is not known whether or not the increased concentration of the dextrose in the substrate causes a sufficient increase in the molecular

concentration of the cell sap, thereby protecting the protoplasm of the cell against coagulation by the heat to account for the raising of the temperature maximum 1° to 1.5° C. The high concentration of sugar in the substrate may also act to lower the water content of the cells of the fungus and thereby increase the concentration of the cell sap which would tend to raise its maximum and lower its minimum. However, the true explanation of this phenomenon must await the accumulation of more experimental data.

DISCUSSION

It is apparent from the foregoing curves and tables that the cardinal temperatures for spore germination, growth, and fruiting of the fungi studied vary somewhat. In general the spores will germinate at a temperature too low for mycelial growth. A higher temperature is required for fruiting than for growth. The same thing holds true for the optimum temperatures, although not to the same extent. The optimum for germination is always higher than for growth and fruiting, while in most cases the optimum for fruiting is about the same as that for growth. The slight differences shown in Table I may be due to the fact that these figures give the optimum temperatures tried rather than the true optimums. The latter probably does not differ from the former more than a plus or minus 1 or 2° C., since the temperature of the incubators usually differed only from 2 to 5°, the maximum differences being between the extreme temperatures. The optimum temperature for fruiting is often not so well defined as that for growth, and the latter less so than for spore germination.

In each case there is a gradual gradation in the maximum from that at which the spores will germinate to that at which fruiting will take place, the maximum for growth being about midway between that for germination and that for fruiting. This fact may be explained in part at least in two ways; first, the spores germinate quickly, probably before the heat acts injuriously; and second, the spores are probably less sensitive to heat than are the germ tubes. Spores which before germination are uninjured by the heat may be readily killed after they germinate. At a slightly lower temperature growth becomes evident but ceases after a time, and the mycelium is killed before sporangia are formed.

The time factor is of greater importance at the minimum than at any other temperature. At low temperatures, germination is greatly retarded, the growth being often so sparse that no measurable felt is produced within the time limit of these experiments.

The genus Rhizopus includes some of the best known, most widely distributed and most destructive of the fungi. *Nigricans* is perhaps the most destructive, since it attacks a wide range of hosts under widely different conditions, being especially destructive to tomatoes, strawberries, and sweet potatoes. However, this species is somewhat limited in its field of destruction by temperature. For example, in these experiments its spores were killed by a comparatively short exposure at a temperature of 34° to 35° C., and it made a very slow growth at 6.5° (35 mm. in 30 days) and no appreciable growth at 1.5° after 30 days. Although some variation in its response to temperature on different substrates may be expected, there is little doubt that decay of fruits and vegetables by this organism can be prevented or retarded by proper cold storage. A temperature as high as 7° or thereabouts will prevent fruiting for several days and hence retard the spread of this fungus. It

must be kept in mind, however, that other species may be destructive at temperatures which are not favorable for the development of *nigricans*, although at the present time little is known regarding the distribution and destructiveness of the other species under natural conditions in the United States.

SUMMARY

(1) The effect of temperatures on the spore germination, mycelial growth, and fruiting of 11 species of Rhizopus has been studied, and the results are presented in this paper.

(2) These 11 species fall into three groups as regards their response to temperature. *Chinensis* has a temperature maximum and optimum several degrees higher than any of the other species and hence can be easily separated from them; *nigricans*, *microsporus*, *reflexus*, and *artocarpi* make up a group having a low optimum and maximum; while the 6 remaining species, namely, *tritici*, *nodosus*, *delemar*, *oryzae*, *arrhizus*, and *maydis* constitute an intermediate group.

(3) In general the spores will germinate at a temperature above the maximum for continued growth.

(4) The optimum for germination for all the species is higher than for growth and fruiting, while in most cases the optimum for fruiting is about the same as for growth.

(5) The temperature at which the spores are produced influences to some extent their rate of germination and the early period of the growth of the mycelium developed therefrom. Spores of *nigricans* produced at 10° C. germinated in 30 minutes less time than those formed at 20° and 26°. Spores of this species from different cultures grown under like conditions germinated equally well, regardless of age, up to 20 days.

(6) Spores of *nigricans* germinate in a considerably shorter time in a nutrient solution than in water. Sweet potato decoction proved to be the best liquid and string bean agar the best solid medium tried. In the comparative tests made this fungus grew nearly twice as fast on string bean agar as on Irish potato agar.

(7) The presence of 20 per cent dextrose in Irish potato agar changed the cardinal temperatures of the strain of *nigricans* studied from 1 to 2° C.

(8) *Nigricans*, which seems to be the most destructive member of this genus, under natural conditions is somewhat limited in its field of destruction by temperature. The spores were invariably killed at 35° C., and growth was very sparse and slow at 6.5°. At 1.5° no appreciable growth was made on Irish potato agar in 30 days.

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NUTRITION OF PLANTS CONSIDERED AS AN ELECTRICAL PHENOMENON [1]

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INTRODUCTION

When wheat seeds are placed in a suitable nutrient solution and germination begins, the demand of the embryo and tiny seedling for food is first upon the stored up material in the seed. As the plumule and radicle develop, however, absorption of food from the nutrient solution begins and gradually increases with increased growth. During the germination process there is first an exudation of plant food, particularly phosphorus and potassium, from the seed, with little or no absorption from the nutrient solution, but, when the plumule has reached a length of 2 or 3 cm. and has broken through its sheath, a distinct demand for food begins to be manifested in the tissues.

It is of much importance in fertilizer investigation to note that this demand of the plant for nutrient material may be measured by its pull upon the nutrient solution, and it is equally important from a physiological standpoint to note that this demand may be modified in certain ways and may even be augmented or built up by withholding any of the food elements. There is a "residual effect" that is very pronounced in plants; the desire for food when not available is carried over a long period of time and it seems to be cumulative. In this respect a plant seems to behave very much like an animal organism, the demand for food increasing as a fast continues. There is another very pronounced phenomenon that might well be termed a "time factor." A plant will not necessarily absorb twice as much nutrient material in two hours as it absorbs in one. It seems to possess the power to prepare reserves—that is, to form compounds in one part of its system which may be translocated to another part when needed.

It seems that this "demand" for food must be taken into account in all studies of absorption.

DEMAND OF WHEAT PLANTS IN THE PRESENCE OF A CONTINUOUS SUPPLY OF PLANT FOOD

About a dozen culture pans were prepared, each pan holding 2,500 cc. of nutrient solution and containing about 500 plants. The plants were grown by sprinkling seed upon floating perforated aluminum disks, and the nutrient solutions, containing 125 parts per million each of nitrogen (N), potash (K_2O), and phosphoric acid (P_2O_5) as sodium nitrate, potassium chlorid, and sodium phosphate, were changed daily. There was thus an abundance of plant food always at the disposal of the plant, and this was determined by measurements made from time to time. A

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concentration of 125 parts per million is just about the concentration producing the best growth under these conditions, as shown by other work; and as will be seen later, there was very little likelihood of the plant absorbing very much more plant food than was actually needed for its development.

Beginning with the fifth day after the seeds were placed in the solution, on every third day enough plants for an analysis were drawn from the pans, care being taken to draw some plants from each pan to equalize variations in the cultures as much as possible. When one of the culture pans fell below the normal it was discarded. In this way a representative set of plants could be withdrawn each time. The analyses of the plants, together with that of the original seeds, are shown in Table I, the results being expressed on the basis of 100 plants.

TABLE I.—*Analyses of 100 wheat seedlings, grown in nutrient solutions, at different stages of growth*

No.	Stage of growth.	Dry weight.	N	K_2O	P_2O_5
		Gm.	*Gm.*	*Gm.*	*Gm.*
1	Original seeds	2. 14	0. 0490	0. 0295	0. 0210
2	5 days old	1. 89	. 0565	. 0272	. 0184
3	8 days old	2. 05	. 0742	. 0489	. 0250
4	11 days old	1. 82	. 0812	. 0869	. 0308
5	14 days old	1. 95	. 0924	. 1203	. 0336
6	17 days old	2. 30	. 1160	. 1311	. 0570
7	20 days old	3. 04	. 1410	. 1466	. 0740
8	23 days old	3. 50	. 1946	. 1610	. 1020
9	26 days old	4. 64	. 2110	. 2018	. 1220

It will be noticed that there is a falling off in dry weight, in potash, and in phosphoric acid, but not in nitrogen, up to the fifth day. The loss in weight is, of course, due largely to the decomposition of starch and sugar of the seeds and to the evolution of carbon dioxid. This loss often amounts to as much as 40 per cent of the dry weight before enough plant food and carbon dioxid are absorbed to balance the loss. Many experimenters with wheat seedlings, when using dry weight as a criterion, fail to realize the fact that in the first stage of growth they are dealing with a diminishing quantity. The loss of potash and phosphoric acid always takes place at the beginning of germination, due to the exudation of these plant foods from the seed. It might be added that these very salts that are exuded from the seeds are absorbed by the seedling in a few days or as soon as the radicle has become sufficiently developed. There is usually little exudation of nitrogen for the reason that the nitrogen of the seed exists in organic combinations, protein, and is not readily dissolved out by water.

These results when plotted are represented by figure 1.

By cutting the curves through any particular date it seems possible to determine the relative demand of the plant at that age for the three important plant foods. It is also evident that the relative demand for food changes very rapidly as the plant develops. Beginning about the fifth day, when the plants begin to feed, the curve for potash rises very rapidly. The little seedling awakes to life with a ravenous appetite for potash, out of proportion to other plant foods. When the seeds are moistened and warmed preparatory for germination, the potash which

is stored up in the seed, and which is fairly evenly distributed through it, begins to move very rapidly toward the end containing the germ or embryo. In the germination, when the embryo has broken through the bran coating and is just large enough to get hold of with the thumb and finger nails, it contains about 50 per cent of the total potash of the seed. The seed does not contain enough potash to satisfy the demand of the little seedling, so it begins early to feed heavily on the nutrient of the solution. Probably, in the natural course of life, the very first food absorbed by the little seedling is potassium. The absorption of nitrogen is steady and comparatively uniform, so after 18 or 20 days the curves for potash and nitrogen cross each other. The absorption of phosphoric acid is regular also.

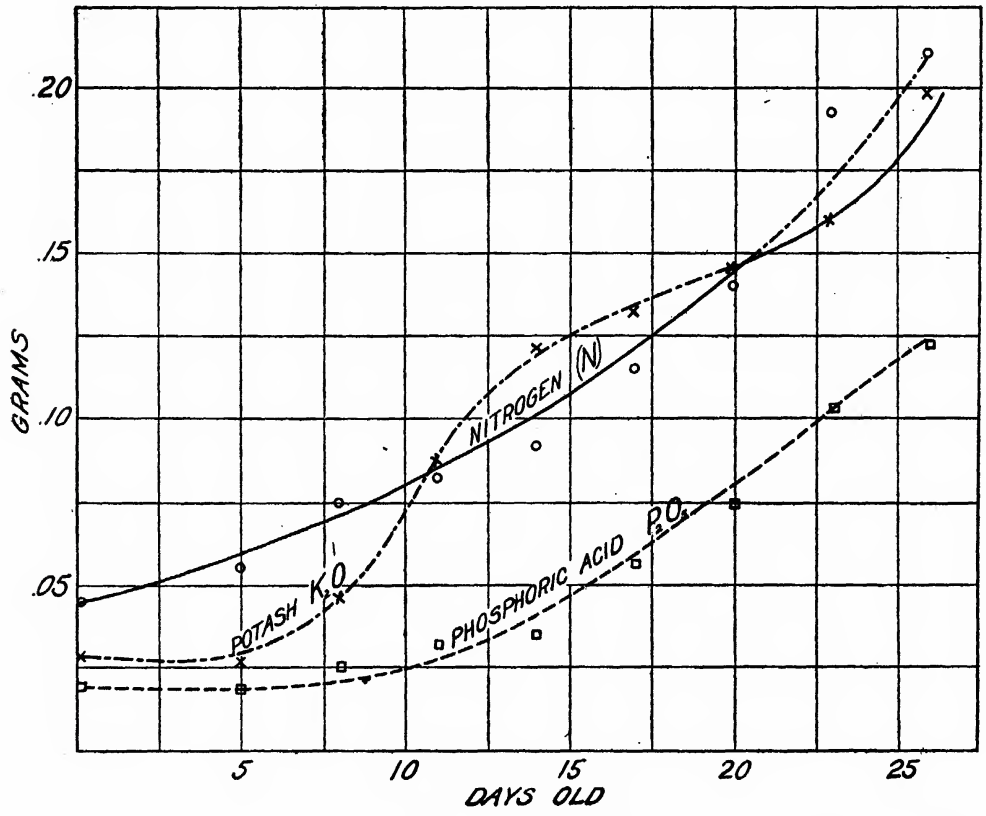

FIG. 1.—Graph illustrating the absorption of plant foods from nutrient solutions by wheat seedlings at different stages of growth.

Without submitting all the data, the curves for a duplicate determination, made about one month later, are given in order to bring out the crossing of the curves of nitrogen and potash (fig. 2).

The data presented give an idea of the nutrition of a plant when feeding full time and under favorable conditions. But a plant in nature does not necessarily have ideal conditions in which to grow. A low moisture content of the soil may temporarily put a stop to nutrition or the fluctuation of plant food, particularly nitrates, in the soil solution may also slow down, or even stop, absorption. It is, therefore, important to know what percentage of the time is actually necessary for absorption and whether a plant can absorb enough plant food in one period of time to last it over another.

DEMAND OF WHEAT PLANTS GROWN IN NUTRIENT SOLUTIONS FOR FRACTIONAL PARTS OF A DAY

Seedlings were placed in culture pans containing a full nutrient solution of a concentration of 125 parts per million each of nitrogen, potash, and phosphoric acid, as before described, and allowed to feed for fractional parts of the day. At the end of the period allotted to each lot to remain in the nutrient solution, the disks with the seedlings were taken up and washed off and placed in distilled water for the remainder of the day. This process was repeated daily for 10 days, and the plants then analyzed for nitrogen, potash, and phosphoric acid. In Table II is

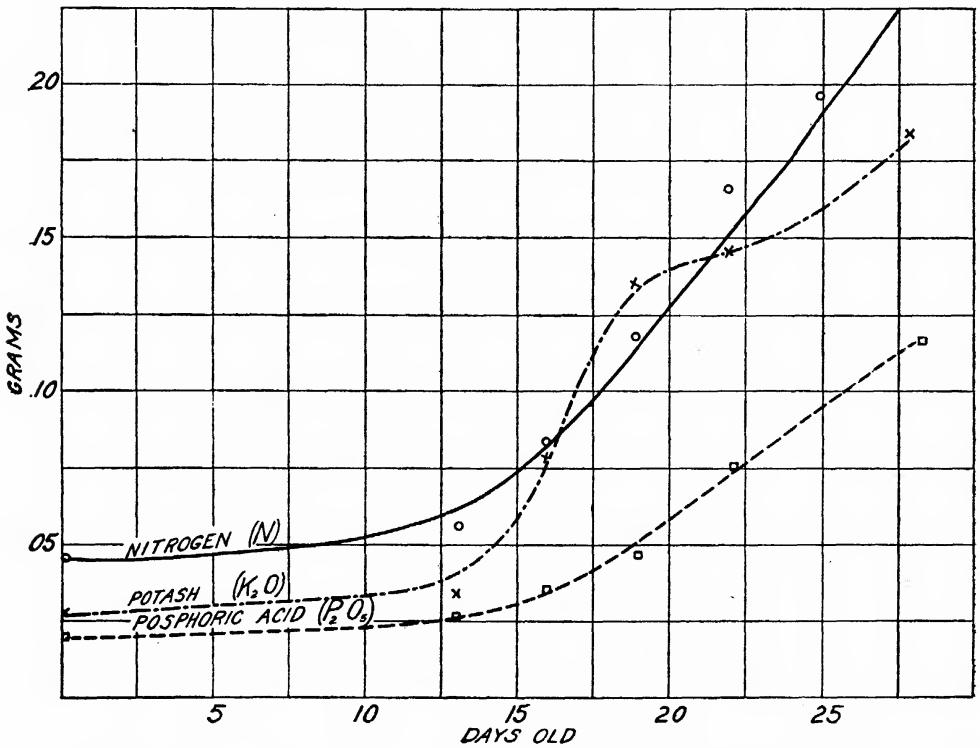

FIG. 2.—Graph illustrating the absorption of plant foods from nutrient solutions by wheat seedlings at different stages of growth.

shown the number of hours each day the plant remained in the nutrient solution and the quantities of plant food absorbed.

TABLE II.—*Analyses of 100 wheat seedlings feeding fractional parts of the day for 10 days*

No.	Hours per day in nutrient solution.	Dry weight	N	K_2O	P_2O_5
		Gm.	*Gm.*	*Gm.*	*Gm.*
1	Control 0	5.32	0.0770	0.0318	0.0420
2	1	5.15	.0882	.0838	.0560
3	2	5.60	.1022	.1047	.0750
4	4	5.20	.1120	.1366	.0840
5	6	5.80	.1540	.1862	.0960
6	8	5.30	.1680	.1940	.1040
7	12	5.73	.2072	.1858	.1060
8	24	6.28	.2352	.2475	.1190

It will be seen by referring to Table II and figure 3 that the absorption of plant food for 1 hour per day, and other fractional parts, is out of proportion to what might be expected if time alone governed absorption. For example, subtracting the amount of potash in the original seed, or in the control, from the analysis of the plants grown 1 hour a day in the nutrient solution, we get 0.0520 gm. of potash actually absorbed by the plants during that time. In the same way by subtracting the control from those grown 24 hours a day in the nutrient solution,

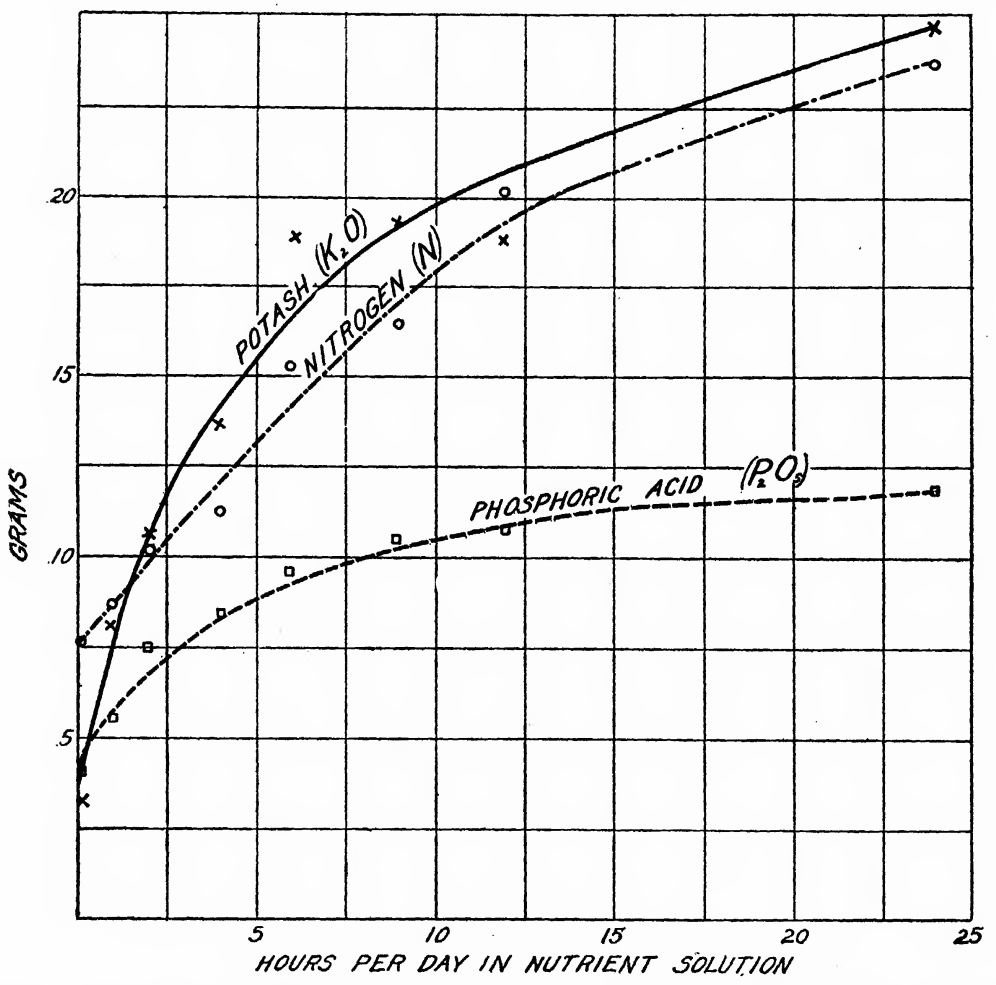

FIG. 3.—Graph illustrating the absorption of plant foods by wheat seedlings when grown for fractional parts of the day for 10 days in nutrient solutions.

we get only 0.2157 gm. of potash actually absorbed in 24 hours. If the rate of absorption of the plants kept in the nutrient solution 1 hour a day had been maintained in the plants kept in 24 hours a day, we would have had an absorption of 1.248 gm. instead of 0.2157 gm. In other words, we get over five times as much potash absorbed in the 1-hour periods as might be expected if time alone governed absorption. This phenomenon is true with other plant foods; the curves rise very abruptly from the start and flatten out as the time increases. The demand or desire for food seems to accumulate until the food becomes available; then an abnormal rate of absorption takes place. The figures, when plotted, are represented in figure 3.

The abrupt rise of the potash curve is noticeable, showing the demand for potash in the seedling was out of proportion to that for other plant foods. The cumulative demand, brought out in this experiment, seems to be true, not only for fractional parts of the day but for much longer periods.

DEMAND OF WHEAT PLANTS AFTER BEING HELD IN DISTILLED WATER FROM 2 TO 17 DAYS

Seven culture pans of soft wheat were germinated and grown in distilled water for 4 days. One pan was then transferred to a nutrient solution of 125 parts per million each, nitrogen (N), potash (K_2O), and phosphoric acid (P_2O_5), while all the others were kept in distilled water. At the end of 3 more days a second pan was placed in the nutrient solution, and this process continued every 3 days until six of the lots had been taken from distilled water and placed in nutrient solution. They were then allowed to grow for 2 more days, when they were taken down and analyzed. Thus the first pan had been feeding from a good nutrient solution for 17 days, while the last pan had been feeding only 2 days. The last pan having been grown in distilled water for 15 days had no increase in growth over the control when placed in the nutrient solution. The results of the analyses are shown in Table III.

TABLE III.—*Analyses of 100 wheat seedlings grown two or more days in nutrient solutions*

No.	Days in nutrient solutions.	Dry weight.	N	K_2O	P_2O_5
		Gm.	*Gm.*	*Gm.*	*Gm.*
1	Control 0	6.28	0.0840	0.0613	0.0400
2	2	6.60	.1410	.1154	.0587
3	5	6.52	.1904	.1685	.0750
4	8	7.12	.2268	.2090	.0813
5	11	6.80	.2674	.2350	.0973
6	14	6.80	.2604	.2361	.0908
7	17	7.00	.2912	.2580	.1440

It will be seen by referring to Table III that with each plant food element the absorption for the 2-day period was out of proportion to that of the 17-day period. By subtracting the quantity of potash in the control, for example, from that of the plants grown for 2 days in the nutrient solution, we get 0.0541 gm. of potash actually absorbed in 2 days. In like manner, by subtracting the control from the 17-day plants, we get only 0.1967 gm. potash actually absorbed in 17 days, when we should get 0.459 gm. of potash if time alone governed absorption. These results plot very well, showing that the demand is fairly regular and cumulative. This experiment was repeated, changing the plants in 2-day periods, with similar results.

If Tables IV and V, showing the rates of absorption of the different plant foods, are studied, other interesting relations are brought out. From Table IV it appears that the absorption of potash for the short period is much more pronounced than the absorption of other plant foods. On the other hand, when seedlings are left in the nutrient solution for a number of days (Table V), the absorption of potash and nitrogen shows a remarkable agreement in the rate. This is brought out in figures 4 and 5.

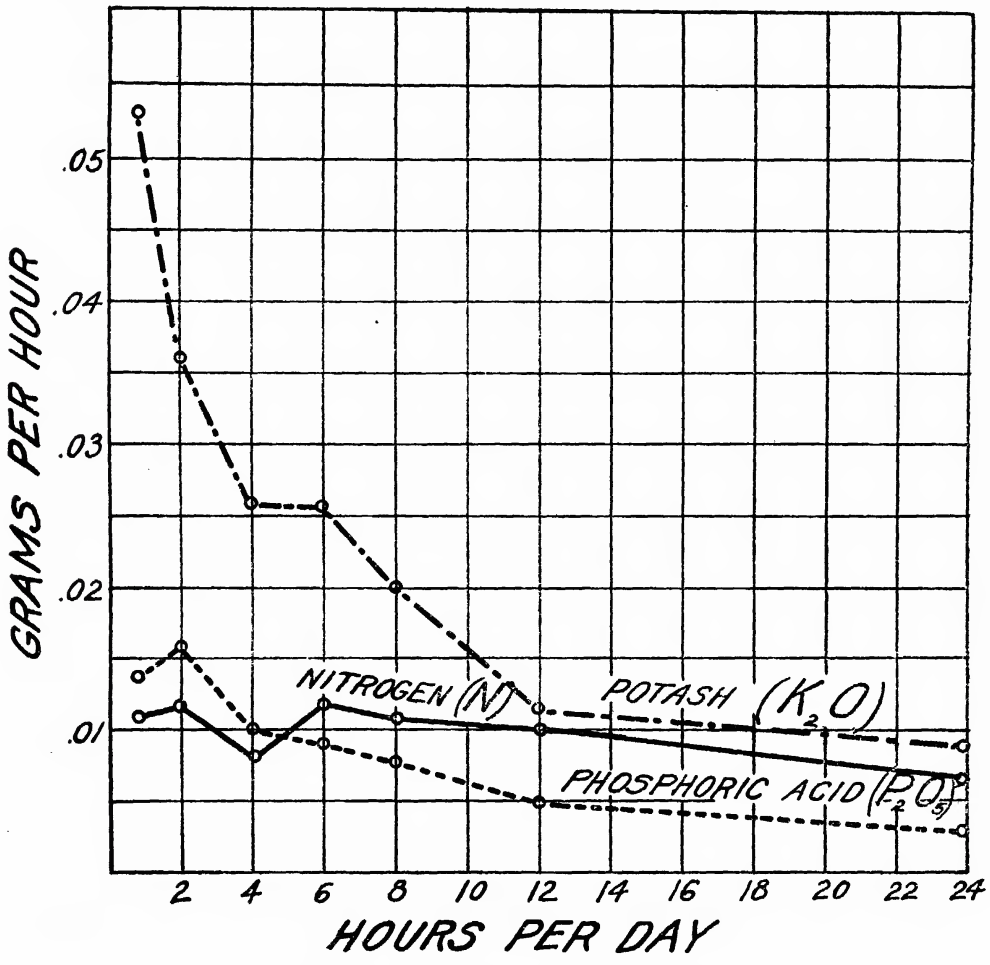

FIG. 4.—Graph illustrating the rate per hour of the absorption of plant foods by wheat seedlings when grown for fractional parts of the day in nutrient solutions.

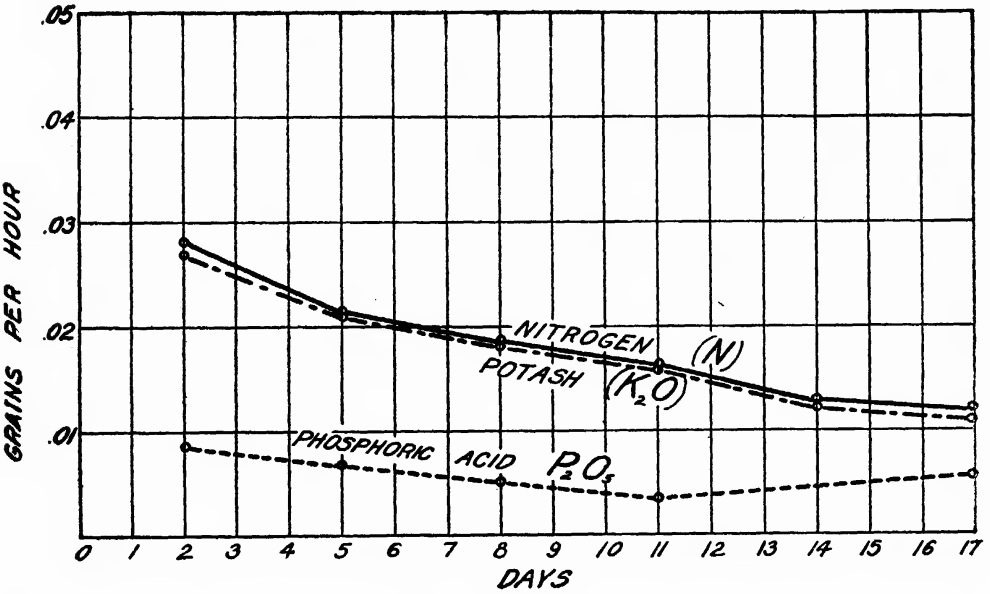

FIG. 5.—Graph illustrating the rate per day of the absorption of plant foods by wheat seedlings when grown for two or more days in nutrient solutions.

TABLE IV.—*Rate of absorption of plant foods per hour when wheat seedlings were feeding fractional parts of day*

[From Table II]

Hours per day.	Rate of absorption per hour.		
	N	K_2O	P_2O_5
	Gm.	*Gm.*	*Gm.*
1	0.00112	0.00520	0.00140
2	.00126	.00364	.00165
4	.00087	.00262	.00105
6	.00128	.00257	.00090
8	.00114	.00203	.00078
12	.00108	.00128	.00053
24	.00066	.00090	.00032

TABLE V.—*Rate of absorption of plant food per day when wheat seedlings were feeding for long periods*

[From Table III]

Days in solution.	Rate of absorption per day.		
	N	K_2O	P_2O_5
	Gm.	*Gm.*	*Gm.*
2	0.0285	0.02705	0.00935
5	.0213	.02144	.007
8	.01785	.01846	.00516
11	.01667	.01579	.00521
14	.0126	.01249	.00363
17	.01219	.01157	.00612

DEMAND OF WHEAT PLANTS GROWN FOR AN INITIAL PERIOD IN A FULL NUTRIENT SOLUTION AND SUBSEQUENTLY STARVED

The second of the two experiments described above was made in an effort to measure the demands for plant food that might be developed in plants that had been grown in distilled water and had never had any food supply except that contained in the seed. It was thought probable that other results might be obtained if the plants were first fed heavily, then allowed to fast, and the demand brought about by this fast measured in a second feeding period. A separate set was started, and when the plumules had reached a length of about 1 cm. all the culture pans were placed in a full nutrient solution of a concentration of 100 parts per million each nitrogen (N), potash (K_2O), and phosphoric acid (P_2O_5) and allowed to remain in this solution with frequent changes of solution for seven days. All were then placed in distilled water and allowed to stand seven days or more, when No. 4 was again placed in the nutrient solution. After one more day No. 3 was placed in the nutrient solution, and after another day No. 2 was put in the nutrient solution. After one more day all four sets were taken down for analysis. In this way, the plants were all given plenty of food for the first period, then allowed to fast and to develop an appetite during the second period, which was measured in the third period. This is shown in Table VI.

TABLE VI.—*Analyses of 100 wheat plants given an abundance of plant food in the first period, placed in distilled water for the second period, and again placed in nutrient solution for two or more days*

No.	Days in nutrient solution in third period.	Dry weight.	N	K_2O	P_2O_5
		Gm.	*Gm.*	*Gm.*	*Gm.*
1	Control 0	3.92	0.1596	0.1500	0.0960
2	1	5.88	.2172	.1998	.1320
3	2	5.68	.2320	.2506	.1400
4	3	5.90	.2605	.2670	.1380

By subtracting the nitrogen in the control, 0.1596 gm., from the nitrogen in the 1-day plant (No. 2), 0.2172 gm., we get 0.0576 gm. nitrogen absorbed in one day. By subtracting the control from the 3-day plants, we get 0.1009 gm. instead of three times 0.0576 gm., or 0.1728 gm., which we might expect if time alone governed absorption. In the K_2O column one determination, the absorption in 2 days, seems to be a little out of line and high. From these experiments one would judge that a demand for any of the plant foods can be developed in the plant, that this demand is cumulative, and that it is possible to measure this demand by analytical means.

DEMAND OF WHEAT PLANTS GROWN IN NUTRIENT SOLUTION FULL TIME AND ALTERNATE DAYS

As nitrates, and possibly other plant foods, are likely to vary in the soil solution from day to day, it was thought of interest to measure the rate of absorption when nutrients were given at varying intervals—that is, to measure the rate of absorption when cultures were placed in nutrient solution one day and in distilled water the next, and in nutrient solution on the third day and so on, feasting for one day and fasting the next. These seedlings were grown both with an abundance of plant food (100 parts per million each N, K_2O and P_2O_5) and also with a limited amount (10 parts per million). Analyses of the 12-day-old plants are given in Table VII.

TABLE VII.—*Analyses of 100 wheat plants grown continuously in nutrient solutions for 12 days, compared with similar series placed in distilled water every other day*

No.	Treatment.	Dry weight.	N	K_2O	P_2O_5
1	Distilled water control	4.44	0.0924	0.0597	0.0720
2	Nutrient solution 100 p. p. m. full time.	4.68	.1988	.2561	.1080
3	Nutrient solution 100 p. p. m. one day, distilled water one day.	5.36	.2100	.2856	.1280
4	Nutrient solution 10 p. p. m. full time.	5.76	.1932	.2273	.1200
5	Nutrient solution 10 p. p. m. one day, distilled water one day.	5.92	.1736	.1940	.1180

This was repeated several times with similar results. The total quantity of plant food absorbed, when plenty of plant food was present, was greater in the plants that had grown in the nutrient solution only half time than in the plants that had grown all the time in the same nutrient. When a limited quantity of plant food was present, the quantity

absorbed was reduced by removing the plants from the nutrient solution for half the time. But when calculated upon the rate of absorption per day, both the plants in the strong solution and those in the weak had a higher rate when grown only half time in the nutrient solution.

It is somewhat surprising to note that not only the rate of absorption but the general appearance of the plants—color, size, root development, and vigor of growth—is usually better when the plants are grown in a good nutrient solution for one period and in distilled water the next. This is not always the case but seems to be characteristic of plants that are grown during warm weather; when growth and absorption are rapid, and is not so likely to be true of plants grown in cold weather, when growth and absorption are relatively slow. Better looking plants are often obtained when they are kept in the nutrient solution at night and in distilled water during the day.

The experiments here described seem to demonstrate that a demand for plant food may exist within the plant, that this demand may be modified in different ways, and that the demand may be determined by analytical methods. The author has demonstrated the fact that it is possible to go out in the field, to take up a plant and put it in a full nutrient solution, and to determine what plant food it is hungry for by the way it feeds upon the nutrient solution.

TRANSFER OF PLANT FOODS WITHIN THE PLANT

The plant seems to feed upon the ions and not upon the molecules, and no plant seems to require a base and an acid in exactly the proper proportion to form a salt. It has been shown by Breazeale and Briggs [2] that a plant is even particular as to the kind of ion that it absorbs. Plants that had a high demand for potassium when placed in a solution of orthoclase were unable to feed upon the dissolved potassium. The solution was dilute, it is true, but not so dilute as to prevent the absorption of potassium. It is probable that the potassium existed in the solution as a double ion in combination with aluminum. The plant did not need and could not utilize the aluminum and therefore would not take up either the potassium or the aluminum.

That the transpiration rate has little to do with absorption can easily be shown by placing a bell jar over a pan of cultures, cutting down the transpiration, and measuring the rate of absorption in comparison with controls. Plants will feed just as rapidly when transpiration has thus been reduced to a minimum as they will when transpiring a maximum amount of water.

One can scarcely conceive of a plant feeding upon ions or exercising selective absorption in such a decided way if the transpiration stream or the osmotic concentration, or any other phenomenon except the specific demand of the plant, dominates the process of absorption.

In the same way, practical field results indicate that all plants do not possess the same ability to feed when placed in competition with each other. We find that if an oat and a mustard plant be grown in the same pot with a very limited supply of nitrogen, the mustard probably will get nearly all of that plant food and the oat very little. Plants vary widely in their ability to cope with each other when placed in keen competition.

[2] BREAZEALE, J. F., and BRIGGS, LYMAN J. CONCENTRATIONS OF POTASSIUM IN ORTHOCLASE SOLUTIONS NOT A MEASURE OF ITS AVAILABILITY TO WHEAT SEEDLINGS. *In* Jour. Agr. Research, v. 20, p. 615-621. 921.

An experiment with corn and kafir seedlings seems to throw some light upon this subject. Six treatments in duplicate were run, 12 pans in all, with corn and kafir seedlings growing upon the same disks and in the same nutrient solutions. The disks were about 12 inches in diameter and were like those used in the wheat culture work except that the perforations were larger. The corn seedlings were planted upon one half of the disk while the kafir grew upon the other. There were about 50 corn to 300 kafir plants. These seedlings were placed in the following solutions:

No. 1. Control, distilled water.
No. 2. 2 parts per million each N, K, and P.
No. 3. 5 parts per million each N, K, and P.
No. 4. 25 parts per million each N, K, and P.
No. 5. 50 parts per million each N, K, and P.
No. 6. 100 parts per million each N, K, and P.

They were allowed to grow for 17 days. In each pan, 2,500 cc. of solution were used and the experiments were run in duplicate. For convenience the average of the duplicates is given in Table VIII.

TABLE VIII.—*Nitrogen absorbed by corn and kafir seedlings competing in nutrient solutions of various strengths*

No.	Strength of solution.	Total N added.	Nitrogen absorbed.	
			By corn.	By kafir.
		Gm.	*Gm.*	*Gm.*
1	Water	0	0	0
2	2 p. p. m.	0.0450	0.0466	[1] 0.0073
3	5 p. p. m.	.1125	.1094	[1] .0091
4	25 p. p. m.	.5625	.2475	[2] .0180
5	50 p. p. m.	1.1250	.3344	[2] .0504
6	100 p. p. m.	2.2500	.3821	([3])

[1] Loss. [2] Gain. [3] Poor plants.

From this one experiment the indications are that when corn and kafir are placed in keen competition as in solutions containing 2 and 5 parts per million the corn may get all the nitrogen and the kafir little or none. An actual loss of nitrogen is noticed in the kafir in the two lowest concentrations, which might be explained by the exudation of this element into the solution or by the probable error of the experiment. The solutions were well mixed and not stirred while the plants drew out the nitrogen. In places the kafir roots were 6 inches away from the corn and in contact with the nitrates in the solution but, the kafir being a sluggish feeder while the corn was a vigorous feeder, the nitrates seem to have gone to the corn and not to the kafir. The absorption of the small quantity of nitrates was so rapid that it does not seem reasonable to assume that diffusion carried it all to the corn side of the pan. The difference is so marked that one must admit that in this case the kafir had no chance in competition with corn and that the nitrogen must have moved as far as 6 inches in a very short time. If, in a soil at optimum moisture content, the plant is dependent upon the soil grains that touch its roots, this competition is rather difficult to conceive of. Practically speaking, the absorbing surface of the roots of different plants would hardly touch the same soil grains often enough to be of serious consideration.

MOVEMENT OF PLANT FOODS IN THE SOIL

Ions are mobile and bear plus or minus charges of electricity. It has long been the opinion of the writer that the demand for food originates in the tissues of the plant and is carried to the absorbing surface of the root by means of an unsaturated carbon compound bearing a plus or minus charge. In the case of potassium, for example, the plant protoplasm in a leaf cell may remove an atom of K from a colloidal compound and use it in building up a permanent compound, which is to be one of the final constituents of its tissues. The removal of the atom K, bearing a plus charge, from its colloidal compound leaves that compound or molecule out of equilibrium and with a minus charge. This charge is transmitted, by replacement and not by bodily movement, down through the cells to the root tips and there appears as a minus charge. If potassium chlorid appears in the nutrient solution ionized as a plus K and a minus Cl, the plus ion will be attracted to the negative charge, and a chemical combination will take place, with the formation of a molecule in equilibrium, with respect to plus and minus electrictity. The potassium could, in this way, be transported from the absorbing surface of the root to the extreme tips of the plant without a bodily movement through the sap. In the case of nitrates the opposite conditions might prevail. A demand for NO_3 might originate in the tissues and be carried to the root as an unsaturated compound bearing a plus charge. This would be neutralized, for example, by the NO_3 ion of $NaNO_3$.

It is possible that the absorbing surface of the root or its walls are actually impermeable to the salts needed in nutrition, and it is possible to assume that food material may be transported from the roots to the rest of the plant without materially affecting the osmotic pressure of the sap. If the food materials are flowing freely in the sap and were it necessary for the plant to remove these materials in the localities where they were needed, it seems probable that there might be times when a high demand and a low supply, or the reverse, might cause considerable fluctuation in the concentration of the sap. As the writer understands it, the sap of plants of like varieties grown in the same localities is fairly constant. The tendency of the plant seems to be to keep its solutions in equilibrium, and the writer can conceive of a plant acting much like the battery of an automobile—the needle of the indicator may register a charge at one instant and a discharge at the next. The plant probably vibrates around the equilibrium point as closely as possible, taking up a plus charge at one time and a minus charge at another. The ordinary plant uses more of the mineral bases than it does of the mineral acids, and the writer is fairly well convinced that in order to maintain equilibrium the plant can absorb otherwise useless acids or bases, use them as ions when necessary, and eliminate them in various ways. With certain plants, if the system is basic, they seem to absorb CO_3 as an acid radical for the purpose of maintaining equilibrium; and in case the system becomes too acid they seem to possess the power of exuding the carbonates as CO_2. The absorption of calcium by certain plants and its elimination as an oxalate might be explained in this way. The absorption of silica in large quantities may probably be traced to the presence of an excess of basic radicals in those plants. The plants that have this characteristic have usually had waterlogged marsh lands for their habitat, and in the ages of their adaptation have had a large quantity of soluble silica and a small quantity of carbon dioxid at their

disposal and as a matter of necessity have adapted themselves to the former.

The plant seems to feed upon ions, and these ions are certainly mobile. If an ionic movement takes place between the root tips and the rest of the plant, it seems equally reasonable to assume the possibility that a similar movement might exist outside the plant and that the plant might draw its food from relatively long distances.

The potassium concentration of young plants, sometimes having small root systems, often runs very high. The soil solution is relatively low in this element, and water movement and diffusion are neglible factors. The absorbing surface of the root is small, and, assuming that the root is obliged to come in contact with a soil grain before it can draw upon the potassium, it is rather difficult to account for the high potassium content of the plant. In the same way the writer has analyzed many samples of Australian saltbush that ran 8 per cent or more of sodium chlorid upon the basis of their dry weight. These plants had grown in a soil that was very low in sodium chlorid and in a semiarid climate where the soil moisture was very low for the greater part of the year. It seems hardly possible for a plant with growing habits like that of the saltbush to be able to absorb such quantities of salt as it does, if obliged to feed in the manner usually attributed to plants. It also seems hardly possible to conceive of this plant being forced to absorb sodium chlorid against its will, so to speak, because the salt is carried into the system by the transpiration stream or other agencies. A noticeable feature of this sodium-chlorid absorption is that the sodium (Na) is absorbed in much larger quantities than are necessary for combination with chlorin (Cl) in the formation of sodium chlorid (NaCl). The excess sodium exists in the plant in organic combinations and is broken down into carbonates upon ashing. Evidently there exists in the Australian saltbush a demand for sodium and chlorin, and the demand for sodium is greater than the demand for chlorin. This absorption of the sodium ions in excess of the chlorin, from a soil solution where the source of supply of these ions is sodium chlorid, which is in equilibrium, seems to eliminate the idea of "forceful feeding," as applied to this plant. The high salt content of the plant, with such a low transpiration, would also indicate a wider field of absorption than is usually attributed to it.

If a soil solution is in equilibrium and the salts are ionized and the ions are mobile, if an atom of the K, for example, bearing a plus charge is removed from solution by the root, the writer sees no reason why the position of this ion can not be filled by replacement and the charge carried along through the soil, as it is in a battery, to where the source of supply of potassium exists. If this be true, the plant will not be dependent upon the soil grains that touch the root tips, but it may actually feed at a distance, the distance probably following some well-known physical law. In practical agriculture we, involuntarily, think of the plant as having all the moist soil surface at its disposal. We also think that the water movement in the soil is neglible as far as nutrition is concerned, that the plant has to grow for its water, and that diffusion is also a neglible quantity. With plants that have a limited root system and with the absorbing zone of the root but a small part of the root itself, if we do not attribute to the plant the ability to feed at a distance, we will have to admit that only a small part of the soil is at the disposal of the plant.

CONCLUSIONS

(1) A demand for plant food may be developed in the tissues of plants, and this demand may be measured by analytical methods.

(2) The demand of the plant seems to be for particular foods, and the effect of an application of plant food as a fertilizer seems to be largely a direct action upon the plant itself and not an indirect action upon some constituent of the soil.

(3) Plants probably feed upon ions, and these ions probably penetrate the root membrane and move through the colloids to the tissues as an electrical charge; therefore the feeding of plants may be looked upon as an electrical phenomenon.

(4) Ions are mobile and may move through the soil solution freely as such; and, this being the case, the plant may not be dependent upon the soil grains that touch its roots for nutrient material but may feed at a distance from the source of supply.

INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE UPON THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS [1]

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INTRODUCTION

The pioneer investigations concerning the influence of soil temperature upon cabbage yellows caused by *Fusarium conglutinans* Wollenw. were conducted in Wisconsin by Gilman (*8*).[3] His experiments were conducted in greenhouse rooms with rather wide fluctuations of air temperature, where the soil temperature could not be held constant for any great length of time. Consequently, Gilman did not determine the complete range of soil temperature for the occurrence of the disease, nor did he inquire into the possible influence of soil moisture.

Since the publication of Gilman's paper, plant pathologists have continued to make observations on the influences of air and soil temperatures upon the growth of the plants and upon the occurrence of yellows in the Wisconsin cabbage fields. During the midsummer months when the soil is dry and hot, cabbage plants begin to languish and assume a pale, lifeless color. Growth is markedly checked, especially when these conditions obtain for two or three consecutive weeks. It is during this trying period that yellows develops in its most destructive form on the "cabbage sick" [4] soils. During very dry hot seasons even the resistant strains of cabbage, such as the Wisconsin Hollander, may show a considerable percentage of incipient disease, but upon the return of more favorable weather conditions (rain and lower temperature) they usually overcome the attack and produce marketable heads. Such field observations soon convince one that the occurrence and severity of yellows are closely correlated with the influences of soil temperature and soil moisture, and the presumption would seem to be that these influences relate both to host and to parasite.

The writer undertook to learn more exactly the importance of these factors as they affect young seedlings, beginning the work in the summer of 1917. The purposes outlined were: (1) To determine the range of soil temperature for the occurrence of yellows in cabbage seedlings; (2) to study the influence of such soil temperatures upon the normal growth of cabbage seedlings in noninfested soil; (3) to study the influence of high soil temperature upon the relative susceptibility shown by the resistant strain, that is, upon the possible "breaking down" of resistance; (4) to determine in a like manner the influence of soil moisture both upon the growth of cabbage plants and upon the occurrence of yellows in them.

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[2] The writer wishes to make grateful acknowledgment to Prof. L. R. Jones, of the University of Wisconsin, for supervision and helpful suggestions during the progress of this work.

[3] Reference is made by number (italic) to "Literature cited," p. 86–86.

[4] The term "sick" or "cabbage sick" soil as used in this paper indicates soil infested with *Fusarium conglutinans*.

Cabbage seedlings have been found to lend themselves fairly well, distinctly better than the larger plants, to these studies. The reasons for this are that (1) the symptoms of the disease become evident very soon after the seedlings emerge from the soil and diagnosis is easy and sure for the experienced observer; (2) the experiments can, therefore, be carried to definite conclusions within a relatively short time; (3) for such a brief period the seedlings develop well in fairly crowded plantation so that adequately large numbers can be used in pot culture; (4) the seedlings behave well under a reasonably wide experimental range of variation in soil temperature and soil moisture.

INFLUENCE OF TEMPERATURE UPON THE GROWTH OF FUSARIUM CONGLUTINANS IN CULTURE

A partial knowledge of the influence of temperature upon the growth of *Fusarium conglutinans* has already been obtained. Gilman (*8*) in his early work found that the conidia did not germinate within 72 hours when exposed at 8° to 10° C. in Van Tieghem cells, while only 3 hours were required for germination at 33° and 8 hours at 21°. His study of the growth of the mycelium was limited to a narrower range of temperature. The mycelium grew slowly at 8° to 10° and most vigorously at 25°, the highest temperature used in his series. An intermediate growth rate was obtained at 18° to 22°. It is seen that data on the upper limits of temperature were still lacking; therefore, it seemed advisable to determine these limits before taking up a study of the relation of soil temperature to the occurrence of yellows.

A fragment of mycelium from a young culture of *Fusarium conglutinans* was placed in the center of plates (10 by 100 mm.) of 2 per cent potato-dextrose agar, titrating + 10 Fuller's scale. The plates were then placed in incubators at a series of temperatures ranging from 7° to 37° C., those at the higher temperatures being inclosed in moist chambers to guard against inhibiting desiccation. Two plates were carried at each temperature, and measurements were made daily for seven days. The results at the end of four days and those at the end of seven days are given in Table I and are also shown graphically (fig. 1) for comparison with the percentage of yellows.

TABLE I.—*Growth of Fusarium conglutinans at different temperatures on potato agar*

Temperature.	Diameter of colony at various ages.	
	4 days.	7 days.
°C.	*Cm.*	*Cm.*
7 to 8	No growth.	Slight growth.
11 to 12	0.5	1.6
17 to 18	1.3	2.9
21 to 22	1.6	3.5
24 to 25	3.2	6.0
27	3.3	6.0
30	1.2	2.0
35	.3	.6
37	No growth.	No growth.

It is readily seen that if diameter of colony is used as a criterion, the optimum temperature for growth on potato agar plates for brief periods of time is found to be between 24° and 27° C., with a rather sudden dropping off toward the extremes. There was no growth at 37°, but the fungus was not killed at this temperature, as was shown by transferring the plates to an incubator held at 24°. After exposure for 10 days at 24° the colonies on the plates which had previously been exposed at 8° and 37°, respectively, showed vigorous growth.

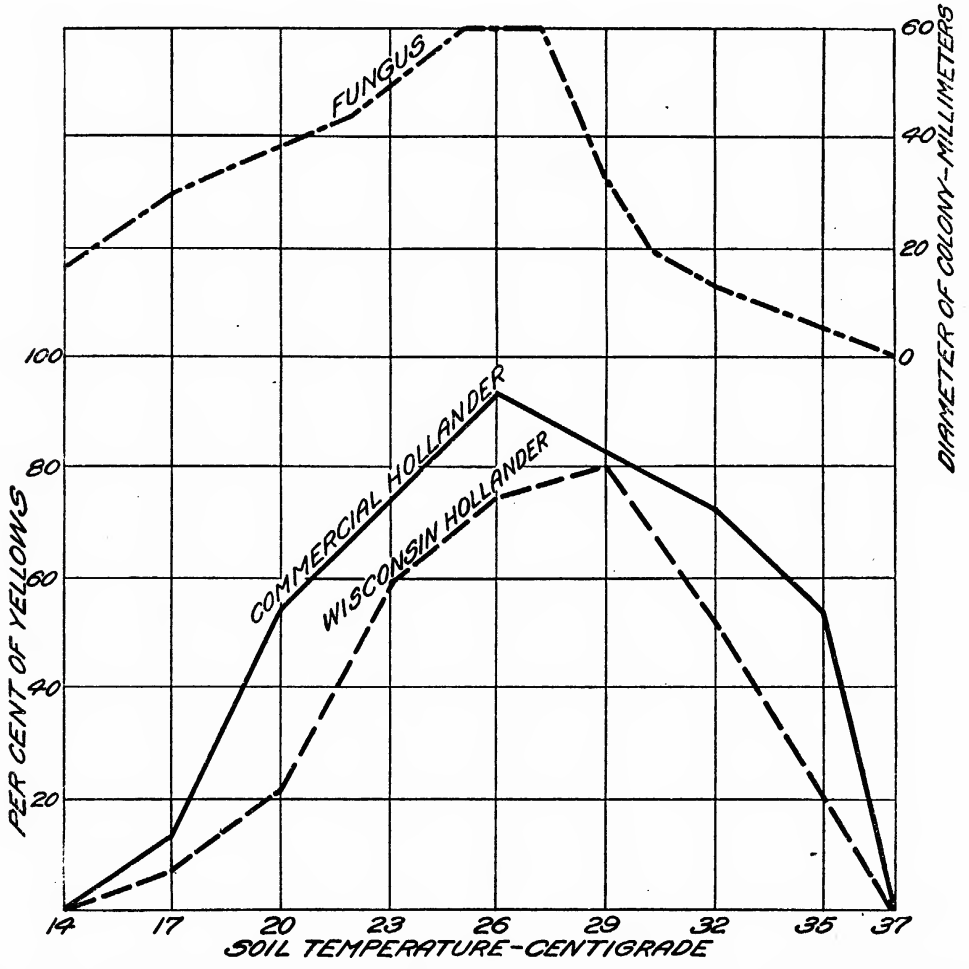

FIG. 1.—Comparison of rate of growth of *Fusarium conglutinans* on agar plates with development of yellows in cabbage seedlings given in Tables I and V; plants grown 20 days from seed in naturally infested soil.

In addition to these differences in vegetative growth, the fungus exhibited a difference in sporulation at the different temperatures. At 14° C. the colonies were raised, with a leathery stroma, and produced abundant conidia. This character became less manifest up to 24°, where there was very little aerial growth and there were practically no conidia. Above 29° the colonies were again raised and produced abundant chlamydospores but no conidia. Table I shows that the growth of the fungus dropped off more suddenly above 27° than below, yet it will be seen (fig. 1) that the percentage of yellows was higher at 35° than at 17°. It is evident, therefore, that rate of growth of the fungus in culture is not directly proportional to percentage of disease at all temperatures.

Although the vegetative growth of the parasite is retarded at the higher temperatures, the data do not indicate that the pathogenicity is reduced in like proportion.

INFLUENCE OF SOIL TEMPERATURE UPON THE GROWTH OF CABBAGE SEEDLINGS

Inasmuch as it was presumed at the outset that soil temperature affects both host and parasite, it was considered of much importance to study separately its effect upon the cabbage plants in noninfested soil as well as upon the fungus in culture. The results of the studies of the influence of temperature upon the fungus have already been presented.

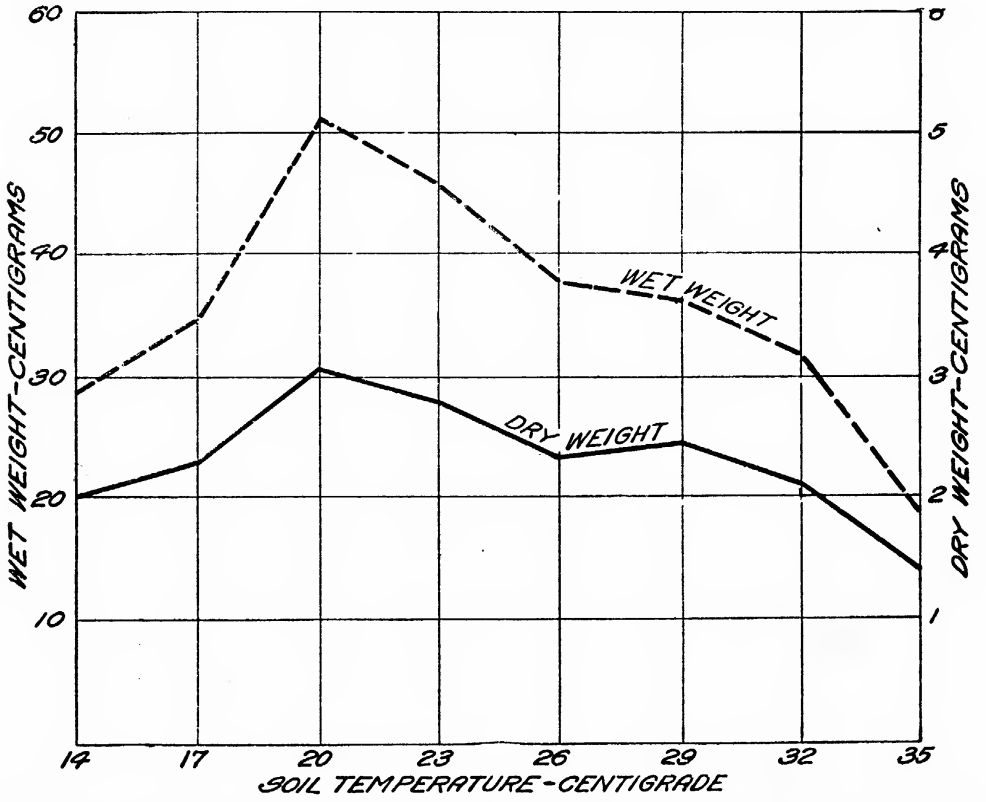

FIG. 2.—Comparison of wet weights and dry weights of shoots of healthy Wisconsin Hollander cabbage seedlings given in Table II, grown 20 days from seed during February and March.

In the experiments conducted for the purpose of determining the influence of soil temperature upon the seedlings, the general appearance of the plants and the dry weight of the shoots and roots were used as bases for comparison. For preliminary data use was made of the Wisconsin Hollander plants which had been grown 20 days in sterilized soil as controls on the relation of soil temperature to the occurrence of yellows. The methods of planting and controlling the soil temperature will be explained in connection with the experiments for studying the influence of soil temperature upon the occurrences of yellows. At the end of 20 days six plants were taken for each temperature and both wet and dry weights were determined. The tops were cut off at the surface of the soil and dried in a constant-temperature oven for 18 to 24 hours at 95° C. The average results of two such series are given in Table II and shown graphically in figure 2. These first trials were conducted during February and

March when the light was weak and the day short. Other experiments were carried out in May and June when the light was stronger and the plants were grown for a longer period of time.

TABLE II.—*Weights of Wisconsin Hollander seedlings grown 20 days from seed at different soil temperatures*

Soil temperature.	Wet weight per plant.	Dry weight per plant.	Ratio of wet weight to dry weight.
°C.	Gm.	Gm.	
14. 5	0. 29	0. 020	14. 5
17. 0	. 34	. 023	14. 7
20. 0	. 51	. 031	16. 4
23. 0	. 45	. 029	15. 5
26. 0	. 37	. 023	16. 0
29. 0	. 36	. 024	15. 0
32. 0	. 32	. 022	14. 5
35. 0	. 18	. 014	12. 8
38. 0	. 06	. 006	10

It will be seen from Table II that 20° C. proved to be the optimum soil temperature for the growth of these cabbage plants for the first 20 days. At soil temperatures above 20° there was a gradual decrease in weight up to 35°, where there was a sudden drop; at 38° the plants soon died. Accompanying this difference in weight was a marked difference in the appearance of the plants grown at low and at high temperatures. At 23° and below they were stocky and had a dark green color. There was also a slight difference in the height and size of the plants at the different temperatures. From 26° to 32°, inclusive, the plants became more strict; that is, the petioles were proportionately longer and approached a vertical position. The color also graded into a lighter shade of green. At 35° the plants were decidedly stunted, and they assumed a distinctly pale green color.

Moreover, if we consider the ratio of wet weight to dry weight, a marked difference will be noticed at the different soil temperatures. At the intermediate temperatures, where the greatest wet and dry weights developed, the ratio of the wet weight to dry weight was greatest. This simply means that the plants were most succulent at these temperatures, and it seems possible that for this reason they may have offered a more favorable medium for the invasion of the parasite than was offered by the plants at the extreme temperatures. This suggestion is favored to some extent by the occurrence of a higher precentage of yellows in plants growing in naturally infested soil at these temperatures. On the other hand, as will be seen later, the highest percentage of yellows occurs in plants grown in artificially inoculated soil above 26° C., where the moisture content of the plant is lower and the plants must be in an abnormal condition. These results indicate that some factor other than succulence of the host plant must be concerned in determining the degree of infection.

In order to determine whether similar temperature relations obtained for longer periods, the seedlings used as controls in the third experiment in studying the disease were allowed to grow at the different soil temperatures for 46 days before the weight determinations were made, as compared with 20 days in the former series. The roots were then washed

out of the soil and dry weights were made separately for roots and shoots. The results, consisting of the average per plant for the nine plants from each temperature, are shown in Table III. This experiment was conducted in May and June, 1919, when the air temperature ranged from 18° to 20° C., with a rise of 10° to 12° during the middle of the day.

TABLE III.—*Dry weights of cabbage plants grown 46 days from seed at different soil temperatures*

Soil temperature.	Dry weight per plant.			
	Wisconsin Hollander.		Commercial Hollander.	
	Shoot.	Root.	Shoot.	Root.
°C.	Gm.	Gm.	Gm.	Gm.
14	0.245	0.029	0.185	0.027
17	.316	.033	.306	.048
20	.281	.042	.262	.041
23	.272	.044	.223	.037
26	.254	.041	.192	.025
29	.302	.052	.252	.035
32	.236	.030	.260	.036
35	.207	.025	.204	.017

It may be seen from Table III that the weights are not consistent at all temperatures with those of plants 20 days old, although the temperature for optimum growth remains the same. The weight at 26° C., is less than that at 23° and 29°, thus giving a distinct bimodal curve when the weights are plotted against temperature. This condition exists alike with roots and shoots of both strains.

Inasmuch as the moisture content of the soil was not kept constant by weight, it was thought that a difference in moisture, provided there were such, might have been responsible for the irregular growth of the plants. Therefore, another experiment was conducted, during the winter of 1919, in which the whole soil mass was kept up to a uniform moisture content by weight. The soil was made up of three parts of fairly rich, virgin loam and one part of clean sand. The water-holding capacity of this soil was 39.2 per cent (in 1 cm. tube), or 28 per cent calculated on a wet-weight basis, and the moisture was kept at about 15 per cent by weighing the receptacles at the higher temperatures every day and less frequently at the lower temperatures and restoring the lost moisture. In place of the cinders used in the other experiments, a 3-inch pot was inverted in the bottom of the receptacle, a glass tube inserted in a hole in the bottom of the pot, and the soil filled in around this and tamped fairly firmly. All of the water was supplied through the tube. The seed was planted as previously described. After the plants were about 5 days old, the stand was thinned to three in each receptacle, and the soil surface was covered with mineral wool. Both the Wisconsin Hollander and the Commercial Hollander were used, the receptacles being handled in duplicate at each temperature.

The experiment was begun October 22 and concluded December 15, 1919, a total of 53 days. The air temperature during this period ranged from 14° to 18° C., with a daily rise of about 5° in the middle of the day.

The roots were washed out of the soil and dry weights made of both shoots and roots in the usual way. The results in Table IV show the average of six plants from each temperature. These results are shown graphically in figures 3 and 4.

TABLE IV.—*Dry weights of cabbage plants grown 53 days from seed at different soil temperatures*

Soil temperature.	Wisconsin Hollander.		Commercial Hollander.	
	Shoot.	Root.	Shoot.	Root.
°C.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
14	0.230	0.034	0.267	0.039
17	.305	.036	.313	.046
20	.302	.036	.313	.040
23	.239	019	.236	.020
26	.258	.021	.255	.019
29	.200	.020	.309	.024
32	.185	.017	.233	.012
35[a]	.021	.006	.053	.005

[a] The temperature rose to 38° C. during two successive nights soon after the plants appeared above ground; this may account for the extremely low weights occurring at this temperature.

This table shows that 17° to 20° C. was also the optimum soil temperature for the growth of cabbage seedlings for this longer period. The dry weights obtained at some temperatures were even lower than the weights

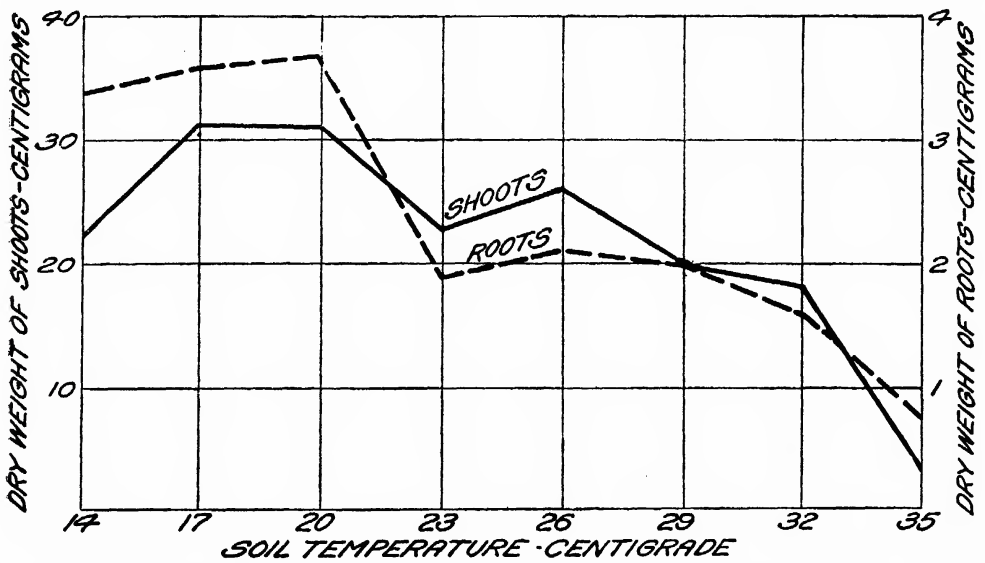

FIG. 3.—Comparison of dry weights of shoots and roots of healthy Wisconsin Hollander seedlings given in Table IV, grown 53 days from seed during November and December.

obtained for plants only 46 days old, but the difference in the composition of the soil was probably sufficient to account for the difference in weight. The bimodal condition was evident in this experiment also, but in this case the lowest weight occurred at 23° instead of 26°, as in the previous experiment. The bimodal condition in weight and the difference in color were probably due to the effect of temperature upon the physiological balance of the plants, but since no analyses have been made of plants

grown at the different temperatures, it is impossible to offer any definite explanation for these differences. However, by comparing figure 1 with figures 3 and 4, it will be seen that the higher percentages of yellows occurred at and slightly above the temperature at which the drop in dry weight of the plant occurred, which was also the temperature at which the fungus grew most rapidly in culture. It is probable that both reduced vigor of the plant and optimum temperature for growth of the fungus played a part in producing a higher percentage of yellows at these temperatures.

There is a marked difference in the external characters of the root systems of the plants grown at different temperatures. Roots grown at 14°, 17°, and 20° C. are coarse, light in color, have a thick cortex, and spread uniformly throughout the soil mass. Beginning at 23° the roots

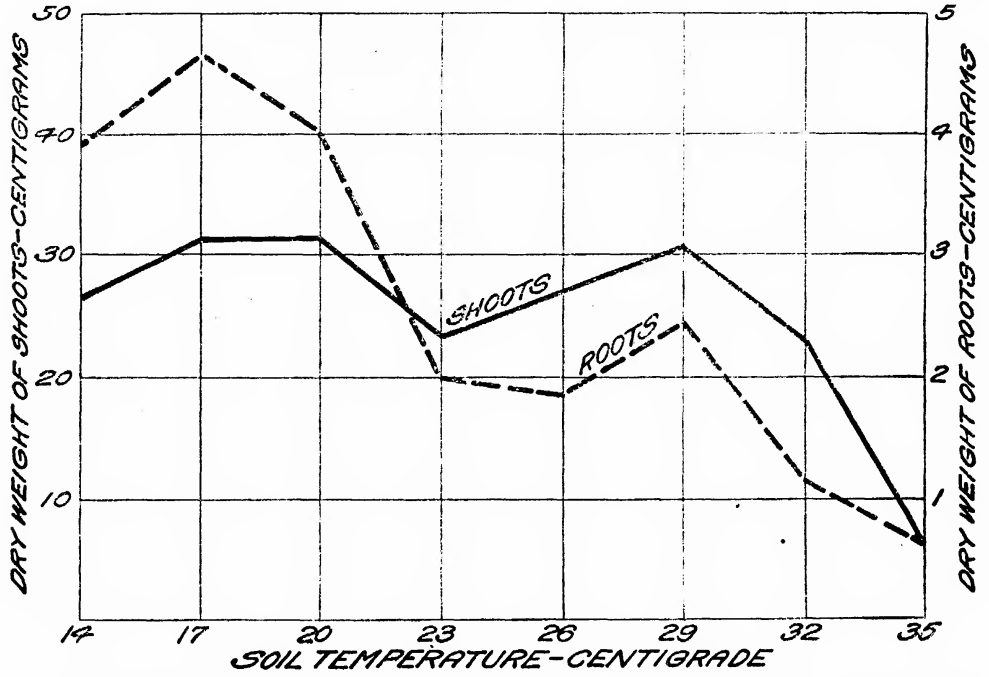

FIG. 4.—Comparison of dry weights of shoots and roots of healthy Commercial Hollander seedlings given in Table IV, grown 53 days from seed during November and December.

are finer, darker in color, and shorter, until at 35° the number of roots and the extent of the system are greatly reduced and the color becomes dark brown. A comparison of the shoots and roots of plants grown at 20°, 26°, and 32° is shown in Plate 1. All of these variations suggest that fundamental differences occur in the anatomical structures of the tissues involved and possibly in their chemical composition, but analytical studies on these differences have not as yet been undertaken. It seems evident, however, that such variations in external appearance of parts of the host at different temperatures may be regarded as indices of the more important internal effects of temperature. They are probably accompanied by a difference in the rate of manufacture of carbohydrates and proteins and in the disposition of these products. In turn, these differences in assimilation and physiological balance could affect the cell walls and protoplasm in a manner which would make the plant more susceptible to the attacks of the fungus.

INFLUENCE OF SOIL TEMPERATURE UPON THE OCCURRENCE OF THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS

GREENHOUSE EXPERIMENTS

METHODS AND APPARATUS.—The apparatus employed for controlling the soil temperature in these experiments was what is termed the Wisconsin soil-temperature tank. Since it differs only in detail from that used by Johnson and Hartman (*9*) and described by Jones (*10*), further description is considered unnecessary in this connection. A series of nine separate tanks or compartments was employed, with extreme temperatures of 14° and 38° C. and a difference of 3° between successive tanks, thus giving the graduated series 14°, 17°, 20°, 23°, 26°, 29°, 32°, 35°, and 38°. The temperatures below 23° were regulated by running in a small amount of cold water two or three times a day as the case required, while the temperatures from 23° to 38° inclusive were maintained through the use of carbon electric lamps under thermostatic control. During the earlier experiments some of the temperatures were regulated through the use of steam, but this method was abandoned when the electric lamps were installed. With personal attention two or three times every 24 hours, the temperatures were kept fairly constant, rarely varying more than 1° from that desired.

The air temperature in the tank room was kept constant within a range of a few degrees. During the course of the first experiments it ranged from 18° to 22° C. with a maximum rise of 5° to 10° for one or two hours on bright days. During the spring of 1919 the air temperature range was from 14° to 18° with a similar rise at midday on bright days.

The surface of the soil in the receptacles was covered with a half-inch layer of mineral wool to insulate it from the air temperature and to reduce evaporation. Even with this protection, the surface inch of soil at the higher temperatures usually registered from 1° to 1½° lower than the water in the tanks. However, the deeper layers of soil registered the same temperature as the water, and the plant roots were found to be distributed largely in the layers below the first inch. Therefore the temperatures recorded for the different series are those at which the water was maintained in the tanks in which the receptacles were suspended.

RECEPTACLES.—The receptacles or culture pots were made of galvanized sheet iron, cylindrical, 6 inches in diameter and 10 inches deep. They were made ready for use by placing a layer of fine coal cinders about 2 inches deep on the bottom with soil on top of this up to within 1½ inches of the top. All of the water was supplied through a glass tube 12 mm. in diameter which was placed in the center of the receptacle of soil with the lower end inserted in the cinders. After the receptacles were filled with soil, they were so placed in the tanks that the surface of the soil was level with the surface of the water in the tank. They were then allowed to remain in the tanks one or two days before the seed was planted.

SOIL.—The naturally infested soil used in all the experiments was obtained from a uniformly "cabbage sick" field in Kenosha County, Wis. It was a dark clay loam containing some gravel. Before being used the soil was screened and uniformly mixed with coarse sand in the ratio of five parts of soil to one of sand. After the sand was added, the portion to be used as control was autoclaved at 5 pounds pressure for two hours and then allowed to stand for one week before it was used.

HISTORY OF SEED AND METHODS OF PLANTING.—The seed used in all the experiments was from the same lots of the Late Wisconsin Hollander and Commercial Hollander strains. The Late Wisconsin Hollander was the strain of Hollander which has proved to be highly resistant to the Fusarium disease under field conditions, as recently described by Jones, Walker, and Tisdale (*12*). This seed was grown in 1917 by S. B. Walker at Racine, Wis. The seed of the commercial strain was obtained from the L. L. Olds Seed Co., Madison, Wis. No information could be had as to where and when it was grown, but it was considered a good representative of the Commercial Hollander type. It had been proved to be about 99 per cent susceptible when grown in "sick" soil.

In the first and third tests, three receptacles in each tank were planted with Wisconsin Hollander and three with the Commercial Hollander. Two of the three receptacles in each set contained "sick" soil and one contained sterilized soil. In the second experiment only two receptacles were used for a strain in each tank, one containing "sick" and the other sterilized soil. When the plants were well above ground, the stand was thinned to 10 plants in each receptacle, except in certain cases, and the surface of the soil was covered with a half-inch layer of mineral wool.

THE EXPERIMENTS.—Four separate tests were conducted for measuring the influence of soil temperature upon the occurrence of yellows in cabbage seedlings, three with naturally infested soil and one with artificially inoculated soil. Three of the tests were conducted during the winter of 1917 and the spring of 1918, and one in the spring of 1919. In all three experiments with naturally infested soil the behavior of the host plant was similar, and the percentage of disease checked fairly closely. Of course, the intensity of light was greater in the spring than during the winter, but it was reduced in the spring by nailing cheesecloth on the inside of the roof of the greenhouse above the tanks. The final data were recorded 20 days after the seed was planted; they show the percentage of yellows and the percentage of plants dead by this time. This length of period was chosen because in the first experiment all of the plants of the commercial strain at 32° C. had developed yellows within 20 days.

METHODS OF EXPRESSING DATA.—The data in the greenhouse experiments were taken daily after the disease began to develop. In the first four experiments the task of taking data consisted merely in recording the number of diseased plants and the number which died as a result of the disease. The percentage of yellows includes the plants which showed the disease in incipient stages as well as those which died from the disease. Except in doubtful cases diagnoses of yellowed plants were made from the external symptoms in the leaves; the doubtful cases were cultured on agar plates.

EXPERIMENTAL DATA.—The data given in Table V and figures 1 and 5 show that *Fusarium conglutinans* is capable of producing yellows in both the susceptible Commercial Hollander and the resistant Wisconsin Hollander seedlings over a wide range of temperature, the minimum being 17° and the maximum about 35° C. At the lower temperatures, however, the disease developed more slowly and less destructively than at higher temperatures (fig. 6). The Wisconsin Hollander strain was even less severely attacked at the lower temperatures than the commercial strain. Plate 2, A–C, shows a contrast of the two strains growing in "sick" soil at 17°. At 15° the disease did not develop even in the most susceptible strains. The optimum soil temperature for the occurrence of yellows in the commercial strain in naturally infested soil

was about 26° and in the Wisconsin Hollander about 29°. These temperatures practically coincide with that for growth of the causal organism on potato agar, but are several degrees higher than the optimum for growth of the seedlings. These results differ from those reported by Tisdale (*13*) for flaxwilt and those reported by Clayton (*5*) for tomato wilt only in that the optimum temperature for growth of the cabbage plant is lower than that for development of the disease and growth of the parasite. The same temperature relations do not obtain for other types of diseases attacking the underground parts of plants. Balls (*1*, *2*) reported that the soreshin disease of cotton developed most destructively at 17° to 23° C., while the optimum for the growth of host and parasite was about 28°. Johnson and Hartman (*9*) reported quite similar relations for the rootrot of tobacco.

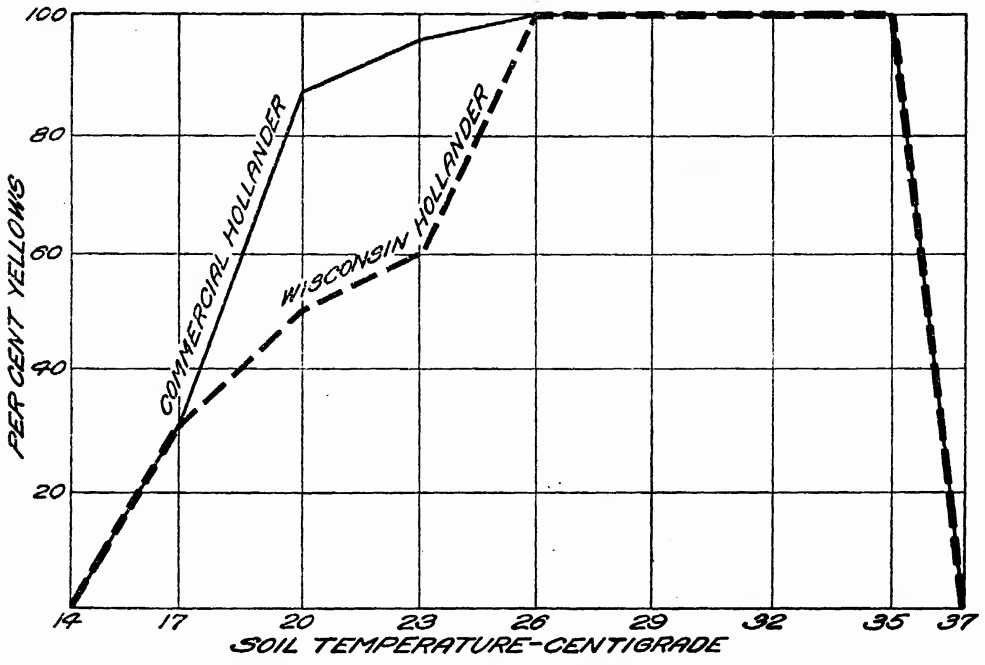

FIG. 5.—Comparison of development of yellows in Commercial Hollander and Wisconsin Hollander seedlings given in Table V, grown 20 days from seed in artificially inoculated soil.

Although the percentage of yellows in the resistant Wisconsin Hollander was rather high during the early period of growth, it was lower at all temperatures than that of the susceptible commercial strain. The greatest differences in percentage of yellows between the two strains occurred at 20° and at 35° C., where they amounted to about 35 per cent. In artificially inoculated soil at 26° and above the two strains seemed to be equally susceptible (fig. 5). The cause for such a wide difference between the pathogenicity of *Fusarium conglutinans* in naturally and in artificially inoculated soil has not been determined. It is apparent, however, that this difference is in part due to the direct relation of the higher temperatures to the stimulation of the Fusarium. This relation becomes especially evident when the fungus is growing in pure culture free from the complicating relations with the normally associated soil flora.

Other workers have obtained similar results with vascular parasites. Edgerton (*6*, *7*) found that a much higher percentage of Fusarium-wilt of tomato developed in the sterilized, reinoculated soil than in the unsteril-

ized soil, but at the same time the resistant tomato was very resistant in the seedling stage. He did not state the temperature at which the plants were grown, however, and temperature is a potent factor in the development of cabbage yellows. At 23° to 32° C. seedlings of the resistant strain of cabbage show a high percentage of yellows, but the plants which escape the disease usually remain healthy. W. H. Tisdale (*14*) found the resistant strain of flax to be highly resistant even in the seedling stage.

The upper limit for the growth of *Fusarium conglutinans* in pure culture is about 35° C., yet it produced 100 per cent yellows in plants grown at this temperature in artificially inoculated soil. As previously stated, the temperature of the first inch of soil at 35° registers from 1° to 1½° lower than the water in the tank, and it may be that the fungus made its successful attack upon these roots in the first inch of soil.

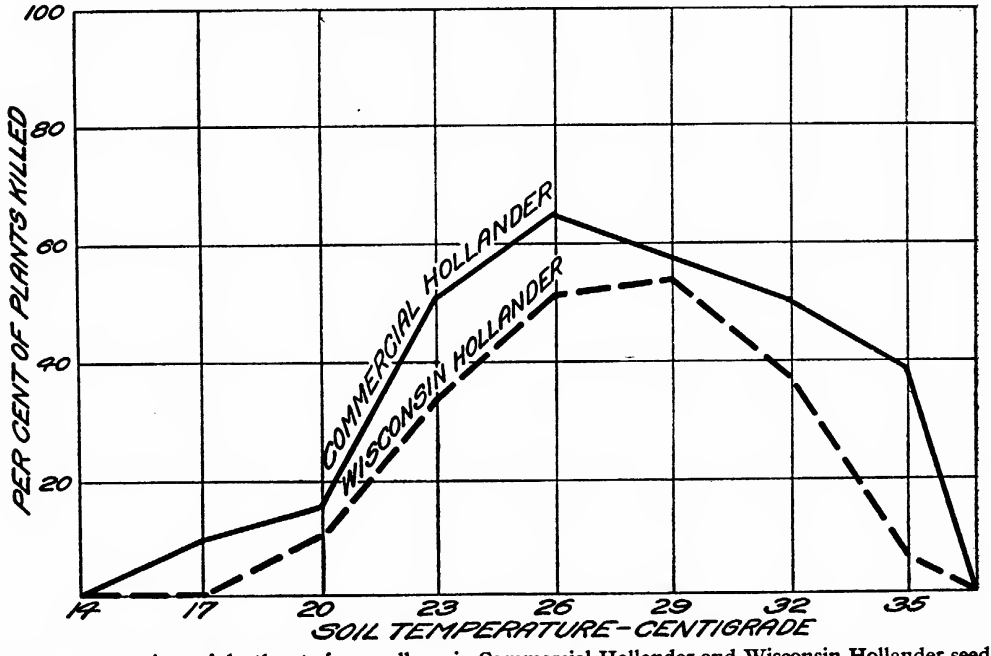

FIG. 6.—Comparison of death rate from yellows in Commercial Hollander and Wisconsin Hollander seedlings given in Table V, grown 20 days from seed in naturally infested soil.

The data presented in Table V and figure 6, especially those for artificially inoculated soil, show that the highest percentage of yellows occurred at temperatures above that most favorable for the seedling. This indicates that resistance is probably broken down at these higher temperatures. However, the chief difficulty in determining to what extent the increase in percentage of yellows at higher temperatures is due to "breaking down" of resistance is the fact that the nature of the resistant character has not been analyzed. Until its true nature has been determined, it will be impracticable to learn by the common method of testing for resistance in plants, that is, by subjecting both host and parasite to the different temperatures, just how much temperature affects the resistant character. However, the results obtained with artificially inoculated soil indicate that either the host becomes less resistant or the fungus more pathogenic at the higher temperatures. The latter condition was not in evidence with susceptible plants, but the decided stunting of the plants would seem to indicate that they may be less resistant.

TABLE V.—*Percentage of cabbage seedlings showing yellows at different soil temperatures after growing 20 days from seed*

Condition of soil.	Strain.	Date planted.	Soil temperature. 14° C.			17° C.			20° C.			23° C.			26° C.			29° C.			32° C.			35° C.			38° C.		
			Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.[a]
Naturally infested...	Wisconsin Hollander....	Feb. 1	20	0	0	19	11	0	19	22	11	20	80	60	20	80	50	20	90	60	20	70	50	20	10	0	20	0	50
		Mar. 2	18	0	0	20	0	0	20	20	10	20	50	20	20	80	80	20	50	20	20	60	20	19	20	0	17	0	30
		Mar. 29	10	0	0	10	10	0	10	20	0	10	50	30	10	70	20	10	100	80	10	40	40	10	30	20	10	0	40
	Commercial Hollander...	Feb. 1	19	0	0	17	14	14	18	75	13	17	71	57	19	78	56	20	80	50	13	100	100	17	57	29	15	0	60
		Mar. 2	16	0	0	17	14	0	19	67	11	19	78	67	17	100	100	18	75	50	22	59	42	21	36	18	24	0	50
		Mar. 29	10	0	0	10	10	10	10	20	10	10	70	30	10	100	50	10	90	70	10	60	40	10	70	70	10	0	40
	Av. Wisconsin Hollander		48	0	0	....	7	0	....	21	7	....	60	37	....	76	50	....	80	53	....	57	37	....	20	7	....	0	40
	Av. Commercial Hollander		45	0	0	....	13	8	....	54	11	....	73	50	....	93	65	....	82	58	....	73	48	....	54	40	....	0	50
Artificially inoculated	Wisconsin Hollander....	Mar. 29	10	0	0	10	30	0	10	50	10	10	60	40	10	100	100	10	100	100	10	100	100	10	100	100	10	0	50
	Commercial Hollander...	May 5	18	0	0	13	31	0	16	88	44	24	96	83	18	100	100	18	100	94	16	100	100	23	100	91	11	0	50

[a] The death of the plants at this temperature (38°) was evidently due to the effect of high temperature alone. No culture of Fusarium was ever obtained from the plants.

INCUBATION PERIOD OF THE FUSARIUM DISEASE IN CABBAGE SEEDLINGS AT DIFFERENT TEMPERATURES

In all of the experiments reported in this paper a great difference in the incubation period of the disease was observed in the plants grown at the different soil temperatures, and also to some degree between plants in the same receptacle. The incubation period, as spoken of in this connection, was reckoned from the time the seed was planted rather than from the date the plants emerged from the soil, because at temperatures below 23° C. two or three days more were required for the plants to emerge from the soil than at the higher temperatures, although the seed coats ruptured practically as soon at the lower temperatures. Because of this fairly uniform breaking of the seed coats, the seedlings at the different temperatures were exposed to the fungus for about the same length of time.

In every case the disease appeared first at 26° to 32° and last at 17° C., with a gradation between these extremes. The incubation periods at these temperatures were 7 days and 18 days, respectively. At 26° to 32° the disease appeared in some plants within 1 or 2 days after the plants emerged from the soil, while others in the same receptacle appeared perfectly normal and healthy for several days longer. At the lower temperatures the disease was much longer in becoming manifest but at the same time showed a similar variation in length of incubation among individuals. The variation among plants grown in artificially inoculated soil was not as great as that among plants grown in naturally infested soil. A comparison of the results in the two cases is shown in Tables VI and VII. Gilman (*8, p. 43, Table VI*) also showed that, although inoculations were made with parts of the same culture on plants from the same pot and under conditions as nearly identical as possible, the incubation period varied widely. The cause of this variation has not been definitely determined, but the results so far obtained indicate that it is a difference in the genetic composition of the individuals. Under the conditions of these experiments the plants must have been exposed to essentially like opportunities for attack by the fungus.

When the plants were grown in "sick" soil for 20 days at 14° and then transferred to 26° C., the incubation period was only 4 to 6 days, although a smaller percentage of disease developed in the resistant seedlings than when the plants were grown from seed in "sick" soil at 26°. This short incubation period is very probably due to the fact that the root hairs were in close contact with the fungus mycelium at the low temperature, and when subjected to a more favorable temperature the fungus immediately began invasion of the roots through the root hairs. The Wisconsin Hollander plants treated in this manner showed the disease mostly in incipient form, whereas the susceptible commercial strain was as susceptible as in the earlier stages of development.

When the plants were grown for 30 to 36 days in noninfested soil and then transplanted to "sick" soil the incubation period was longer in all cases than when the seed was planted in "sick" soil. Under these conditions the incubation period was shortest at 26° C. and longest at 17°, being 11 and 17 days, respectively. It is probable that this longer period was due to the inability of the fungus to make a successful attack until new root hairs developed on the roots.

A COMPARISON OF SUSCEPTIBILITY IN THE WISCONSIN HOLLANDER AND COMMERCIAL HOLLANDER SEEDLINGS

In all of the experiments conducted under controlled temperature and moisture conditions variations in individual susceptibility among plants have been very noticeable in both susceptible and resistant strains. Data were obtained from experiments previously reported by recording each day the percentage of plants in each can showing the disease at the different temperatures. These data are shown for both naturally and artificially infested soils in Tables VI and VII.

TABLE VI.—*Variations in susceptibility among cabbage seedlings grown in naturally infested soil at different soil temperatures*

Soil temperature.	Total number of plants.	Percentage of diseased plants in each receptacle at the end of different periods after planting.									
		Commercial Hollander.					Wisconsin Hollander.				
		10 days.	12 days.	15 days.	18 days.	20 days.	10 days.	12 days.	15 days.	18 days.	20 days.
°C.											
17	10	0	0	0	10	10	0	0	0	10	10
20	10	0	0	0	10	20	0	0	0	10	20
23	10	0	30	30	40	50	0	20	30	40	50
26	10	10	50	60	70	100	20	80	80	80	80
29	10	30	60	60	70	90	10	20	20	30	50
32	10	20	20	30	50	60	10	20	30	50	60
35	10	10	40	50	70	70	0	0	0	20	20
38	7	0	0	0	0	0	0	0	0	0	0

TABLE VII.—*Variations in susceptibility among cabbage seedlings grown in artificially inoculated soil at different soil temperatures*

Soil temperature.	Percentage of diseased plants in each receptacle at end of different periods after planting.											
	Commercial Hollander.						Wisconsin Hollander.					
	Total number of plants.	10 days.	12 days.	15 days.	18 days.	20 days.	Total number of plants.	10 days.	12 days.	15 days.	18 days.	20 days.
°C.												
17	13	0	0	0	0	31	10	10	10	10	30	30
20	16	0	0	0	69	88	10	0	0	20	30	50
23	24	0	21	44	92	96	10	0	10	30	50	60
26	18	6	22	67	94	100	10	40	80	100	100	100
29	18	6	28	89	94	100	10	80	90	90	90	100
32	16	50	75	100	100	100	10	80	90	100	100	100
35	23	26	52	87	91	100	10	60	100	100	100	100
37	11	0	0	0	0	0	10	0	0	0	0	0

Examination of the Tables V–VII shows considerable variation in susceptibility as between individuals at all temperatures. This variation is manifest not only by length of incubation period but also by extent of infection and rate of development of the disease once infection has occurred. At and above 26° C. the incubation period is materially shortened for a great number of individual plants, especially those of the Wisconsin Hollander strain. In naturally infested soil Wisconsin Hollander plants which escape the disease for the first 20 days usually remain healthy, even though they are growing at high temperature. In artificially inoculated soil all of the plants become diseased within the first 20 days as a rule.

Plants of the Wisconsin Hollander strain which showed symptoms of yellows even when very young have been observed to overcome the attack and resume vigorous growth. Their ability to do this was less pronounced in plants grown in artificially inoculated soil. It will be seen from Table VII that a high percentage of plants were killed in the artificially inoculated soil. The sudden death of some plants, the ability of others to overcome the attack, and the ability of still others to escape the disease entirely, furnish evidence of a variation in degree of resistance of the individual plants during the early stage of development. The same condition has been known for several years to exist among varieties. However, still more significant is the fact that such variations in degree of resistance continue to appear as between individuals of the most resistant and most stabilized of the Wisconsin strains.

Our main interest lies, therefore, in the variations in resistance as between individual plants. The facts cited as bearing on this variation indicate that Fusarium resistance in cabbage is due to hereditary factors, probably multiple, and that many of the plants are entirely lacking in one or more of the factors for resistance or possess them in a heterozygous condition. The Wisconsin Hollander strain was selected to resist the Fusarium disease under certain conditions to which the plants are normally subjected in the field. Plants which resist under these conditions may not possess sufficient factors for resistance to enable them to resist under more severe conditions, as evidenced during very hot summers. Because of the fact that the seed plants are grown under conditions which permit free intercrossing, the tendency is for them to remain in this heterozygous condition even when grown in "sick" soil. When the seed plants are grown on "sick" soil, however, the plants which possess an insufficient number of factors for resistance develop the disease and are discarded. Thus, by selecting seed plants from "sick" soil, it is possible to maintain a resistant strain, under the conditions selected, even though it may not be homozygous for resistance.

RELATION OF AGE OF SEEDLINGS TO EXPRESSION OF THE RESISTANT CHARACTER

It has been shown (Table V) that during the first 20 days of development the Wisconsin Hollander plants are practically as susceptible to yellows at the higher temperatures as the susceptible commercial strain. On the other hand, the Wisconsin Hollander strain shows a high degree of resistance by the time the plants are old enough to be transplanted into the field. Since this condition has manifested itself for several seasons and since the plants are usually grown in soil with a fairly low tem-

perature before they are transplanted, there appear to be only two possible explanations for the low degree of resistance in the young seedlings, namely: (1) the resistant character is not fully expressed in the young seedlings and becomes more manifest as the plants grow older, or (2) the high temperatures inhibit or modify the expression of the resistant character in seedlings. Experiments have furnished some evidence favorable to both hypotheses. Data supporting the second have already been presented and discussed.

The initial experiments for testing the first theory were conducted by transferring the receptacles to the 26° C. tank after the plants had grown 20 days in infested soil at 14°. Plants of the commercial strain grown under similar conditions were transferred at the same time for controls. The air temperature was the same as that recorded for other experiments conducted at the same time, 14° to 18°. In Table VIII the results obtained at the end of 10 days are compared with the results obtained with seedlings which had been growing at 26° continuously for 20 days.

Because the number of plants used in these tests was small, the infection of one or two more plants in one test than in another made a considerable difference in the percentage of yellows. Even so, the results are consistent enough to indicate that the Wisconsin Hollander plants are more resistant after 20 days than when younger. In Table VIII it may be seen that there is an average difference of 54 per cent of yellows between the older plants of the Wisconsin Hollander and the Commercial Hollander strains at 26° C., while with the younger plants there is a difference of only about 14 per cent. At the same temperature the difference in the percentage of plants killed is equally striking. With the younger plants there is a difference of only 18 per cent, while with the older ones it amounts to 67 per cent. The disease developed very slowly in the Wisconsin Hollander seedlings, and at the conclusion of the experiment most of the affected plants showed only slight symptoms of yellows, while the reverse condition was manifest in plants of the commercial strain.

These investigations were carried further by planting both kinds of seed in sterilized soil and later transplanting the seedlings to "sick" soil. The plants for the first experiment were grown for 30 days in sterlized soil in flats. The temperature of both air and soil ranged from 18° to 22° C. during this period. In the first experiment, which was conducted during May and June, 1918, only three different soil temperatures were used in the series, but the complete series of temperatures from 14° to 35° was employed for the second. Three plants were set in each receptacle, and after transplantation on May 10 the receptacles were exposed to room temperature (18° to 22°) for three days in order to give the plants an equal chance to recover from the shock of being transplanted. The surface of the soil was covered with mineral wool and the receptacles were placed in the tanks at 15°, 17°, and 26°. The final results, recorded 30 days later, are given in Table IX. The air temperature during this experiment ranged from 18° to 22°, with a sudden rise of 10° to 15° for a few hours during the middle of the day. A contrast of the susceptible plants grown in "sick" soil at 15° and at 17° is shown in Plate 2, D, E.

TABLE VIII.—*Influence of age of cabbage seedlings upon the occurrence of yellows*

Date planted.	Condition of soil.	Wisconsin Hollander.						Commercial Hollander.					
		Grown in "sick" soil 20 days from seed, at 26° C.			Grown in "sick" soil 20 days at 14° C., then transferred to 26° for 10 days.			Grown in "sick" soil 20 days from seed, at 26° C.			Grown in "sick" soil 20 days at 14° C., then transferred to 26° for 10 days.		
		Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.
Feb. 1, 1918	Naturally infested	20	80	50	10	50	0	19	78	56	9	89	44
Mar. 29, 1918	do	10	70	20	10	30	10	10	100	50	10	100	100
Average results of two experiments.	Naturally infested soil	30	75	35	20	40	5	29	89	53	19	94	72
Mar. 29, 1918	Artificially inoculated	10	100	100	10	50	10	....	....	....	....	....	....
May 5, 1919	do	....	....	....	....	....	....	18	100	100	18	100	100

TABLE IX.—*Number of cabbage plants which showed yellows at the end of 30 days after transplantation to "sick soil"*

Temperature.	Number of plants.	Wisconsin Hollander.			Commercial Hollander.		
		Number healthy.	Number yellow.	Number dead.	Number healthy.	Number yellow.	Number dead.
°C.							
14	3	3	0	0	3	0	0
17	3	3	0	0	0	3	2
26	3	3	0	0	0	3	3

All of the Wisconsin Hollander plants remained healthy throughout this experiment, whereas all of the susceptible commercial plants at 17° and 26° C. developed typical yellows symptoms. At 26° yellows began to appear after 11 days and at 17° after 17 days. All three plants at 26° were dead after 17 days.

In the second experiment, plants were grown in sterilized soil 36 days from seed before they were transplanted into "sick" soil on May 24, 1919. The air temperature ranged from 22° to 25° C. during this period, but the subsequent treatment was similar to that in the preliminary experiment. The final results, recorded on June 13, are shown in Table X.

TABLE X.—*Number of cabbage plants which showed yellows at the end of 20 days after transplantation to "sick" soil*

Temperature.	Wisconsin Hollander.			Commercial Hollander.		
	Number healthy.	Number yellow.	Number dead.	Number healthy.	Number yellow.	Number dead.
°C.						
14	3	0	0	3	0	0
17	3	0	0	2	1	0
20	3	0	0	0	3	0
23	2	[a] 1	0	0	3	2
26	1	[a] 2	0	0	3	3
29	1	[a] 2	0	0	3	3
32	2	1	0	1	2	0
35	3	0	0	0	3	0

[a] The Wisconsin Hollander plants showed yellows in none but the lower leaves, and there only in incipient form.

Here again, all three of the Commercial Hollander plants developed typical yellows at 23° to 29°, inclusive, whereas only one or two of the Wisconsin Hollander plants showed any symptoms of the disease at all and then only in incipient form in the lower leaves. The number of plants is too small to warrant definite conclusions, but the evidence from both field and greenhouse experiments seems sufficient to indicate that older plants are more resistant than younger ones, and also that resistance becomes more stable as the plants grow older. There is also some indication here that resistance was weakened by starting the plants at a higher temperature, but the experiment needs to be repeated with larger numbers of plants before final conclusions on this point are justified.

The fact that a higher percentage of yellows develops at temperatures unfavorable for the growth of the seedlings is further evidence that the resistance is "broken down" at these temperatures. It has been reported that hereditary characters in several different organisms have been modified by changing the environment (including temperature) of the individuals. Baur (*3, p. 8–9*) found that flower color, an inherited character of the Chinese primrose (*Primula sinensis rubra*), was conditioned upon temperature. The plants were grown by the usual method until about one week before the blossoming stage; then some of them were put into a warm room (30° to 35° C.) and the others into a cool room (16° to 20°.) The plants at the higher temperature produced pure white flowers, while those at the lower temperature produced flowers of the normal red color of the variety. Biffen (*4*), in his work on the inheritance of resistance to yellow rust in wheat, concluded that any factor altering in any way the metabolic processes of the plant in turn alters the degree to which it is attacked by yellow rust and probably other fungi as well. He also found that a variety of wheat which under ordinary conditions of cultivation would be classed as moderately susceptible may be severely attacked when large amounts of nitrates are added to the soil.

FIELD EXPERIMENTS

FIELD OBSERVATIONS

Jones and Gilman (*11*) first recorded the fact that severe attacks of cabbage yellows are associated with hot, dry weather. Gilman (*8*) analyzed this evidence in detail and showed that the incubation period of the disease under field conditions is also materially influenced by soil temperature. The incubation period was about 14 days in 1912 when the mean daily temperature 6 inches below the surface was about 23° C. and 20 days in 1914 when the mean daily temperature at the same depth was about 20°.

Similar observations were made during the summers of 1917 and 1919 in the same field by the writer. In 1919 the mean daily soil temperature 4 inches below the surface was 25° C. at the time of transplantation. Thirteen days later 30 per cent of the Commercial Hollander plants showed typical yellows. This high percentage of diseased plants, together with the different stages of symptoms at that time, indicated that the disease had been present in some of the plants at an earlier date. According to these observations, the incubation period of the disease may vary under field conditions from about 12 days with a

mean soil temperature of about 25° to 20 days with a mean soil temperature of about 20°. It has also been repeatedly observed (*11, 8*) that the plants which escape or survive the disease for the first month may remain healthy during the remainder of the season.

These observations gave sufficient evidence to prove that soil temperature is an important factor in the development of yellows in cabbage plants transplanted into "sick" fields. However, they do not satisfactorily show what results might be obtained with younger seedlings planted at intervals throughout the season. Data upon experiments of this nature were considered of distinct value for comparison with results obtained in the greenhouse at different temperatures. Therefore, experiments were begun in 1917 for obtaining such data. Both the resistant Wisconsin Hollander and the susceptible Commercial Hollander were used. By beginning to plant early in the season and continuing until late in the summer, it was possible to have each successive crop of seedlings exposed to a different range of soil temperature. It was the original plan to plant the seed at intervals of 7 to 14 days and record the final data 20 days later, as was done in the greenhouse, but for various reasons this schedule could not at all times be very closely adhered to. Consequently, the data obtained in these experiments are not altogether comparable with those obtained in the greenhouse. Even so, as will be shown later, the two sets of data coincide closely enough to justify the conclusion that under field conditions soil temperature is one of the chief limiting factors in the development of yellows in young seedlings as well as in the older plants after transplantation.

EXPERIMENTAL METHODS

The soil temperature was recorded in 1917 in the experimental plot at 1 inch below the surface throughout the growing season and at 4 inches for a part of the season. In 1919 the temperature was recorded only at 4 inches below the surface. Fries kerosene bulb thermographs were used for this purpose. The instruments were checked once a week against a standardized thermometer to insure accuracy.

The seed was sown in short rows and observations were made every few days for a period of 20 to 30 days. In a few cases more than 30 days intervened between observations. During the driest part of the season it was necessary to water the soil at the time the seed was planted in order to insure germination. The seedlings were counted soon after they emerged from the soil, and this number was used as a basis for calculating the percentage of disease. The results of the experiments of 1917 and 1919 are given in summarized form in Table XI; the percentage of yellows and the range of the soil temperature are shown graphically in figures 7 and 8. In these figures is shown the correlation between the percentage of yellows and the curves which represent the range of mean daily soil temperatures. The mean is an average of the temperature readings taken at 2-hour intervals during the day. This is only a relative temperature, of course, because it gives no notion of the duration of the extremes, which is undoubtedly an important factor with diseases of this type.

EXPERIMENTAL DATA.—An inspection of Table XI and figures 7 and 8 shows that seedlings started early in the season may remain absolutely free of the yellows disease for the first 30 days, while the successive later plantings, up to about August 15, show a progressive increase in

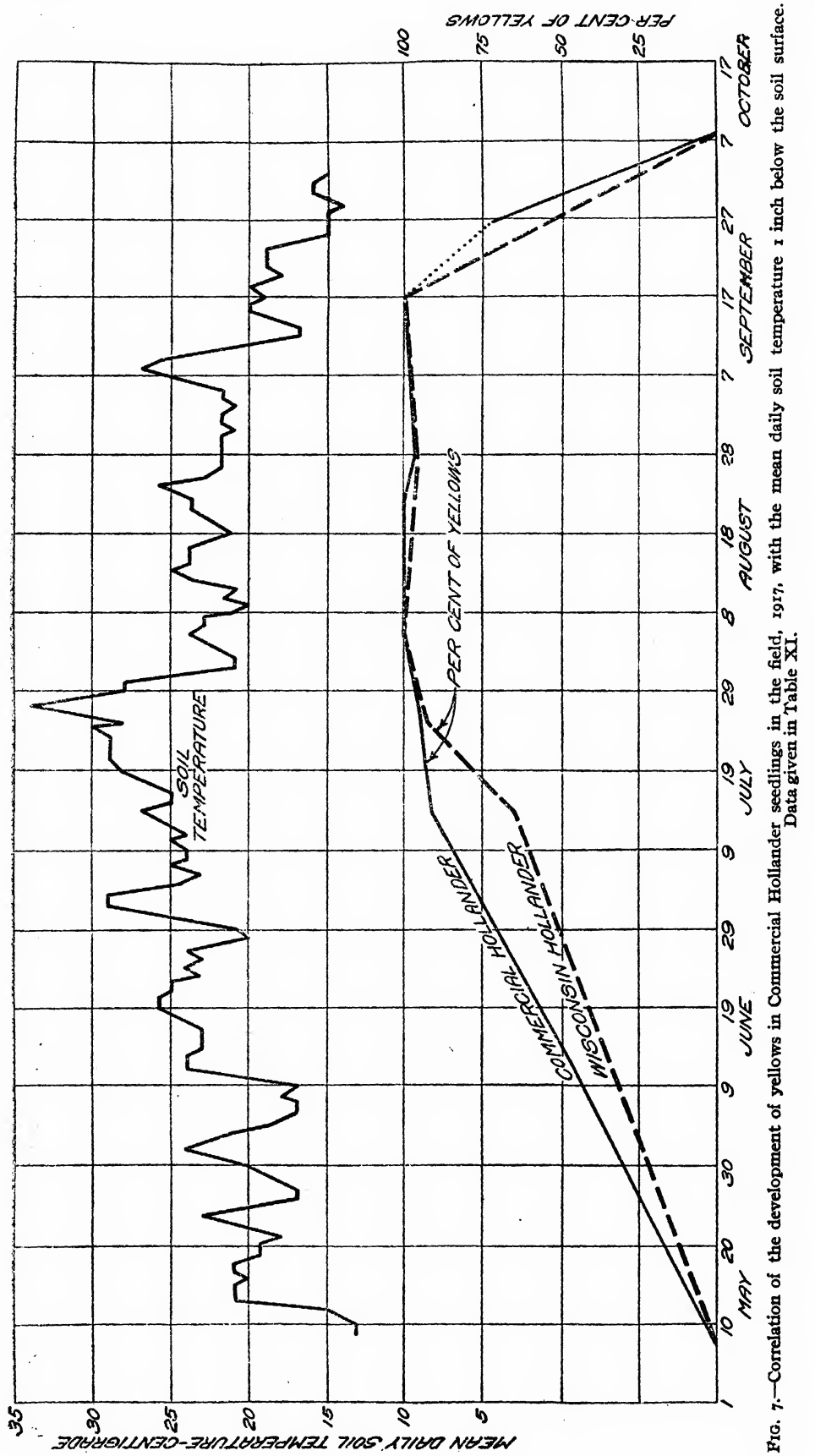

FIG. 7.—Correlation of the development of yellows in Commercial Hollander seedlings in the field, 1917, with the mean daily soil temperature 1 inch below the soil surface. Data given in Table XI.

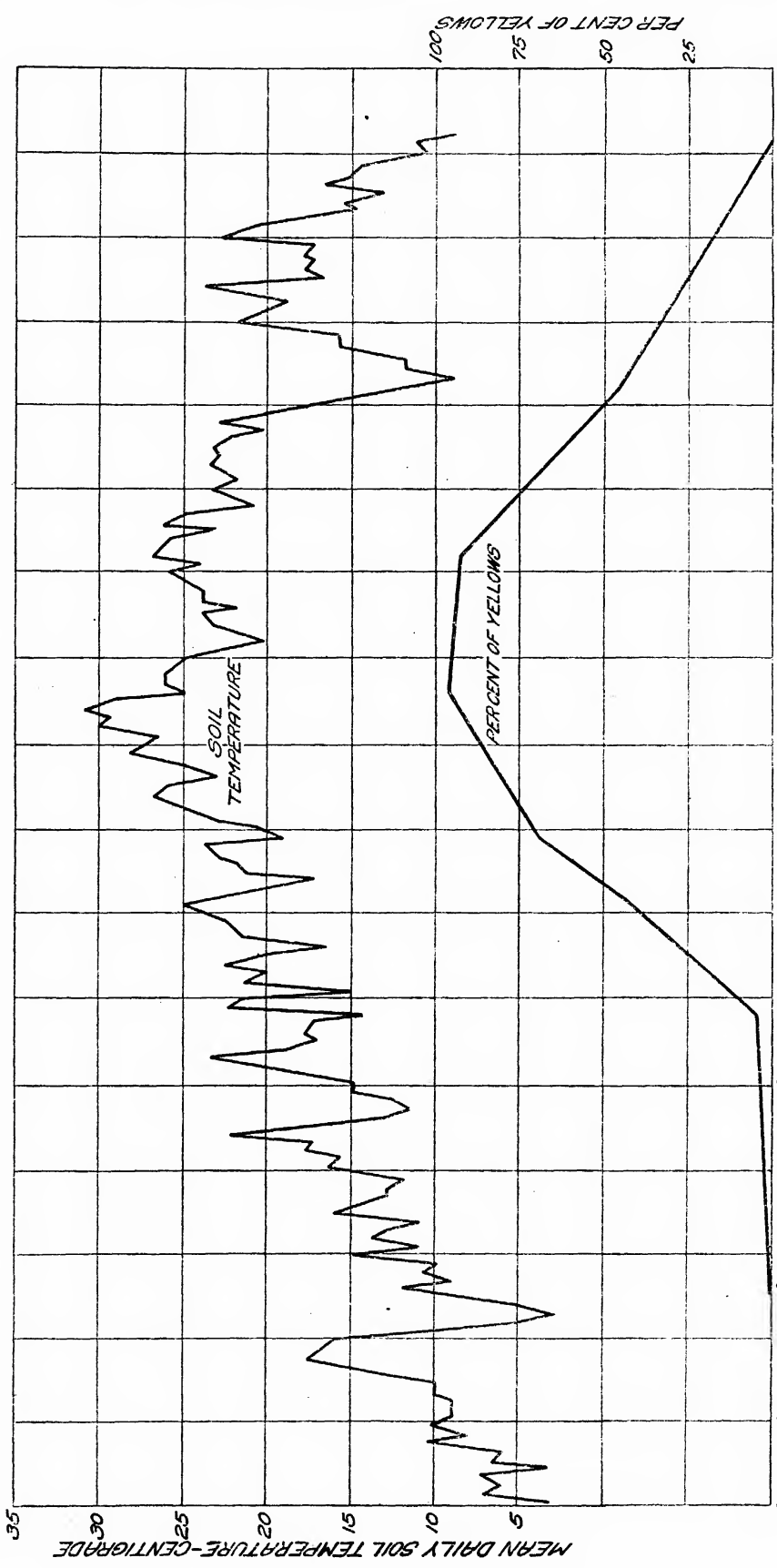

FIG. 8.—Correlation of the development of yellows in Commercial Hollander and in Wisconsin Hollander seedlings in the field, 1919, with the mean daily soil temperature 4 inches below the soil surface. Data given in Table XI.

the percentage of disease. Plantings made after the latter date showed a rapid decrease in percentage of disease until about September 15, after which the disease did not develop. It may also be observed (fig. 7 and 8) that this rise and fall in the percentage of disease correlates in general with the soil-temperature curve. The disease did not appear in the early part of the season until the soil temperature had remained above 17° C. for several days, and it ceased to develop in the latter part of the summer as soon as the temperature dropped below this point. By comparing the temperature curves in figures 7 and 8, it may be observed that the soil did not warm up as early in 1917 as in 1919 and that the development of yellows was deferred in a corresponding manner.

During the hot, dry period, which included the greater part of July and the first half of August, the disease developed as quickly as, and even more destructively than, at the high temperatures in the greenhouse. This result was probably due to the combined influences of high soil temperature and low soil moisture, each of which was found to favor rapid development of the disease under greenhouse conditions. On the other hand, the disease was found to develop more slowly in the field at the lower temperatures than in the greenhouse at constant similarly low temperatures. This difference was probably due to the effect of the wide daily fluctuation of the soil temperature upon the fungus under field conditions—that is, the short period of higher temperature might have been insufficient for the fungus to recover from the effect of the longer duration of low temperature. Often when the mean temperature was about 20° C. the range was probably below 20° all night and a large portion of the day, while it remained above the mean only a few hours.

In several instances, during both 1917 and 1919, the plants were left in the beds for the greater part of the season and observations were made upon them from time to time. By this method it was learned that most of the Wisconsin Hollander plants which were healthy at the end of the first 30 days remained so throughout the experiment. The number of plants thus escaping the disease was greater when they were started in May than when started in July or in August when the soil was hot and dry. The Wisconsin Hollander plants which were started on May 15, 1917, showed no yellows at the end of the first 33 days, and only 15 per cent 62 days after planting. There was no increase in percentage of disease between July 18 and July 26. Conversely, the plants which were started on July 14, 1917, showed 96 per cent of yellows on August 4, 21 days after planting. Sixteen days later the disease had increased to 98 per cent. These data confirm the results obtained in the greenhouse, that plants which were permitted to establish themselves before being exposed to the Fusarium at a temperature favorable for its attack were more resistant than plants started in "sick" soil at the optimum temperature for the fungus. In other words, the plants became more resistant with age.

TABLE XI.—*Percentage of plants developing yellows when planted in the field on different dates during the season*

Date of planting.	Date of observation.	Number of days between time of planting and final observation.	Total number of plants.		Percentage of yellows.	
			Wisconsin Hollander.	Commercial Hollander.	Wisconsin. Hollander.	Commercial Hollander.
1917.						
May 25	June 27	33	100	100	0	5
June 21	July 11	20	100	100	15	57
June 27	July 18	21	100	100	22	70
July 14	Aug. 4	21	140	104	96	97
July 26	Aug. 20	25	89	205	94	94
Aug. 18	Sept. 8	21	400	120	48	48
Sept. 22	Oct. 8	16	100	100	0	0
1919.						
Apr. 6	May 8	32	200	200	0	0
May 8	June 12	35	200	200	35	45
June 18	July 14	26	256	173	64	92
June 30	July 25	25	191	96	94	96
July 14	Aug. 6	23	111	93	100	100
July 30	Aug. 22	23	269	140	97	100
Aug. 6	Aug. 28	22	210	266	96	97
Aug. 12	Sept. 16	35	390	360	100	100
Aug. 20	Sept. 16	26	28	131	100	100
Sept. 7	Sept. 27	20	152	134	53	70
Sept. 16	Oct. 8	22	213	280	0	0

INFLUENCE OF SOIL MOISTURE UPON THE OCCURRENCE OF YELLOWS IN CABBAGE SEEDLINGS

EXPERIMENTAL METHODS

The experiments for measuring the influence of soil moisture upon the occurrence of yellows were conducted during the winter of 1918 and the spring of 1919 simultaneously with, and under the same atmospheric conditions as, the soil-temperature experiments. The same kind of soil and receptacles were used, but no cinders were placed on the bottom of the receptacles. The moisture-holding capacity of the soil was found by means of 5 by 20 cm. tubes to be 46 per cent. When based upon wet weight, this gave approximately 31 per cent moisture.

The moisture content of the soil was kept constant during the experiments through the use of one Livingston cylindrical auto-irrigator, 5 by 15 cm., in each receptacle. The cup was imbedded vertically in the soil, and the water reservoirs were so placed that the moisture content became adjusted at 14.5, 19, 23, and 26 per cent, respectively, in the four sets of duplicate receptacles. The receptacles with 14.5 per cent moisture were kept in the series for only about two weeks when the water columns broke and they were not restored in time to keep them in the series. Moisture determinations were made just before the seed was planted and at the conclusion of the experiment. The percentage of moisture at the conclusion was only a few tenths of 1 per cent lower than at the beginning. The soil temperature was kept constant at 22° to 23° C. throughout the experiment.

After the soil moisture had become constant, seed of the Commercial Hollander strain was planted in the usual manner, and the final data were recorded 28 days later. A few days after the plants emerged from the soil, they were thinned to the desired stand and a half-inch layer of mineral wool was placed over the surface of the soil to equalize surface temperatures. In Table XII the average results from the duplicate receptacles are shown separately for each experiment at the end of different periods of time. The average results of the two experiments are given in Table XIII and shown graphically in figures 9 and 10.

TABLE XII.—*Influence of soil moisture upon the development of yellows in cabbage seedlings, shown at end of different periods after seeding*

FIRST EXPERIMENT, BEGUN FEBRUARY 28 AND CONCLUDED MARCH 27

Number of days after planting.	19 per cent moisture.			23 per cent moisture.			26 per cent moisture.		
	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.	Total number of plants.	Percentage yellow.	Percentage dead.
10	20	0	0	20	0	0	20	0	0
12	.....	20	0	.....	0	0	.....	3	0
15	.....	50	0	.....	5	0	.....	20	0
17	.....	85	30	.....	45	0	.....	35	5
20	.....	95	50	.....	80	15	.....	70	25
24	.....	95	95	.....	95	55	.....	95	60
28	.....	100	95	.....	100	80	.....	100	75

SECOND EXPERIMENT, BEGUN APRIL 23 AND CONCLUDED MAY 22

10	17	0	0	27	0	0	31	0	0
12	.....	40	20	.....	0	0	.....	3	0
15	.....	70	30	.....	4	0	.....	52	0
17	.....	70	40	.....	15	0	.....	65	0
20	.....	80	60	.....	63	7	.....	81	29
24	.....	100	60	.....	63	15	.....	87	42
28	.....	100	90	.....	78	48	.....	94	61

TABLE XIII.—*Percentage of cabbage plants developing yellows in soil with different moisture contents—average results of the two experiments shown in Table XII*

Number of days after planting.	Percentage of plants yellow.			Percentage of plants dead.		
	19 per cent moisture.	23 per cent moisture.	26 per cent moisture.	19 per cent moisture.	23 per cent moisture.	26 per cent moisture.
10	0	0	0	0	0	0
12	30	0	3	10	0	0
15	60	5	36	15	0	0
17	78	30	50	35	0	3
20	88	72	75	55	11	27
24	98	79	91	78	35	51
28	100	89	97	93	64	68

EXPERIMENTAL DATA.—From Table XIII it may readily be seen that with favorable temperature yellows develops at any percentage of soil moisture permitting growth of the cabbage seedlings. However, it

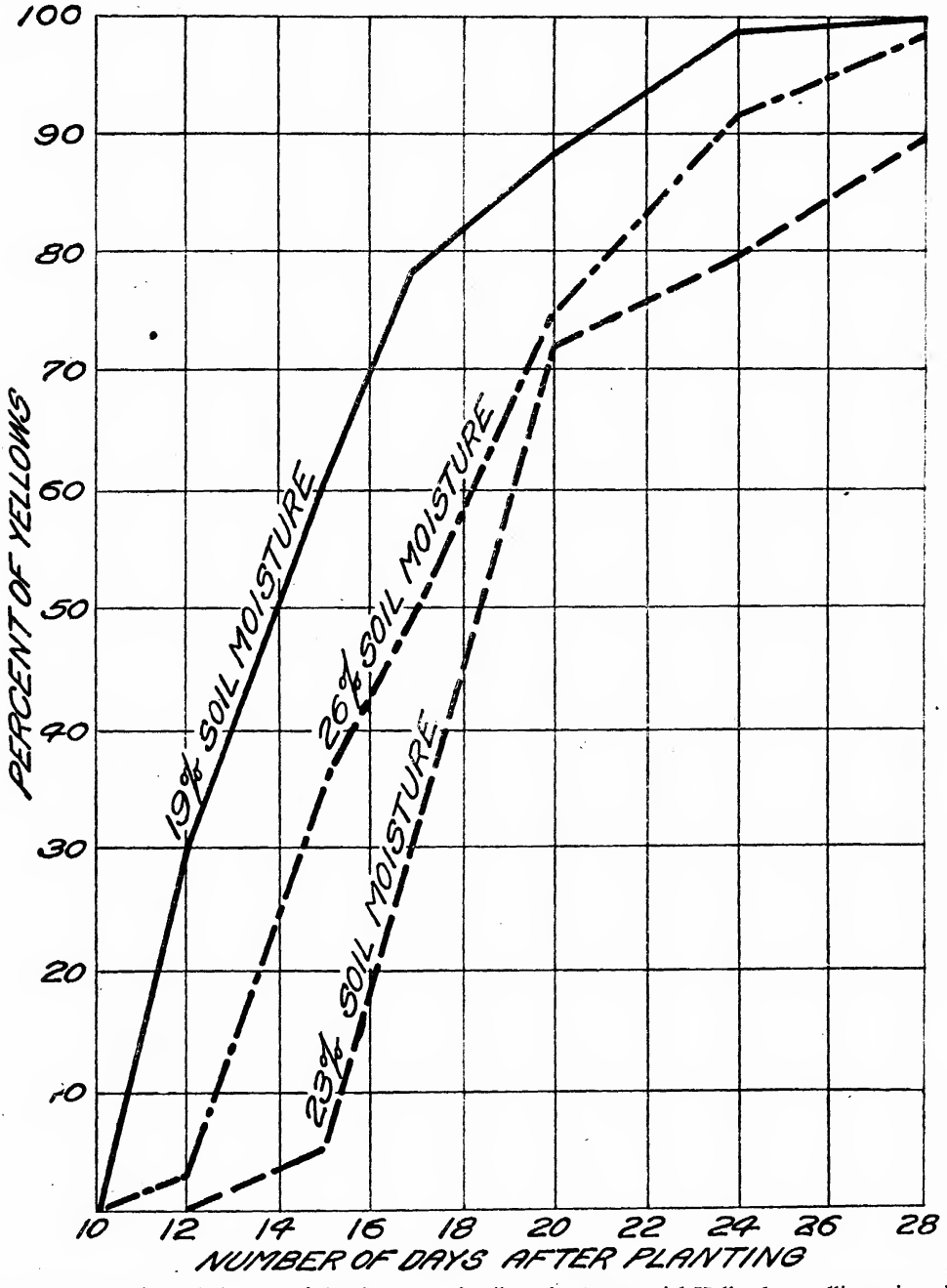

FIG. 9.—Comparison of the rate of development of yellows in Commercial Hollander seedlings given in Table XIII, grown 28 days at 23° C.

developed most rapidly and destructively in soil with 19 per cent moisture, even though, as will be shown later (Table XIV), this percentage of moisture was most favorable for the growth of the seedlings. This was a case, then, where the most vigorous plants were the least resistant to the disease.[5] The development of the disease with 19 per cent moisture

[5] Plants were grown long enough with about 15 per cent soil moisture to show that the yellows developed even more rapidly with this amount than with 19 per cent moisture. This percentage of moisture was about as low as would permit growth of the seedlings.

was very rapid until most of the plants were affected, after which the remaining plants showed infection more slowly. Conversely, the disease developed slowly but at a fairly uniform rate with 23 and 26 per cent moisture, and at the conclusion of the experiment the percentage of

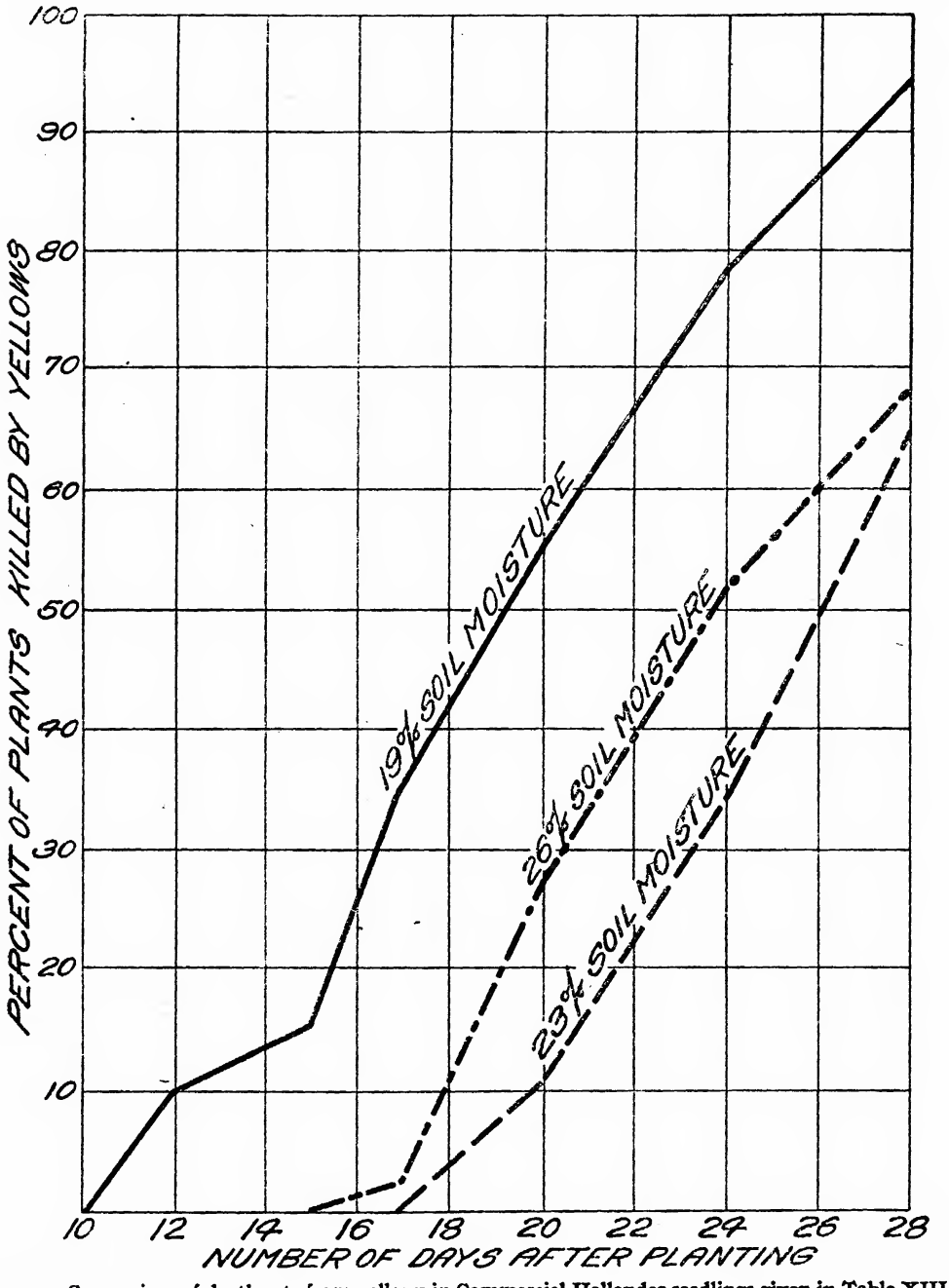

FIG. 10.—Comparison of death rate from yellows in Commercial Hollander seedlings given in Table XIII, grown 28 days at 23° C.

disease was noticeably lower, especially with 23 per cent moisture. It was rather surprising to find that this comparatively slight difference in percentage of moisture made such a significant difference in the rate of development of yellows.

The difference in death rate of the affected plants is even more striking. At the conclusion of the experiment the number of plants killed by yel-

lows at 19 per cent moisture was 29 per cent greater than at 23 per cent moisture and 25 per cent greater than with 26 per cent moisture. At first sight, this condition suggests that because of the reduced functional root system the affected plants were unable to take up the necessary amount of water from the soil with 19 per cent moisture. These results are probably partially explainable on this basis, but the more rapid death rate with 26 per cent moisture than with 23 per cent and the general appearance of the plants in the former case suggest the operation of some factor in addition to deficient water supply. The fact that beyond a certain point both the general vigor of the highly susceptible plants and the percentage of yellows in them decreased with an increase in percentage of soil moisture suggests a reduction in the virulence of the Fusarium. It is possible, therefore, that the reduced oxygen supply in the soil due to increased water content affects both host and parasite.

INFLUENCE OF SOIL MOISTURE UPON THE GROWTH OF CABBAGE SEEDLINGS

It is interesting to note in this connection the influence of soil moisture upon the growth of cabbage seedlings themselves. This investigation was made simultaneously with the study of the influence of soil moisture upon the occurrence of yellows. The dry weight of the plants was used as an index of growth in this case, as previously, and the plants grown in sterilized soil as controls for the second test in the study of the disease were used for the determinations. The plants were cut at the surface of the soil 35 days after seeding and dried for 16 hours at 95° C. Six plants from each of the two receptacles were used. The average weight per plant of the 12 plants is given in Table XIV.

TABLE XIV.—*Dry weight of cabbage seedlings grown in soil with different moisture contents*

Soil moisture (per cent)	19	23	26
Dry weight per plant (gm.)	0.478	0.343	0.251

It will be seen from the foregoing table that the soil with 19 per cent moisture was the most favorable for increase in dry weight. Also, the vigor of the plants, rich leaf color, and extensive root system indicated that the plants were in a healthy, flourishing condition. Contrasted with these, the plants growing in soil with 26 per cent moisture showed decided stunting, yellowish-green leaf color, and a greatly reduced root system. In addition, the roots were brown and small. Both the discoloration and the disintegration were even more pronounced in roots grown in the "sick" soil, probably because of the action of secondary organisms following the Fusarium. The plants grown in the soil with 23 per cent moisture exhibited characters between these extremes.

The results of these experiments show that cabbage is not a high-moisture-loving plant and that the Fusarium is more pathogenic in soil with low moisture content.

SUMMARY

(1) Cabbage yellows, a disease caused by the vascular parasite *Fusarium conglutinans*, has been observed to develop in its most destructive form in southeastern Wisconsin only under conditions of hot, dry weather,

whereas there is little or no development even in the "sickest" soils during moist, cool weather.

(2) Hot, dry weather also retards the growth of cabbage plants.

(3) During such critical periods they become pale green in color and show a general lack of vigor; even the resistant Wisconsin Hollander shows a considerable percentage of incipient yellows. With the return of favorable rainfall and lower temperature the resistant plants overcome the attack of yellows and produce marketable heads.

(4) Such field observations soon convince one that the occurrence and severity of yellows are closely correlated with the influence of soil temperature and soil moisture, and the question arises as to how far and in what way these influences relate to the host on the one hand and to the parasite on the other.

(5) The commercial varieties have shown considerable differences in degree of resistance to Fusarium. Also, within any one variety there is always a variation in resistance as between individuals. During severe seasons most of the plants of the standard commercial varieties quickly succumb to the disease; others linger along in a dwarfed condition, slowly shedding their lower leaves, whereas a few scattered individual plants in the field usually remain healthy and produce marketable heads. This variation in individual susceptibility or disease resistance has been the basis for developing the resistant Wisconsin strains.

(6) The preceding observations and experiments were made upon plants which had been transplanted into the field.

(7) Gilman (*8*), in his greenhouse experiments, showed that yellows did not develop below 17° C. while it developed quite destructively at 23° to 25°. He also studied the effect of certain temperatures upon the growth of the fungus in culture, but he did not define the upper temperature limits either for its growth or for the occurrence of the disease.

(8) The writer undertook to learn more exactly the factors regarding these questions, using seedling cabbage plants. The purposes outlined were: (1) To determine the range of soil temperature for the occurrence of yellows in cabbage seedlings, the air temperature being kept constant; (2) to study the influence of soil temperature upon the growth of cabbage seedlings in noninfested soil; (3) to determine in like manner the influence of soil moisture both upon the growth of cabbage seedlings and upon the occurrence of yellows in them; (4) to study the influence of high soil temperature and soil moisture upon the susceptibility of the resistant strain, that is, upon the "breaking down" of resistance. These experimental investigations have justified several conclusions bearing upon these questions as follows.

(9) Pure cultures of *Fusarium conglutinans* on potato agar plates showed the following relations to temperature: (1) The organism grew at temperatures ranging from 7° to 35° C.; (2) the optimum temperature, using diameter of colony as a criterion, at the end of 7 days was 25° to 27°; (3) although no growth took place in 7 days at 37°, the organism was not killed at this temperature.

(10) Cabbage yellows develops in seedlings growing in "sick" soil at soil temperatures ranging from 17° to 35° C. At 17° it develops very slowly even in the most susceptible strains. In naturally infested soil the disease appears first and develops most rapidly in both resistant and susceptible strains at 26° to 29° and in sterilized artificially inoculated soil at 29° to 32°.

(11) It thus appears that the optimum temperature for the vegetative growth of the fungus in culture practically coincides with the optimum for the development of yellows in seedlings. This is above the optimum for the growth of the host plant.

(12) Cabbage seedlings grew at all temperatures from 14° to 38° C., but only very poorly at the latter. At 38° the seedlings emerged from the soil, but most of them died before developing any true leaves. The optimum soil temperature for seedling growth was found to be about 20° when the Wisconsin tank method was used.

(13) Soil temperature greatly influences the length of the incubation period for the disease. Under controlled conditions the incubation period varied from 18 days at 17° C. to 8 days at 29° to 32°.

(14) With constant favorable temperature yellows developed at any percentage of soil moisture permitting growth of cabbage seedlings. In soil with a moisture-holding capacity of 46 per cent the yellows developed more rapidly and destructively in susceptible plants when the moisture was held at 15 per cent than at 19, 23, or 26 per cent. At 19 per cent moisture the disease appeared two days later than at 15 per cent, but once it had started the subsequent rate of development was about the same in both cases.

(15) Nineteen per cent soil moisture was the most favorable for the growth of cabbage seedlings when the soil temperature was held at 23° C. and the air at 14° to 18°. The growth of plants was materially checked at 26 per cent and also at 15 per cent soil moisture. At these less favorable moistures the color of the foliage was quite abnormal. Plants grown at 19 per cent soil moisture had healthier color and a more extensive root system, and the dry weight was almost twice that at 26 per cent moisture. Thus it is evident that the soil moisture (15 per cent) which was too low for good growth of the host plant was most favorable for the development of yellows, while the soil moisture (19 per cent) which proved almost equally as stimulating to the disease was highly favorable for normal development of the host plant.

(16) The preceding conclusions relative to the relation of soil temperature and soil moisture to the occurrence of yellows in cabbage seedlings were first worked out in the greenhouse under experimentally controlled conditions.

(17) Soil temperature and soil moisture influenced the occurrence of yellows in the field in a manner similar to that in the greenhouse. With seedlings started in May, June, and September when the soil temperature was low, the disease showed a lower percentage and was slower in appearing than with those started in July and August when a high soil temperature prevailed.

(18) These facts, no doubt, are of significance in the geographical distribution of the disease. In the Southern States, where cabbage is generally grown commercially as a winter or early spring crop, the soil temperature is very probably too low for the organism to gain a foothold on such plants. Reports from these sections indicate, however, that where once introduced the Fusarium establishes itself on summer-grown cabbage or other related hosts and may be expected to persist and attack the crops whenever soil temperature is favorable.

(19) The foregoing conclusions are based on the general or average behavior of the cabbage seedlings. It is in this connection that noteworthy variations in the incubation period as between certain individual plants in the same receptacle may be as great as between some plants

grown at 17° and at 29° C. This variation occurs not only with seedlings of the susceptible strain but also with those of the Wisconsin Hollander.

(20) The great difference in the individuality of plants, as shown by the variation in the length of incubation period of the disease and degree of infection of plants, is conclusive evidence of a variation in degree of resistance. This variation in degree of resistance is, no doubt, due to a lack of factors or to a heterozygous condition of some of the plants for the factors for resistance.

(21) The degree of resistance shown by a strain of cabbage depends to a considerable extent upon the environmental conditions under which the plants are grown. In our trials all plants remained healthy in "sick" soil below 17° C.; many were resistant at 17° to 23°, and a small number were resistant at the higher temperatures. At all temperatures within the infection range some plants showed intermediate degrees of resistance, whereas others were entirely susceptible. Somewhat similar results were obtained with different percentages of soil moisture.

(22) Fusarium resistance in cabbage becomes more pronounced with increasing age of the plant. Young seedlings of the resistant strain, Wisconsin Hollander, developed a relatively high percentage of yellows when started in "sick" soil at high temperatures, whereas plants grown for 30 days or more in noninfested soil, or even in "sick" soil below 17° C., when transferred to "sick" soil at high temperatures developed only a low percentage of yellows and then usually only in an incipient form.

(23) This fact has practical significance in predicting, interpreting, or improving the performance of this and the other resistant Wisconsin strains in the following ways:

(*a*) In the first place, it indicates that the strains will give the best results commercially when started in a noninfested seed bed during cool spring weather. This accords with the best practice of commercial cabbage growers in the Northern States.

(*b*) In the second place, it indicates that these resistant strains may safely be recommended for trial in Fusarium "sick" soil in all geographic localities where the prevailing temperatures at the different early stages of development of the cabbage plants are not distinctly higher than those in Wisconsin.

(*c*) Finally, the resistance of these Wisconsin strains may be expected to "break down" in some degree proportional with the elevation of temperature above this point. However, in the Northern States, even in the warmer seasons, this usually stops with the incipient stages of the disease and leaves the crop commercially successful. If more trying conditions are met with elsewhere, it seems probable that through further selection strains showing a correspondingly higher degree of resistance may be secured.

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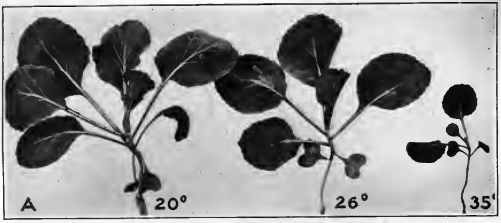
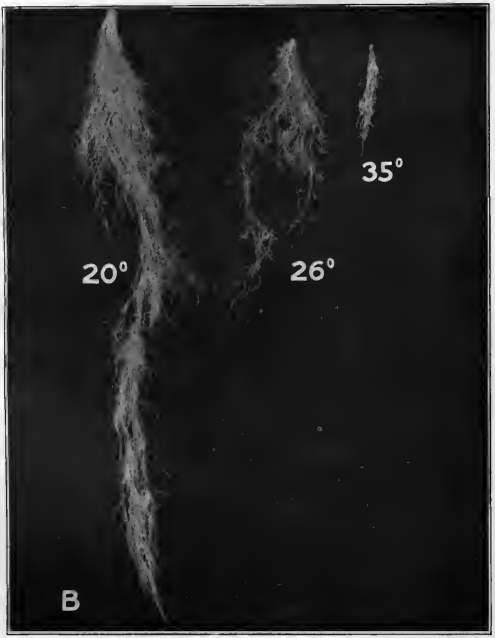
PLATE 1

A.—Comparison of shoots of Wisconsin Hollander cabbage seedlings grown during November and December for 53 days from seed in noninfested soil at the temperatures indicated.

B.—Comparison of root systems of plants shown in A.


A
20°
26°
35°


35°
20°
26°
B


A
SUSCEPTIBLE.
STERILE SOIL.
HEALTHY.
B
SUSCEPTIBLE.
SICK SOIL.
DISEASED.


C
RESISTANT.
SICK SOIL.
HEALTHY.


D
SUSCEPTIBLE.
SICK SOIL.
15° HEALTHY.
E
SUSCEPTIBLE.
SICK SOIL.
17° DISEASED.

PLATE 2

A.—Commercial Hollander plants grown 30 days from seed in sterilized soil at 17° C. All 10 plants healthy.

B.—Commercial Hollander plants grown 30 days from seed in "sick" soil at 17° C. Three of the 10 dead; all others yellowing and stunted.

C.—Wisconsin Hollander plants grown 30 days from seed in "sick" soil at 17° C. All healthy.

D.—Susceptible Commercial Hollander plants grown 36 days from seed in non-infested soil, then 30 days after transplanting to "sick" soil at 15° C. All healthy.

E.—Plants of same variety as those in D and grown under same conditions but transplanted to "sick" soil at 17° C. Two dead; the other yellow and stunted.

ACTION OF SOAP UPON LEAD ARSENATES[1]

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Soap is sometimes added to the water used for applying lead arsenate as an insecticide. Few authorities advise its use on apple trees or tender vegetation, but it is occasionally advised for use upon hardy and smooth-leaved crops, such as cabbage and sugar beets. It is said that the use of soap with arsenates is increasing.

THE ADVANTAGES

Several advantages are to be gained by the use of soap. The lead arsenate remains in suspension longer in soap solution than in pure water, hence is more easily and evenly applied, especially when hand-operated sprayers are used. The soap also helps to spread the arsenical. J. R. Parker (*3*) [2] found that soap retarded the settling of lead arsenate and stated that it also improved the spreading upon smooth-leaved plants.

THE DISADVANTAGES

The disadvantage to be feared is that of burning the leaves of the crop sprayed by arsenic dissolved by the action of the soap upon the lead arsenate.

Tartar and Bundy (*6*) in 1913 reported that fruit trees were injured by spraying with soap and lead arsenate and showed that the use of the soap increased the quantity of soluble arsenic in the liquid. They also noted that acid arsenate was much more soluble in soap solution than neutral arsenate. Headden (*1*) had already pointed out the danger in using water containing alkali salts for arsenical sprays. It was therefore natural that some presumed that the solubility of lead arsenate in soap solution was due to free sodium carbonate in the soap, and that the damage could be avoided by using only neutral soaps. However, it has been commonly known to chemists for a long time that lead readily forms insoluble soaps. Therefore it is to be expected that neutral soaps might undergo double decomposition with the lead arsenate, forming lead soap and alkali arsenates which would be soluble. This possible reaction may be represented thus:

Sodium soap + lead arsenate = sodium arsenate + lead soap. As the sodium compounds are soluble and the lead compounds are insoluble, it is not to be expected that the reaction will go to completion in either direction. This double decomposition, however, is not the only imaginable reaction which might take place between the soap and lead arsenate; and, in fact, others have been reported by some investigators and will be discussed later. So far as observed by the writer, no one has heretofore reported whether or not soaps of different fatty acids behaved differently toward lead arsenates.

[1] Accepted for publication May 29, 1922.

[2] Reference is made by number (italic) to "Literature cited," p. 95.

FORMULAS OF LEAD ARSENATES

The composition and naming of lead arsenates is a matter that was at one time in great confusion. It has been held by most chemical workers in this field, however, that the lead arsenates on the market fall into three classes:

1. Triplumbic ortho arsenate ($Pb_3(AsO_4)_2$), called by manufacturers "ortho," "triplumbic," "normal,," or "neutral" lead arsenate.
2. Diplumbic ortho arsenate ($PbHAsO_4$), called "monoplumbic," "diplumbic," "acid arsenate," or simply "lead arsenate."
3. Mixtures of these two compounds.

Tartar and Robinson (*4*, 7) believe that the substances here designated as class 1 are really of much more complicated structure and suggest the formula

$$21Pb_3(AsO_4)_2.Pb(OH)_2.10H_2O.$$

They also state that the diplumbic arsenate, $PbHAsO_4$, can be readily transformed into this basic compound by treating with ammonia and that by this treatment, a definite quantity of arsenic is dissolved and may be recovered in the ammonia solution. It thus is an accurate method for the determination of the quantity of diplumbic arsenate in class 3.

Their formula for this basic compound was refuted in two papers appearing at the same time by McDonnell and Smith (*2*) and by G. Ennis Smith (*5*), both suggesting the same formula, $Pb_5OH(AsO_4)_3$, and G. Ennis Smith suggesting the name lead hydroxy arsenate. By this treatment with ammonia, 40 per cent of the arsenic present in the diplumbic goes into solution and the rest remains insoluble as the basic compound.

All investigators agree that the diplumbic arsenate is the more active compound of the two and that where conditions are such as to cause any apprehension of damage from burning the normal compound should be preferred. On the other hand, the acid arsenate contains a greater percentage of arsenic; hence the consumer can secure the required quantity of arsenic with a smaller number of pounds to pay freight and profits upon. The acid arsenate is sold in much greater quantity in many markets.

G. Ennis Smith, in the paper already referred to (*5*), states that soap acts upon diplumbic arsenate in the way that Tartar and Robinson reported for ammonia (*4*, 7)—transforms it into the more basic lead arsenate and dissolves some of its arsenic but sets free an equivalent quantity of the fatty acid, which is insoluble. Thus assuming that the end product is triplumbic arsenate, the reaction is:

$$3PbHAsO_4 + 2NaC_{17}H_{35}CO_2 = \underset{\text{(insoluble)}}{Pb_3(AsO_4)_2} + \underset{\text{(insoluble)}}{2C_{17}H_{35}COOH} + \underset{\text{(soluble)}}{Na_2HAsO_4}.$$

In this case 33⅓ per cent of the arsenic of the diplumbic arsenate is made soluble. In case we consider the end product to be lead hydroxy arsenate, the reaction is:

$$5PbHAsO_4 + 4NaC_{17}H_{35}CO_2 + H_2O = \underset{\text{(insoluble)}}{Pb_5OH(AsO_4)_3} + \underset{\text{(insoluble)}}{4C_{17}H_{35}COOH} + \underset{\text{(soluble)}}{2Na_2HAsO_4}.$$

In this case 40 per cent of the arsenic of the diplumbic arsenate goes into solution. Under this theory there should be no reaction possible

between soap and the lead hydroxy arsenate; hence treating that compound with soap should not bring arsenic into solution.

McDonnell and Smith (*2*) state that triplumbic arsenate is formed under certain conditions, but that it has a very limited range of existence and is readily changed over to the more basic lead arsenate (lead hydroxy arsenate) by treatment with ammonia. By that change 10 per cent of its arsenic should go into solution; hence that should be the maximum possible to obtain by treatment with soap if the end product is in fact lead hydroxy arsenate.

On the other hand, if the reaction between soap and lead arsenate is of the simple double decomposition type, it might be possible for diplumbic arsenate to yield more than the 33⅓ or 40 per cent above quoted, and it might be that the lead hydroxy arsenate would yield soluble arsenic. Furthermore, whichever the type of the reaction, its completeness might be different with soaps of different fatty acids. With these points in view, the writer's materials were selected as follows:

THE SOAPS

The soaps were prepared in the laboratory from c. p. sodium hydroxid and the best grade of the free fatty acids, stearic and oleic. Palmitic was omitted, as no satisfactory palmitic acid was at hand. After the soaps had been prepared they were analyzed for free alkali and for moisture. The stearic soap contained 83.1 per cent moisture and 0.85 per cent free alkali, computed as sodium carbonate. It was weighed in this form for the first series of trials but is recorded on the dry basis. For the second series this soap was dried to 4.3 per cent moisture and weighed in that form.

The oleic soap contained 83.5 per cent moisture and no free alkali. Though it was not found practicable to dry the oleic soap, on the tables it is reported on the dry basis.

THE LEAD ARSENATES

The lead arsenates were selected from a large number of samples that had been furnished by manufacturers for comparison and are designated in this paper as "M" and "T". "M" was labeled by the manufacturer "Monoplumbic Lead Arsenate" and contained at the time of the first series of tests 41.9 per cent water. When dry, it contained arsenic equivalent to 31.53 per cent As_2O_5, and lead to 65.4 per cent PbO. When received, it was analyzed for water soluble arsenic and found to contain, on the dry basis, 0.85 per cent As_2O_5. This was taken to indicate that this arsenical was a practically pure diplumbic hydrogen arsenate. A later test on the dried material with 4 per cent ammonium hydroxid, in the manner described by Tartar and Robinson (*4*, 7), dissolved 8.85 per cent As_2O_5, indicating that only about 67 per cent of the material was really diplumbic arsenate ($PbHAsO_4$) and that 30 per cent of the arsenic present was in some other form.

"M" was used in paste form for the first series and in dry form for the second.

"T" was labeled "Triplumbic Ortho Lead Arsenate" and contained 46 per cent water, and in the dry material arsenic to the amount of 25.75 per cent As_2O_5 and lead to 72.10 per cent PbO. The water-soluble arsenic when received was equivalent to 0.43 per cent As_2O_4, on the dry basis. This was supposed to indicate that the paste was as

labeled, a pure triplumbic arsenate; but since the authorities already noted cast considerable doubt upon the existence of this salt in commercial lead arsenates, it might have been a mixture. The dry matter when treated with 4 per cent ammonium hydroxid, yielded but 0.22 per cent of soluble As_2O_5, indicating but 1.70 per cent $PbHAsO_4$.

BRINGING THE MATERIALS IN CONTACT

Preliminary work indicates that when the soap solution is mixed with the lead arsenate, either as paste or dry, it is difficult to get concordant results for the quantity of arsenic made soluble. It was also noted that when finely divided lead arsenates were shaken with soap solutions the resulting precipitates were bulky and the individual curds frequently large. Besides, as would be expected of either lead soaps or free fatty acids, they were water-repellent. It seemed probable, therefore, that the particles of lead arsenate, when brought into contact with the soap solution, were at once acted upon at the surface and surrounded with an envelope of the water-repellant reaction product, which in turn protected the lead arsenate at the interior of the curd from further action. This reasoning led to the following procedure.

In each case, the treatment was carried out with 2 liters of soap solution in a 2½-liter acid bottle. The soap to be used for each sample was dissolved in 1 liter of distilled water, to be later mixed with a second liter of distilled water, as follows: The water was placed in the acid bottle and the weighed sample of lead arsenate was placed in a porcelain mortar where it was ground with successive portions of the soap solution. The soap and finely divided material was poured into the liter of water, taking care that the coarser particles were left in the mortar to be ground with the next portion of the soap solution. When all the lead arsenate had become so fine as to pass easily into the water with the soap solution, the remainder of the liter of soap solution was poured into the bottle, making 2 liters. The bottle was stoppered and shaken at intervals for five days, then filtered through paper, using suction. One half the samples were treated as stated; the other half were ground in the water and poured into the soap solution, the only difference being the fluid used for the grinding.

The lead arsenates in the first series were weighed in paste form, 2 gm. of "M" amounting to 0.346 gm. of As_2O_5 and to 0.707 gm. PbO, and 2.15 gm. of "T" amounting to 0.289 gm. of As_2O_5 and to 0.805 gm. PbO.

The soaps were used in two concentrations, 0.6 gm. and 1.8 gm. (dry basis) per 2 liters of solution.

The filtrate thus secured was analyzed for arsenic, while the insoluble material was dried, removed from the filter, and preserved for analysis for lead and for total arsenic.

It was found advisable to remove the soap that remained dissolved in the filtrate. This was accomplished as follows: The measured aliquot to be analyzed was placed in a beaker and a few drops of barium chlorid solution were added, just enough being used to clear the solution. The insoluble barium soap separated at once and was removed by filtering, leaving a clear solution that could be reduced and titrated with iodin in the usual Gooch and Browning method (*8, p. 239*).

The quantity of arsenic rendered soluble and found in the filtrate is given in Table I.

TABLE I.—*Percentage of arsenic made soluble by treatment of lead arsenate paste with soap—first series*

No.	Soap.		Lead arsenate.		As_2O_5 in 200 cc.	As^2O^5 rendered soluble.	Solution in which lead arsenate was ground.
	Quantity. (dry).	Kind.	Quantity.[1]	Brand.			
	Gm.		*Gm.*		*Gm.*	*Per cent.*	
1	0.6	Sodium stearate	2.0	"M"	0.01760	50.8	Water.
2	1.8	do	2.0	"M"	.01870	54.0	Do.
3	.6	do	2.0	"M"	.01424	41.1	Soap.
4	1.8	do	2.0	"M"	.01704	49.2	Do.
5	.6	do	2.15	"T"	.00884	32.7	Water.
6	1.8	do	2.15	"T"	.00573	19.8	Do.
7	.6	do	2.15	"T"	.00670	23.2	Soap.
8	1.8	do	2.15	"T"	.00752	26.0	Do.
9	.6	Sodium oleate	2.0	"M"	.00335	9.7	Water.
10	1.8	do	2.0	"M"	.00817	23.6	Do.
11	.6	do	2.0	"M"	.00251	7.3	Soap.
12	1.8	do	2.0	"M"	.00726	21.0	Do.
13	.6	do	2.15	"T"	.00111	3.8	Water.
14	1.8	do	2.15	"T"	.00111	3.8	Do.
15	.6	do	2.15	"T"	.00084	2.8	Soap.
16	1.8	do	2.15	"T"	.00084	2.8	Do.

[1] Pastes contained:

	"M"	"T"
As_2O_5	17.30 per cent.	13.40 per cent.
PbO	35.35 per cent.	37.45 per cent.

TABLE II.—*Percentage of arsenic made soluble by treatment of dry lead arsenate with soap—second series*

No.	Soap.		Lead arsenate.[1]		As_2O_5 in 200 cc.	As_2O_5 rendered soluble.	Solution in which lead arsenate was ground.
	Quantity (dry).	Kind.	Quantity.	Brand.			
	Gm.		*Gm.*		*Gm.*	*Per cent.*	
17	0.6	Sodium stearate	1.35	"M"	0.01280	30.1	Water.
18	1.8	do	1.35	"M"	.02814	66.2	Do.
19	.6	do	1.35	"M"	.01325	31.1	Soap.
20	1.8	do	1.35	"M"	.03097	72.9	Do.
21	.6	do	1.20	"T"	.00625	20.2	Water.
22	1.8	do	1.20	"T"	.01084	35.1	Do.
23	.6	do	1.20	"T"	.00596	19.3	Soap.
24	1.8	do	1.20	"T"	.00893	28.9	Do.
25	.6	Sodium oleate	1.35	"M"	.00144	3.4	Water.
26	1.8	do	1.35	"M"	.00345	8.1	Do.
27	.6	do	1.35	"M"	.00144	3.4	Soap.
28	1.8	do	1.35	"M"	.00308	7.2	Do.
29	.6	do	1.20	"T"	.00122	3.9	Water.
30	1.8	do	1.20	"T"	.00066	2.1	Do.
31	.6	do	1.20	"T"	.00122	3.9	Soap.
32	1.8	do	1.20	"T"	.00081	2.6	Do.

[1] Dry arsenate contained:

	"M"	"T"
Moisture	0	0.24 per cent.
As_2O_5	31.50 per cent.	25.75 per cent.
Lead (PbO)	65.40 per cent.	72.10 per cent.
As_2O_5 soluble in 4 per cent ammonia	8.85 per cent.	0.82 per cent.

The second series was in most respects a repetition of the first, except that the lead arsenates were dry and used in slightly larger quantities per sample. The sodium stearate soap was also almost dry in this case, but the quantity of soaps used (dry basis) was the same as before.

The samples, treatment, and soluble arsenic found are given in Table II.

The grinding in the water or in the soap solution was perhaps more thorough than in the first series, but the shaking was continued for only four days.

It will be noted that the pair of tests 5 and 6, in Table I, are not in agreement with other pairs of similar tests. The general tendency is, the greater the concentration of soap, the greater the quantity of soluble arsenic found, but in this one pair this tendency seems to be reversed. It is believed this indicates some mistake, possibly in the numbering of the bottles, or of the record. The corresponding pair in Table II, No. 21 and 22, is in accord with the tendency noted.

EFFECT OF THE LIQUID USED FOR GRINDING

Comparison of the quantity of arsenic made soluble in pairs of tests otherwise identical, but in one of which the grinding was in the presence of pure water and the other in the presence of soap solution, shows that the quantity of arsenic made soluble is seldom the same. It frequently varies by several per cent of the arsenic present, but does not always vary in the same direction. The cause of this lack of uniformity was not determined, but conceivably might be as follows: The shells of lead soap formed about the small solid particles of lead arsenate are somewhat plastic and the real effect of the grinding might be a "churning" one that would serve to stick some of the particles together and increase the protective action of the shells of lead soap. In other cases the grinding might remove the coating of lead soap, as was the intent when the work was done.

SOLUBLE ARSENIC DUE TO ACTION OF FREE ALKALI DEDUCTED

Since the free alkali in the sodium stearate soap would probably exert some solvent action, it is worth while to compute how much of the soluble arsenic found might be due to that cause. Starting with the reaction

$$Na_2CO_3 + PbHAsO_4 = PbCO_3 + Na_2HAsO_4,$$

the highest ratio possible between sodium carbonate and soluble arsenic pentoxid is 1 to 1.085. On this basis Table III is computed from the data already given, showing the quantity of sodium carbonate present, the equivalent quantity of As_2O_5 that might be made soluble, the quantity of soluble arsenic as actually found, and, by difference, the quantity that must be made soluble by the neutral soap, the quantity of As_2O_5 present in the lead arsenate used, and the percentage made soluble by the action of the soap aside from the free alkali.

Since the oleic soap contained no free alkali, no similar table is presented for correction of the results on oleic soap in Tables I and II.

TABLE III.—*Quantity of As_2O_3 that might be made soluble by the free (excess) sodium carbonate with stearate soap (4.72 per cent)*

No.	Brand of lead arsenate.	Weight of free Na_2CO_3	Equivalent weight of As_2O_5	As_2O_5 found in 2 liters.	Quantity due to soap (by difference).	Total As_2O_3 in system.	Arsenic made soluble by neutral soap.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent.*
1	"M"	0.0095	0.0103	0.1760	0.1657	0.3460	47.89
2	"M"	.0285	.0309	.1870	.1561	.3460	45.12
3	"M"	.0095	.0103	.1424	.1321	.3460	38.18
4	"M"	.0285	.0309	.1704	.1397	.3460	40.37
5	"T"	.0095	.0103	.0884	.0781	.2890	27.02
6	"T"	.0285	.0309	.0573	.0264	.2890	9.13
7	"T"	.0095	.0103	.0670	.0567	.2890	19.62
8	"T"	.0285	.0309	.0572	.0261	.2890	9.02
17	"M"	.0095	.9103	.1280	.1177	.4050	29.06
18	"M"	.0285	.0309	.2814	.2505	.4050	61.85
19	"M"	.0095	.0103	.1325	.1222	.4050	30.17
20	"M"	.0285	.0309	.3097	.2788	.4050	68.84
21	"T"	.0095	.0103	.0625	.0522	.3090	16.89
22	"T"	.0285	.0309	.1084	.0775	.3090	25.08
23	"T"	.0095	.0103	.0596	.0493	.3090	15.95
24	"T"	.0285	.0309	.0895	.0586	.3090	18 96

It is plain from Tables I, II, and III that "M" is very much more acted upon by both soaps than is "T" and that sodium stearate is very much more (two to seven times) effective in dissolving arsenic from lead arsenates than is sodium oleate. It follows that if it is desired to use soap with lead arsenate in spraying, the danger of injury from arsenic in solution can be diminished in some degree by securing triplumbic arsenate but could be almost entirely overcome by using only sodium oleate as the soap.

It is also clear that sodium stearate at least does not act as stated by G. Ennis Smith (5), but dissolves arsenic from the basic arsenate and dissolves much more than the 40 per cent that should be made soluble in transforming diplumbic into lead hydroxy arsenate. This behavior is exactly in accord, however, with the supposition that the reaction is of the double decomposition sort, giving insoluble lead soaps as one of the end products, instead of free fatty acids as Smith states. In order to confirm this supposition, the insoluble residues from the first series were analyzed for arsenic and for lead.

In case the reaction stops as soon as the lead hydroxy arsenate is reached, the ratio of lead oxid to arsenic pentoxid could never be greater than that in the lead hydroxy arsenate, 1 to 3.23, and would usually be lower than that, as there would be some material unacted upon. On the other hand, if the reaction is of the double decomposition sort, it might be possible to carry the reaction far beyond that ratio, as in fact occurs in six of the eight samples that were acted upon by sodium stearate. When oleate soap was used, the reaction was so incomplete that the ratio never approaches even 1 to 3. It may be that soap first transforms diplumbic arsenate into lead hydroxy arsenate and then undergoes a double decomposition with that salt, but it is hard to explain the results on the supposition that the action stops at the lead hydroxy arsenate. The results of the analysis of the dry insoluble residue and the ratio of As_2O_5 to PbO are given in Table IV.

TABLE IV.—*Analysis of 0.1 gm. dry insoluble residue from treatment of lead arsenate with soap solution*

No.	Soap.		Lead arsenate.		Weight of As_2O_3.	Weight of PbO.	Ratio of As_2O_3 to PbO.
	Quantity.	Kind.	Quantity.	Brand.			
	Gm.		*Gm.*		*Gm.*	*Gm.*	
1	0.6	Sodium stearate....	2.00	"M"	0.0101	0.0437	1 : 4.3
2	1.8	.....do.....	2.00	"M"	.0065	.0274	1 : 4.2
3	.6	.....do.....	2.00	"M"	.0122	.0460	1 : 3.8
4	1.8	.....do.....	2.00	"M"	.0065	.0283	1 : 4.3
5	.6	.....do.....	2.15	"T"	.0132	.0475	1 : 3.6
6	1.8	.....do.....	2.15	"T"	.0092	.0310	1 : 3.4
7	.6	.....do.....	2.15	"T"	.0140	.0468	1 : 3.3
8	1.8	.....do.....	2.15	"T"	.0086	.0257	1 : 3.0
9	.6	Sodium oleate.....	2.00	"M"	.0256	.0538	1 : 2.1
10	1.8	.....do.....	2.00	"M"	.0211	.0482	1 : 2.3
11	.6	.....do.....	2.00	"M"	.0322	.0552	1 : 1.7
12	1.8	.....do.....	2.00	"M"	.0204	.0470	1 : 2.3
13	.6	.....do.....	2.15	"T"	.0244	.0635	1 : 2.6
14	1.8	.....do.....	2.15	"T"	.0242	.0610	1 : 2.5
15	.6	.....do.....	2.15	"T"	.0221	.0564	1 : 2.5
16	1.8	.....do.....	2.15	"T"	.0185	.0482	1 : 2.6

Theoretical for diplumbic ortho arsenate ($PbHAsO_4$) 1 : 1.94.
Theoretical for triplumbic ortho arsenate ($Pb_3(AsO_4)_2$) 1 : 2.91.
Theoretical for lead hydroxy arsenate ($Pb_5OH.(AsO_4)_3$) 1 : 3.23.

SUMMARY

The data exhibited make the following conclusions plain:

(1) That pure soaps dissolve arsenic from both samples of lead arsenate and therefore might cause injury to foliage.

(2) That sodium stearate is much more effective in dissolving arsenic from both "M" and "T" than is sodium oleate, from two to six or even seven times as much soluble arsenic being found in the solution of the former as in the latter.

(3) That, as Tartar and Robinson point out (*4*, 7), the arsenic of diplumbic arsenate is much more acted upon than the more basic compound.

(4a) Increasing the concentration of the stearic soap solution increased the amount of arsenic made soluble.

(4b) Increasing the concentration of the oleic soap made more arsenic soluble from the acid lead arsenate "M" but did not increase the amount made soluble from the basic lead arsenate "T."

(5) The extent of the action was sometimes greater when the lead arsenate was ground in water than in the soap, sometimes less. No definite statement can be made as to which is most effective.

(6) Sodium stearate dissolves far too much arsenic from diplumbic arsenate to confirm the supposition that its action stops with the conversion of diplumbic into lead hydroxy arsenate.

(7) Both sodium stearate and sodium oleate dissolve arsenic from basic lead arsenate, the stearic soap in large quantities (as much as 25 per cent).

(8) Numbers 6 and 7 together indicate that the action of soaps upon lead arsenates is of the double decomposition sort.

(9) Danger of injury from soluble arsenic in spraying with lead arsenates and soap can be largely eliminated if the soap is entirely made from oleic acid.

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PHYSIOLOGICAL REQUIREMENTS OF ROCKY MOUNTAIN TREES[1]

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I. INTRODUCTION

The most casual observer ascending any mountain range in the western United States can hardly fail to be impressed with the fact that there is a sharp line of demarcation between the grassy plains at the base of the mountain and the wooded slopes of the mountain proper. As one ascends farther, gradual or sometimes very abrupt changes are noted in the character of the forest cover. With increase in altitude the forest generally becomes more dense, trees of greater stature are observed, and those who are able to distinguish note the occurrence of new species in each altitudinal zone. To a lesser degree the same differences in the forest cover may be noted on opposing slopes at the same elevation—that is, slopes facing the south bear forests similar to those at lower elevation, while those facing the north have the character of higher altitudes. The vegetation of ridges is always different from that of ravines at the same altitudes.

The laymen will recognize that these differences in the forest cover are the result of different "growing conditions" at different elevations, just as a person who had lived in the South would recognize, almost instinctively, that the growing conditions of the mountain valleys of Colorado could not possibly be suited to the cultivation of corn or cotton.

While the intensive study of the relations of plants to the soil and climatic conditions which comprise their environment goes under the formidable name "ecology," ecological knowledge is not confined to scientists and, in fact, has been common property for ages. The writer has had opportunity to observe the first impressions of a great many people who were visiting the western mountains for the first time, and has been impressed by the amount of logic exhibited in relating cause and effect in the matter of forest distribution. Why does this southerly exposure bear an open, scrubby forest of yellow pine, and that northerly exposure, directly opposite, bear a much more dense stand of vigorously growing firs? Often the people who know no botany and much less ecology take in the situation at a glance, at least so far as it is possible to do so from superficial evidence.

To the forester such questions are of the utmost practical importance. Not only is it the forester's business to know the trees with which he

[1] Accepted for publication July 6, 1921.

[2] This project has been under the direction of the writer since its inception in 1910, and he assumes full responsibility for the quality of the work done and for the conclusions deduced from the data. It is, however, a pleasure to acknowledge the great efforts which have been required of a number of observers in the accumulation of the records. The records furnished by the Weather Bureau are duly acknowledged, and the efforts of all those numerous and changing observers who have created these records. The records of the Wagon Wheel Gap and Fremont Experiment Stations are the result of the concerted effort of many regular observers, all of whom deserve credit. The original installation of instruments at the Wagon Wheel Gap Experiment Station was made, and the records were obtained for two years, under the direction of B. C. Kadel, of the Weather Bureau.

is daily working, their relative and absolute demands for moisture, light, heat, and soil fertility, but he must be ready to make practical use of such knowledge in formulating his plans for reforestation and in deciding upon the amount and kind of timber which may safely be cut from a given area. Consciously or unconsciously he is daily making use of whatever knowledge he may possess as to the physiological requirements of each species in his region—that is, those properties of the tree which determine that it will grow best under certain conditions of soil and atmosphere. It is of the utmost importance that this knowledge should not be superficial and that through the increase in scientific facts forestry should be removed from its empiric basis.

Unfortunately, much of our knowledge of tree physiology and tree requirements is still very vague, and it is the aim of forest ecology to increase, systematize, and analyze such knowledge. As a means of systematizing the knowledge of trees, in this report we speak of an area of forest of essentially uniform composition as a forest type; and it is assumed, since the composition of the forest is uniform over a given area, that the environmental conditions which have brought this uniform forest into existence must be just about the same over the whole area, or at least must have been the same at the time when the forest started. Usually a forest type is given the name of the tree species which predominates and gives it its essential character, even though a great many other species may occur in the same stand in lesser numbers. Thus a forest in which Douglas fir is the most prominent tree, with occasional neighbors of spruce and pine, would be spoken of as a "Douglas fir type." The word "type" is somewhat loosely used to refer to the ground occupied as well as to the forest itself.

A broader use of the word "type" is as a synonym for forest zone or altitudinal zone. It is true that the character and composition of the forest changes gradually with a change in altitude, and, for example, most of the ground between elevations of 8,000 and 10,000 feet, in a given region, might be occupied by Douglas fir stands. But, from the lower extremity, a great many strips of yellow pine forest might extend into this zone on the warmer, southerly exposed areas, and likewise from the upper edge there would extend belts of spruce. As a general term, therefore, the word "zone" is far preferable to "type," and the latter will be used in this report only for specific areas bearing forests of uniform character.

As a further distinction between forest areas, the forester has brought into use the word "site" to describe the producing qualities of the ground with respect to any particular species. Thus among many Douglas fir types, all of which were characterized by the predominance of Douglas fir over other species, it might be desirable to distinguish those of the best quality as "Douglas fir sites I" and those of very meager producing qualities as "Douglas fir sites III." Such distinctions are usually based upon the evidence of the tree growth itself—that is, either the apparent rate of growth determined, for example, by the general vigor of the stand, or the growth rate actually measured, is the best possible evidence of the quality of the ground. But there is every reason to believe that the productive capacity of the ground can be measured, sooner or later, in terms of the soil quality and the atmospheric conditions which simultaneously affect tree growth, so that any systematic effort to study forest types and to describe those qualities which distinguish them must inevitably be at the same time a study of sites.

OBJECTS OF THE PRESENT STUDY

Having now shown in a general way the nature of the problem which is constantly under consideration by students of ecology and forestry, the purpose of the study (one phase of which is covered by the present paper) may be definitely stated. As a matter of fact, it has several purposes, which are by no means distinct, but worthy of individual enumeration.

1. To compare the environments of the several species of trees with respect to each condition which may be separately measured, in order to determine what particular conditions have the most important bearing on the initiation of new stands, favoring one species rather than another.

2. After noting the differences in the conditions of the various types, which really indicate differences in the physiological functioning of the species, to determine experimentally the degree of such differences as may exist between the species, and, as a result, the degree of difference in the actual requirements of the species for optimum growing conditions. This second object can hardly be attained in the field without most extensive long-term study, and necessarily resolves itself into experiments under controlled conditions of temperature, light, moisture, etc. The present paper deals largely with the results of such experiments.

3. To describe the conditions of the various forest types of the region in such a manner as will explain most clearly the reasons for the success or failure of artificial forestation, so far as these may result from the environment, and the conditions and means necessary for successful natural regeneration after fires or cutting. Here the object is to lead away from empiric silvicultural "systems" and toward the attainment of definite environmental conditions in all silvicultural practice.

4. To convey a conception of the conditions under which the Rocky Mountain forests exist—that is, a view of the climatic and soil conditions of the forest region as a whole.

It is needless to state that not all of these objects have been attained in the present work, which should be considered as something of a pioneer effort, merely blazing the way for much greater efforts and more refined methods which are necessarily for the future to bring forth. The hope may be justified, however, that the results and conclusions of this study will add somewhat to the information on the general subject and encourage the doing of more intensive work.

SCOPE OF WORK

The entire work, of which this paper represents only a part, is mainly a study of the physical environments of a number of different forest types in Colorado and Wyoming. The detailed study of the composition of these several types has not been given a great deal of attention. Since the forest conditions are common ones and are frequently encountered in the region, it is believed that the indications of prevailing composition and of changes in composition, as they may be expressed in general terms, will be as valuable for present purposes as tabulated statements of the number, size, and kind of trees found at the several stations. The observations of meteorological conditions and of certain soil conditions thought to be fully as important, have, therefore, comprised the major part of the field work.

However, the early efforts (*1*) [3] to summarize and compare these site conditions, as given in meteorological and soil records, and to deduce

[3] Reference is made by number (italic) to "Literature cited," p. 163–164.

therefrom the full reasons for the presence or absence of a given species on the various sites were so far from satisfactory that the need for controlled experiments to indicate the comparative reactions of the several species has long been apparent. The amount accomplished in this line is not insignificant, and, since the results aid very materially in interpreting the field conditions, we have chosen to present, first, all of the physiological data that are available. In other words, we shall first consider the manner in which the several species are affected by more or less controlled conditions in the laboratory or greenhouse; then, perhaps, we may weigh more precisely the importance of each condition encountered in the field, and especially avoid the almost universal and inevitable error of placing reliance upon the measurement of a condition which has not really been measured or expressed in the proper terms.

Of the meteorological records which are used in this report the larger share have been obtained by the Forest Service at the Fremont Experiment Station, near Manitou, Colo., and by the Weather Bureau and Forest Service, cooperating in an intensive project at the Wagon Wheel Gap Experiment Station, near Wagon Wheel Gap, Colo. It has been the aim to conduct the meteorological work at both these stations on a high plane of accuracy, with equipment as complete as was thought necessary. At both places the observations have covered elevations from the station headquarters at about 9,000 feet to timber line at 11,000 or 12,000 feet. In both cases, too, soil moisture and soil temperature data have been secured during most of each period of atmospheric observations.

In addition, the Forest Service has equipped and operated from the Fremont Station three subsidiary stations at which the Weather Bureau had previously started the usual observations of precipitation and air temperature. These stations were manned by forest officers, and a very diligent effort has been made to obtain continuous records for them. The stations in question are at the Monument Nursery, in the yellow pine type near Monument, Colo., at the Foxpark Ranger Station, lodgepole pine type, at Foxpark, Wyo., and in the Nebraska sand hills, where the planting of yellow pine and jack pine has reached such large and successful proportions. The first station is only about 20 miles north of the Fremont Station and for all practical purposes may be considered as belonging in the Pikes Peak altitudinal series.

Although the Weather Bureau records of precipitation and air temperature, secured through cooperative observers, were available for a large number of stations in the region covered by this report, and many such stations are within the mountain forest zone, only limited use has been made of such records, and for the following reasons:

(*a*) Many such observation points are situated, with the small towns, in deep valleys where the conditions met with, especially those of air drainage and soil composition, are not at all the conditions of the adjacent forested slopes. In fact, nearly all such valleys, especially if they possess a distinct flood plain, are devoid of forest cover, and to use their weather records in this study might lead to very erroneous conceptions though the temperature conditions alone are probably not responsible for the absence of forests.

(*b*) The records obtained at all such mountain stations are only those of precipitation and air temperature. No soil data whatever are secured.

The special work in this project dates from January 1, 1910, when the control station at Fremont was equipped. Records obtained up to 1918

have, in general, been employed, the summarizing of data having been started at that time. However, some records particularly needed have been added since 1918. The period of operation, as well as the equipment of the several other stations, is given under the respective descriptions.

METHOD OF STUDY

As has already been indicated, the primary data to be presented in this study are records of climatic and soil conditions in different forest types, and the main object of such presentations must be to show that differences in climatic and soil conditions, between the forest types, either do or do not exist in sufficient degree to account for the varying phenomena of occurrence and growth.

Since the special data collected by the Forest Service in no case cover periods long enough to establish the "normal" conditions of any of the forest types (and by this we mean the average conditions for a period of at least 10 years), and since even "normal" conditions as established by 10 years of observation are certain always to be changed by the addition of further records, it is necessary that we adopt short-period records as a basis for comparison. Such adoption can not be fraught with any serious dangers when the forest types to be compared are in the same general locality, that is, in the path of the same air currents, storm centers, etc. Any considerable separation of the stations, however, especially in a rugged mountainous region, is likely to introduce temporary variations in certain conditions which are not "normal," and particularly in those factors which are most directly influenced by the paths of storm centers. Thus, at the moment of writing this statement, it appears that the storm centers have for some time been passing considerably to the north of the Pikes Peak region, giving that locality rather unusual westerly winds and leaving it with a dearth of moisture, so that at the end of May an unprecedented shortage of water exists. Scarcely 100 miles to the north, unusually large accumulations of snow are reported at the same moment. Again, the moisture factor is most variably influenced by the restricted character of many of the summer showers; especially are the heaviest downpours in a given locality likely to affect only a very small area.

Temperatures are generally not so directly affected by local conditions. Thus the month of December, 1917, was not only an unusually warm month at the Fremont Station but showed the same character over a large part of the western United States, and January, 1918, was, likewise, generally cold to an unusual degree.

We may, therefore, feel safe in comparing the records of any two near-by stations for short periods, whatever the factor under consideration, and we shall demand an increasing period as the distance between stations increases.

Fortunately, the dozen or more stations located in the vicinity of Fremont all come under the same general influences. This is true of the entire area from the plains to the summit of Pikes Peak, with the exception that summer rains frequently fall in one part of the area without wetting other parts. Winter snows may also be so localized, but usually in conformity with altitudinal zones. It is true, two stations not 100 yards apart may on a given day have temperatures varying by a couple of degrees in one direction and on the next day varying in the

opposite direction. Such variations from a consistent relation are, however, always small, and there is every reason to believe that the means of a single month usually express essentially the normal temperature relation between two stations for that month of any year.

Therefore the method of study and the method of presenting results is that of comparing each factor for any station with the corresponding factor at the control station for whatever period observations may have been taken at the outlying station. As a basis for this comparison we have the record of each factor at the control station, measured with practically no variation in method from January, 1910, to date. Exception should be made here to the measurement of evaporation, in which a satisfactory method was not attained until 1917.

The detailed methods of taking meteorological observations, so far as they vary from the standard methods of climatologists the world over, will be described in connection with each condition measured.

REVIEW OF OTHER WORK ALONG SIMILAR LINES

Although much systematic ecological study has been attempted in the United States and other countries, and the western portion of our own country has offered an especially attractive field for ecologists because of the sharp contrasts in vegetation and causative factors which are found in relatively small areas, still the main field with which foresters are concerned has barely been scratched. Several studies, which might have been very productive, scarcely satisfy the forester's requirements because of the lack of long-term records.

Be that as it may, a number of authors have obtained facts and deduced conclusions as to the distribution of our Central Rocky Mountain forest trees which we can not afford to overlook. No attempt will be made at this stage to introduce these facts, which may better be mentioned in connection with my own discussions and conclusions. I shall merely list here the works which have a direct bearing on the major problems, and with no attempt to cover the general or specific physiological studies.

Clements (*7*, *8*, *10*) in three of his works presents many valuable ideas regarding the relation of Rocky Mountain vegetation to environmental conditions, and with particular reference to the Pikes Peak region, in which much of his investigation has been conducted. The latest of Clements's books, "Plant Succession" (*10*), published in 1916, may be said to cover the entire ground of the earlier works, bringing all of his observations under one comprehensive theme, namely, the changes which occur in the character of the vegetation of a given area as the result of reactions of the plant forms upon the environment and of gradual changes in the climatic and edaphic (soil) conditions.

In a more specific work Clements (*9*) gives to foresters a much more concrete idea of the requirements of an important tree species, lodgepole pine, with lesser data on its common associates.

Ramaley (*16*, *17*), working near Boulder, Colo., and in the deeper mountains at Boulder Park, has likewise made numerous observations on the forest types and zones of Colorado. His papers, however, make no claim of extensive systematic measurement of physical factors and hence can be considered as having only suggestive value in connection with the present work. In both of the papers cited the theme is a classi-

fication of Colorado forests according to moisture conditions and composition.

Shreve (*24*, *25*), working at the Desert Laboratory at Tucson, Ariz., and in the Santa Catalina Mountains adjacent thereto, has in a number of papers published results directly bearing on the subject in hand. "The Vegetation of a Desert Mountain Range" is the most comprehensive of these papers and covers most fully the ultimate problems, beyond pure climatology, with which the forester is concerned. Although the Santa Catalinas are somewhat different from the Central Rockies in being surrounded by desert on nearly all sides and in having a different seasonal distribution of rainfall, yet it is apparent that the limiting factors for the occurrence of a given species must be essentially the same in the two regions; else all attempts to formulate a systematic ecology would be vain. These factors may not, it is true, appear quantatively alike under present methods of measurement, but, if so, we should seriously question the method of measurement.

It will be noted that in the papers referred to Shreve ascribes the main control of the upward extension of desert plants to temperature, and in another paper (*23*) he has quite convincingly shown that the duration of freezing temperatures is all-important with plants accustomed to the ordinarily warm winter air of the desert. With this view we shall have no reason to take issue. The other main conclusion, that lack of moisture limits the downward extension of the forest species, individually and collectively, will, it is believed, be found subject to question or at least modification.

Robbins (*18*) has prepared the most recent and complete summary of Colorado's climatic conditions in relation to native vegetation and agriculture. While, as stated by the writer, this work attempts to show only a qualitative relation between climate and plants, it is, nevertheless, excellent both in the data systematically presented and in the relations described. For the most part these relations are too broadly stated to be of direct assistance in the present study. A quotation from the discussion of the freezing of plants is of considerable technical interest:

> It is a familiar observation that some of the more tender plants are injured by temperatures above the freezing point; and that, on the other hand, there are many plants that may withstand temperatures considerably below the freezing point. This statement may apply not only to dormant plant parts, but to swelling buds, open flowers, and forming fruit as well. The plants at timber line and above are subject to freezing temperatures almost every night in the year. The exact nature of this immunity to low temperatures is not known.

Weaver (*27*) in 1917 studied the desert-to-mountain formations of Washington in a manner not unlike Shreve's, and ascribes the changes in vegetation mainly to increasing soil moisture and decreasing evaporation with a rise in elevation.

Shantz (*20*, *21*) has dealt with problems intimately connected with the factors limiting the downward extension of the Rocky Mountain forests. Particularly is Shantz's work enlightening to foresters in the thorough treatment of the soils problem. He has made it plain that the lighter soils of the plains, characterized by bunch grass, show much less variation in productivity from year to year than the heavier, loamy soils which develop the grama-buffalo-grass association. This difference is due to greater penetration of both moisture and roots in the lighter soil as well as to the greater availability of the moisture when the content becomes low, tending to encourage slow growth, and the longer lived bunch grass.

By analogy we may say that the same relation exists between grassland or sage brush and the lowest type of forest, commonly called "woodland" by foresters, since it is obvious that there can be no important change in climatic conditions in the small space between the centers of development, and that the development of the forest is made possible by the slight soil changes resulting from elevation, surface erosion, and leaching, all of which maintain a younger soil.

The establishment of the forest experiment stations in the western United States, beginning with that at Flagstaff, Ariz., in 1908, gave unparalled opportunity for the collection of forest and climatological data over a period of years. As a result, studies similar to the present one have been initiated in Arizona, California, and Idaho, and the study of forest and herbaceous vegetation has been carried on in connection with a number of experiments at the Utah Station, where grazing problems occupy the attention first. The strictly forest studies, however, are for the most part not yet ready for publication.

Pearson (*14*) at the Flagstaff Station early investigated the effect of yellow pine forests upon local climatic conditions, by securing data in the forest, the edge of the forest, and "parks" (grassy, meadowlike openings) of considerable extent. While the climatic conditions recorded by Pearson are interestingly compared with our own, this study can not be said to throw very much light on the conditions governing different forest types.

However, Pearson (*15*) has recently made available the results of observations at a series of stations in the San Francisco Mountains, in a very comprehensive way, and we shall have reason frequently to compare his conditions with our own.

We have, similarly, had access to an unpublished report by Larsen [4] on the conditions of Montana and Idaho, which has been extremely helpful in giving comparable data.

The problem of the prairies in the Middle West, and their physical relation to the occasional forested areas, has received considerable attention, and this problem is not too remote from our own entirely to lack interest. On this subject may be considered the work of Shimek (*22*), who concludes, regarding Iowa conditions:

> 1. Exposure to evaporation as determined by temperature, wind, and topography is the primary cause of the treelessness of the prairies.

and

> 3. Rainfall and drainage, while of importance because determining the available supply of water in both soil and air, are not a general, determining cause, both frequently being equal on contiguous forested and prairie areas.

Shimek also dismisses fires as a cause of the absence of forests. It is believed that the later conclusions of Weaver and Thiel (*26*, *28*), with reference to Minnesota, are essentially in agreement with this. The point which seems to have been overlooked here, and in all similar discussions, is that forests occur usually on the slopes of ravines or on hillsides, where the old soil is being rejuvenated by a secondary erosion and where, even with less moisture than in the heaviest soils, the availability may be greater. Considered from this angle, the occurrence of forests in the prairie region is exactly parallel to their occurrence on the first mountain elevations at the edge of the plains.

[4] LARSEN, J. A. CLIMATIC STUDY OF FOREST TYPES, DISTRICT 1. U. S. Dept. Agr. Forest Serv., unpublished report, 1918.

It will be fairly evident from the reading of the few treatises which have been mentioned that the region under discussion has not been neglected by ecologists. It will be equally evident that there is still room for much systematic effort in the study of the environmental factors in order that the theories advanced regarding the distribution of mountain forests may be more thoroughly tested by well-established facts. The most apparent fact, after considering all of these regional studies, is that so far ecology has given the physics of the soil-moisture problem entirely inadequate attention.

II. PHYSIOLOGICAL STUDIES LEADING TO AN INTERPRETATION OF THE ENVIRONMENTAL DATA

It is quite generally recognized that the result of studying any condition in nature, even when the method of study is strictly quantitative, should not consist wholly in presenting the accumulated facts but quite as much in placing a logical interpetation on those facts. In the present study we are dealing not only with a great variety of natural conditions which require quantitative expression but with a variety of growing entities whose behavior and reaction to known conditions can not be determined by casual observation. For example, the mere fact of finding a spruce tree growing at the water's edge does not prove that the tree uses or requires an unusual amount of water, much less that it is growing in that particular spot primarily because of the moisture, or even indirectly because of the moisture. It would be as logical to say that because the aligator spends a good deal of his time in the water, he must drink and must require for physiological processes an extraordinary amount of water. This may not be true at all; he may be a most abstemious animal.

The point is that in ecology we dare not take the conditions of growth as prima facie evidence of the requirements of growth, even though it be true that none of the conditions can be altered without affecting the character of the growth. This is especially true when we are compelled, as in the present instance and in most ecological studies so far made, to speak of requirements in a relative rather than an absolute sense; that is, when we are simply trying to compare the requirements of several species rather than determine them absolutely for any species. This may be illustrated by a point which has appeared very forcefully in the present study. Taking the superficial appearance of soil conditions as a measure of relative requirements, foresters have repeatedly stated that the moisture requirements of spruce were greater than those of yellow pine. Now, there could be no objection to saying, and probably no error in saying, that spruce requires or at least develops best in a fresh, moist soil of high water-holding capacity. This would be an absolute expression which would simply gain in accuracy as the soil conditions were further analyzed. We might infer, and would be likely to do so, because of the character of the soil occupied, that spruce must use a great deal of water in its development. Such an inference would be unwarranted, but would be especially dangerous if we should say, comparatively, that spruce uses more water than pine. Here we are treading on absolutely unsafe ground. On the face of it there is no scientific basis for such a statement, if we use simply the evidence of the field conditions observed. And even if this were true of the spruce forest in the aggregate, that bespeaks nothing as to the individual.

It has been, therefore, in the hope of partially overcoming the inherent weakness of a comparative field study that certain observations have been made under laboratory conditions, permitting a better knowledge of the trees themselves, hence a safer interpretation of the environmental conditions which surround them in the field, and, perhaps, a clearer conception of how those conditions should be measured in the future to express a logical relation between the environment and growth.

For the most part these special observations have been made upon the four important species which are involved in the field study, namely, western yellow pine, Douglas fir, lodgepole pine, and Engelmann spruce. Two other species, forest "weeds," have been studied to some extent, namely, limber and bristlecone pines (*Pinus flexilix* and *P. aristata*). A few observations have also been made on the Lake States pines and other conifers not indigenous to the Rocky Mountains.

In the interest of brevity, some details of the conditions of these experiments may have been omitted which might be considered as having important bearings. Anyone wishing to investigate these details will be given all possible assistance.

TRANSPIRATION TESTS IN 1917

To establish the water requirements of some of the Rocky Mountain trees in the same terms as used by Briggs and Shantz (*6*) for agricultural crops, and to determine the relative transpiration rates of the species as a basis for gauging their moisture requirements in the field, transpiration tests were conducted in the greenhouse of the Fremont Experiment Station for a period of about six months in 1917. The experiment was repeated in 1920.

It should be recognized at the outset that the greenhouse did not present natural conditions for the growth of any of the species, the air temperatures being higher than commonly occur except possibly in the lowest zone of the region, and the air movement considerably less than the wind which would occur in any situation out of doors. For these reasons, though we may speak of the "absolute water requirements" of the trees in this particular test, these requirements are not an indication of what the water use might be under any other conditions; and it would be best, as Briggs and Shantz have done, to assume only that we have established relative requirements of the several species for one set of conditions. These relations may or may not hold good under other conditions. Briggs and Shantz found that relative water requirements of different species did not vary much under different conditions, though the absolute requirements of all might be twice as great during a dry season as during a moister one. Thom and Holtz (*26*) found that the physical conditions might vary sufficiently to change even the relative requirements of different species, but their more important result was to show that the absolute water requirement increases with the availability of moisture.

It would appear that the high temperatures and low wind velocities occurring during these tests should tend to stimulate assimilation rather more than transpiration, so that the absolute water requirements here would be less than under normal field conditions for any one of the species. This, however, may not be true. For this and other reasons appearing later it is difficult to compare the absolute requirements with those of agricultural crops.

MATERIAL STUDIED

As the means were not at hand for treating large trees in the intensive manner required in such a study, efforts were confined to nursery specimens, 5 and 6 years old at the outset. These had all been developed in the nursery of the Fremont Station, with practically uniform soil conditions and with no artificial watering except as small seedlings, so that all should have been in much the same condition at the outset.

Two specimens each of yellow pine, Douglas fir, lodgepole pine, Engelmann spruce, limber pine, and bristlecone pine were taken for potting, while a third specimen of each was taken at the same time for drying, in order that the initial dry weight of the specimens to be grown might be computed. This drying and all other dryings required in these studies were done in hot water bath or controlled hot air oven at a temperature of about 92° C. and without vacuo.

To determine the initial green weights, and also the green weights at the close of the test period, each tree was washed to remove adhering soil particles, whipped vigorously through the air to remove free water, and placed immediately on the scales. After this the potting was accomplished as soon as possible.

The initial weights varied from 7 to 14 gm. and heights from 3 to 6 inches, the spruce being, on the whole, best developed for its age. No measurements other than weights were taken at the outset. At the end of the test period the green weights were taken; each tree was photographed to scale, as shown in Plates 1 to 3; measurements were made to determine the mean needle dimensions of each tree and the ratio of surface to volume (the whole volume having been determined by immersing the top in water to the root collar); finally the remains were oven dried, and later the dry material was reduced to ash in a porcelain dish over a Bunsen flame.

From the volume displacement and needle dimensions we are enabled to compute the area of leaf surface in each case, with a very considerable but general error on account of the stem volume included. This will, at least, give some basis for comparison with other experiments in which the leaf surface is the basis for calculations of water loss. Because of the great inaccuracies involved in the method and the practical impossibility of applying it to a large tree, and also because it is believed that transpiration is so largely controlled by the area exposed to insolation and the consequent total absorption of radiant energy, we have also used another basis for expressing leaf area, which we shall call "leaf exposure." This is obtained from the tree photographs, which are against a background of cross-section paper, by estimating the proportion of each square inch which is obscured by the foliage. This method, if carefully followed, gives reasonably consistent results, except in cases like tree No. 8, in which the focus is bad.

It is seen that the "leaf exposure" could not be more than one-third of the whole leaf surface, and owing to a great deal of overlapping of needles, as well as elimination of stem, the data in this case compare generally on a basis of about 1 to 6. But with the limber and bristlecone pines, whose foliage is very compact, the ratio is more nearly 1 to 10.

SOIL

For potting, open-topped galvanized cans were used, 4 inches in diameter and 10 inches deep. No drainage openings were made in the

cans; but, to encourage aeration of the soil, a 2-inch florist's pot was inverted in the bottom of each can, and a glass tube one-eighth inch in diameter was so bent and placed that its lower end opened into the pot and its upper end just above the rim of the can. This tube served for supplying the necessary water and was at all times left open for aeration of the soil. It is believed that the amount of vaporization through the tubes was insignificant, though no control tests were made at the time. We are enabled to approximate the loss from this source by the observations in 1920. However, in 1917, the soils were never allowed to become greatly heated, the potting cans being placed in similar cans having diameters of 6 inches, so that the sun never shone on the lower portions of the pots.

Before weighing and potting, each tree was trimmed so that the longest roots would not be cramped in the can. The longer roots were spread around the porous pot in the bottom of the can, and the others were placed as the pots were filled, so as to be evenly distributed throughout the soil. When the cans were all filled to within a half inch of the tops, they were sealed with a 2 to 1 mixture of paraffin and vaseline, which held very well throughout the season in spite of occasional melting.

The soil used was a specially prepared loamy sand of granitic origin, containing considerable humus derived mostly from leaves of limber pine and *Arctostaphylos* sp. Both sand and humus were sifted through one-eighth-inch screen. The resultant mixture was what would ordinarily be considered a good potting soil. It was thought to be desirable to insure an abundance of nutrient material, and there is no reason for supposing that this was overdone.

None of the soil placed in the pots was oven-dried, but a weighed amount of air-dried soil was used in each, and during the process of potting several samples were taken for the purpose of determining the moisture content. The net oven-dried weight for each pot was then computed.

The saturation capacity of the soil used was originally determined to be about 40 per cent, and, in accordance with Kiesselbach's (*12*) finding that transpiration occurs most freely when the soil is about half saturated and the theory of Hilgard (*11*) that half saturation permits the desired aeration, 20 per cent moisture was adopted as the standard at which the soil would be kept. Later it was found that with greater compactness this saturation might be much less, and after centrifuging, as low as 25.8 per cent. However, the figure 31.9 probably applies most nearly to the condition of the soil in the pots. The corresponding capillarity was 28.2 per cent, and the moisture equivalent at 100 gravity was 11.05 per cent, using the term in the same sense as it is used by Briggs and Shantz (*5*) for the water-holding capacity under a force of 1000 gravity. The mean wilting coefficient was determined in 1920 to be 3.47 per cent for Douglas fir and 3.91 per cent for spruce, or an average value of 3.69 per cent. On this basis, and assuming that the 20 per cent maximum moisture was evenly distributed, we should have as the availability $\frac{20.-3.69}{20}=0.816$. It is more probable that the moisture within reach of the roots, at the bottom of each pot, was 25 per cent or more, making the availability at least 0.850.

Table I shows all of the fundamental data regarding the trees, the amounts of soil used, and the gross weight of the pots as they were maintained throughout the season.

TABLE I.—*Description and condition of trees used in transpiration measurements*

Species	Yellow pine.		Douglas fir.		Lodgepole pine.		Engelmann spruce.		Limber pine.		Bristlecone pine.	
Can No.	1	2	3	4	5	6	7	8	9	10	11	12
Age of trees (years)	5	5	5	5	5	5	5	5	6	6	6	6
Initial green weight (gm.)	10.55	13.48	6.77	8.90	7.52	8.80	14.38	13.46	8.10	14.50	10.85	10.15
Green-dry factor of sample tree	2.45	2.45	2.56	2.56	2.51	2.51	2.32	2.32	2.37	2.37	2.41	2.41
Initial dry weight (computed)	4.31	5.50	2.65	3.48	3.00	3.51	6.20	5.80	3.42	6.12	4.50	4.21
Final weight:												
Green	16.97	20.56	10.05	16.50	13.28	15.20	26.06	28.50	12.15	16.82	15.39	14.26
Dry	5.56	7.46	4.28	5.85	4.32	5.24	10.41	10.58	4.48	6.47	5.95	5.49
Weight gain for season:												
Green	6.42	7.08	3.28	7.60	5.76	6.40	11.68	15.04	4.05	2.32	4.54	4.11
Dry	1.25	1.96	[a] 1.63	2.37	1.32	1.73	4.21	4.78	1.06	[b] .35	1.45	1.28
Ash:												
Weight (gm.)	.31	.30	.20	.49	.25	.34	.50	.64	.29	.33	.39	.35
Percentage of final green weight	1.83	1.46	1.99	2.97	1.88	2.24	1.92	2.25	2.39	1.96	2.53	2.45
Computed leaf surface:												
Volume of top by displacement (cc.)	8.8	11.9	7.4	10.3	7.0	8.7	21.1	19.1	8.5	9.3	9.7	8.8
Length of needles (cm.)	7.23	9.87	2.20	1.90	4.90	5.58	1.83	1.53	3.00	3.37	3.07	3.02
Cross section (mm.)[c]	1.13	1.30	1.23	1.20	1.12	.93	.90	.77	.92	.90	.80	.73
	×	×	×	×	×	×	×	×	×	×	×	×
	.65	.78	.42	.38	.53	.45	.70	.48	.78	.75	.75	.53
Ratio of area (in sq. mm.) to volume (in cu. mm.)	5.22	4.46	6.72	6.92	6.08	7.20	5.08	6.77	6.61	6.91	7.36	8.75
Computed area (sq. cm.)	459	531	497	713	426	626	1,072	1,293	562	643	714	770
Leaf exposures (sq. cm.), from photographs	64	79	64	100	56	66	153	149	57	66	71	71
Net dry-soil weight (gm.)	2,580	2,550	2,540	2,516	2,479	2,562	2,380	2,398	2,566	2,502	2,476	2,405
20 per cent water (gm.)	516	510	508	503	495	513	476	479	513	501	496	481
Tare (gm.)	1,022	980	996	1,001	973	988	1,037	1,012	1,015	977	1,017	1,009
Gross, with tree as above	4,128	4,054	4,051	4,029	3,955	4,072	3,907	3,902	4,102	3,994	4,000	3,905

[a] At end of tests, all root tips are old and lignified.
[b] Root tips very short, showing slow or late development.
[c] Cross sections of yellow pine and lodgepole pine computed as semicircles; limber pine and bristlecone pine as fifth circles, more or less; Douglas fir as elipses; Engelmann spruce as rectangles.

PROCEDURE

The trees were taken from the nursery and potted about April 15, 1917. From that time until April 26 they were kept in a warm room, without sunlight, to encourage root growth and establishment in the soil. On April 26 the cans were first brought to standard moisture content, and measurements of transpiration losses were begun. From that date to June 3 they were kept in the window of a warm room, where they received light for only a few hours each day. The pots were frequently but not regularly shifted in position.

On June 3 they were placed on a revolving table in the greenhouse, where they remained until the close of the test on November 14, with the exception of one day out of doors. This table was handled in several ways, the power available for rotating it at the outset being inadequate. At first a small motor was used, the motor being cut in each minute for a period of a second or more, so as to give the table a fraction of a revolution. For several short periods the table was turned by hand, a quarter revolution about once each hour. A water motor was finally used, which for a time kept the table revolving continuously. This, however, seemed to have a theoretically objectionable feature in that the trees were constantly passing from light to shadow, in a very unnatural manner. The driving belt was therefore arranged so as to move the table a perimetral distance of about 6 inches each minute, or, say, a complete revolution in about 25 minutes.

The pots occupied the periphery of the 4-foot table, various types of evaporimeters being placed between them. Within this circle was placed an air-and-soil thermograph, the arm of the air register being shaded, while the soil bulb was blackened and so placed, with its long axis horizontal, as to receive as much sunlight as the trees. With the assistance of maximum and minimum registering air thermometers and a thermometer attached to the blackened bulb of the soil thermograph, there were thus recorded both air temperatures and "sun" temperatures. In addition, a psychrometer was used during the morning observation each day, giving a rough indication of prevailing vapor pressures.

The most important question of procedure, of course, concerns the method of determining water losses. As shown by Table I, each pot had, at the outset, a known gross weight when its soil contained 20 per cent of moisture. The aim was to keep the moisture to this standard by replacing losses each day. It was only necessary to determine the amount of water required to bring the pot up to standard weight in order to record the loss for the preceding period. This was accomplished by placing the pot on one pan of the scale, the standard weights on the other pan, and filling from a titrating burette until a balance was reached. The amount drawn from the burette was, therefore, the measure of the loss. The measurements of transpiration, it is thus seen, were actually volumetric, even though scales were used. This introduced no error worth considering, as the temperatures at the observation hours varied scarcely at all from about 50° F.

The burette was graduated to 0.1 cc. The scales were barely sensitive to 0.1 gm. under the usual load of 4,000 gm. However, errors from this source should be compensating. Whenever the filling was carried too far, as not infrequently happened, the overload was determined and allowed for, and also carried to the record for the next period.

All observations were made in the early morning and before sunlight had reached the trees, and when, therefore, the transpiration rate would be almost at its minimum. The order of measurements was invariable, and the time rarely varied more than 15 minutes from the standard.

Now, in fact, though it has been stated that the plan was to maintain standard moisture in each can, it is readily seen that the moisture was most of the time below standard. The extent of the ordinary depressions was very small. The largest single loss between fillings, 157.1 gm., would mean a moisture content at the end of the period of 13.9 per cent, or a depression of 6.1 per cent. The average periodic loss of the heaviest water-user was 23.76 gm., and the average depression below standard moisture, therefore, only 0.93 per cent. This average depression would reduce the availibility of the moisture only from the approximated value 0.852 to 0.846.

On the other hand, the distribution of the moisture from top to bottom of each pot, as shown by examination at the end of the tests, was not all that might be desired. The lowest inch of soil was practically saturated, and above this the moisture decreased so that just below the paraffin the soil was only freshly moist. In spite of this, rootlets had penetrated to all sections of the soil. It seems evident that, except with the most extreme depletion noted, there was probably within reach of the longest roots at all times practically saturated soil.

The atmospheric conditions of the greenhouse, as has been stated, were not such as would occur naturally in any of the sites where these species grow. At times the ventilators were kept closed and the air temperatures were allowed to go as high as they would with full sunlight. At other times the ventilators were opened and all possible draft was developed; and, of course, under these conditions the air of the greenhouse did not become so warm. Again, sunlight was excluded on certain days to see what effect this would have on transpiration rates. On two or three occasions when cloudy weather prevailed, an interval of 2 or 3 days was allowed to elapse between measurements, since the losses were very small. On two occasions when the writer was not there to make the measurements, the intervals were considerably longer, the trees being shaded by canvas for the entire period.

No apparent injury resulted from the high temperatures in the greenhouse, except to tree No. 4, Douglas fir. On June 24, which was a clear day of exceptionally low humidity and high evaporation rate, two of the newly formed shoots on this tree wilted and did not recover. Nevertheless the tree continued to function properly. The other Douglas fir (No. 3) followed No. 4 very closely, but after the first of September showed a gradual descrease in its response to transpiration stimuli and when unpotted was found to be deficient in new root growth. In the normal trees it appeared that many of the root tips had continued growth to the end of the season, while in this one growth had evidently ceased much earlier.

Tree No. 10, limber pine, at the end of the season showed very short growing tips on the roots, indicating that root development had been very sluggish or had started very late. This sluggishness is doubtless related to the small weight accretion.

RESULTS

The amount of transpiration for each tree during each period of the study from April 26 to November 14 has been tabulated, and it is found that the tree-to-tree relations hold very closely, from day to day, in spite of great variations in the environmental conditions. It is not relevant to present purposes to present the detailed data. In Table II the transpiration by months is given and the tree performances are summarized. In correcting for the loss of water directly from the soil, the amount of 107 gm. for the season has been arrived at by considering the daily loss the same as in 1920. While in 1917 the pots were more fully protected from the sun, yet considerably higher air temperatures were attained, so that this allowance seems justified and can hardly be enough in error appreciably to affect the results.

TABLE II.—*Summary of transpiration in the test of 1917*

Species	Yellow pine.		Douglas fir.		Lodgepole pine.		Engelmann spruce.		Limber pine.		Bristlecone pine.		All.
Tree No.	1	2	3	4	5	6	7	8	9	10	11	12	
Transpiration by periods:													
April and May—													
Grams	42.1	187.5	143.3	157.1	189.9	221.3	223.6	223.	86.3	86.7	137.5	136.6	1,835.2
Per cent[a]	2.3	10.2	7.8	8.6	10.4	12.0	12.2	12.2	4.7	4.7	7.5	7.4	.....
June—													
Grams	360.8	704.2	337.2	348.8	329.7	411.0	433.5	535.1	265.7	215.2	322.4	388.5	4,652.1
Per cent	7.8	15.2	7.2	7.5	7.1	8.8	9.3	11.5	5.7	4.6	6.9	8.4	.....
July—													
Grams	285.1	427.9	221.5	235.3	182.9	208.9	232.5	354.0	173.5	132.5	175.8	213.6	2,843.5
Per cent	10.0	15.0	7.8	8.3	6.4	7.3	8.2	12.5	6.1	4.7	6.2	7.5	.....
August:—													
Grams	388.4	571.6	293.0	323.8	256.7	296.3	302.6	581.0	244.6	203.1	266.9	297.7	4,025.7
Per cent	9.7	14.2	7.3	8.0	6.4	7.4	7.5	14.4	6.1	5.0	6.6	7.4	.....
September—													
Grams	402.1	515.4	155.8	334.1	208.9	238.9	342.9	578.1	234.0	189.1	260.6	268.7	3,728.6
Per cent	10.7	13.8	4.2	9.0	5.6	6.4	9.2	15.5	6.3	5.1	7.0	7.2	.....
October—													
Grams	382.6	479.5	44.9	260.2	176.1	202.4	303.3	506.4	215.5	171.7	266.8	249.9	3,259.3
Per cent	11.8	14.7	1.4	8.0	5.4	6.2	9.3	15.5	6.6	5.3	8.2	7.6	.....
November—													
Grams	201.4	250.3	10.2	89.2	85.8	92.7	134.2	221.1	100.3	72.5	126.3	115.9	1,499.9
Per cent	13.4	16.7	.7	6.0	5.7	6.2	8.9	14.8	6.7	4.8	8.4	7.7	.....
Total, April to November (grams)	2,062.5	3,136.4	1,205.9	1,748.5	1,430.0	1,671.5	1,972.6	2,999.0	1,319.9	1,070.8	1,556.3	1,670.9	21,844.3
Correction for direct evaporation	107	107	107	107	107	107	107	107	107	107	107	107	1,284
Net loss through leaves	1,955.5	3,029.4	1,098.9	1,641.5	1,323.0	1,564.5	1,865.6	2,892.0	1,212.9	963.9	1,449.3	1,563.9	20,560.3
Transpiration:													
Grams per gram weight accretion—													
Green	305	428	335	216	230	244	160	192	300	415	319	381	263
Dry	1,565	1,545	675	693	1,002	905	444	606	1,144	2,750	999	1,221	880
Grams per gram mean weight	142	178	131	129	127	130	92	138	100	62	110	128	123
Grams per square centimeter leaf surface	4.26	5.7	2.21	2.3	3.11	2.5	1.74	2.24	2.16	1.5	2.03	2.03	2.48
Grams per square centimeter leaf exposure	30.6	38.4	17.2	16.4	23.6	23.7	12.2	19.4	21.3	14.6	20.4	22.0	20.6

[a] Percentage of the transpiration of all the trees.

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Water Requirements

This term, as used by Briggs and Shantz (*6*), denotes the units of water used by a plant for the production of a unit of dry plant material.

The first computation in the lower section of Table II gives corresponding data for the small trees whose transpiration has been measured. Averaging the results for each pair of specimens, we find the species arranging themselves according to Table III. In this table the use per unit of green weight is also given, since the green-weight accretions were directly measured, whereas the original dry weights were obtained indirectly, as explained.

TABLE III.—*Water requirements*[a]

Species.	Water used per unit of—			
	Dry-weight accretion.		Green-weight accretion.	
	Gm.		*Gm.*	
Limber pine	1,947	(678)	358	(49)
Yellow pine	1,555	(8)	366	(52)
Bristlecone pine	1,110	(94)	350	(26)
Lodgepole	954	(41)	237	(6)
Douglas fir	684	(8)	276	(50)
Engelmann spruce	525	(68)	176	(14)

[a] The probable error in the average of two figures is indicated by the quantity in parentheses.

Considering only the first column of figures, it is seen that the probable error in the averages is large in two or three cases, and especially so with limber pine, so that this species might possibly belong after yellow pine in the list. In fact, considering that it is the specimen of high water requirement (Tree 10, 2,750) which showed at the end of the season evidence of lack of vigor, it seems altogether probable that the normal or true water requirement of limber pine should be gauged by the lower figure. Also, from the fact that No. 10 was at the beginning a larger, probably more succulent specimen, we may quite confidently place this species on a par with bristlecone pine.

No other change in the order of arrangement is indicated as probable by the variations in the first column. However, examining the second column of the table, it is seen that the requirement of Douglas fir is greater than that of lodgepole. But, again, it is the Douglas fir specimen of higher water use (No. 3, 335) which behaved abnormally, its activity apparently almost ceasing before the end of the season, so that we must incline toward leaving Douglas fir in the position indicated by the first column of figures.

We shall not attempt here to discuss the cause of these variations, though that, too, is most interesting and will be at least partially clarified later.

It must be recognized that the relative water requirements, or ability to make growth with a given volume of water, while having a direct bearing on the relations of two or more species which compete with each other, may tell very little as to the ability of a tree of a given species to withstand the drought or wind exposure of a given site. The water requirements no doubt explain in some degree the gradual suppression

and crowding out of yellow and limber pines by fir or spruce, the similar elimination of lodgepole when spruce seriously competes with it. The water requirements may explain certain things which we have habitually ascribed to presence of or lack of shade "tolerance." But it does not necessarily mean that yellow pine, for example, might not resist transpiration and survive under rather rigorous conditions where no question of relative growth rate was involved.

Resistance to Transpiration

To obtain a better idea of relative resistance to transpiration we should consider the water losses, under equal conditions, as related to plant mass, leaf area, or leaf exposure. In Table IV the data are summarized on each of these bases, but the species are arranged in the order indicated by the relative transpiration per unit of leaf exposure.

TABLE IV.—*Water losses per unit of leaf area and plant mass*

Species.	Seasonal water loss (grams).		
	Per square centimeter of leaf.		Per gram mean green weight.
	Surface.	Exposure.	
Yellow pine	4.98	34.5 (3.)	160
Lodgepole	2.80	23.6 (0)	128
Bristlecone	2.03	21.2 (0.7)	119
Limber pine	1.83	18.0 (2.8)	81
Douglas fir	2.26	16.8 (0.3)	130
Englemann spruce	1.99	15.8 (3.0)	115

On the basis of the transpiration per unit of leaf exposure (which is believed to be the safest basis we have) or per unit of mass, the order of arrangement is essentially the same as in Table III. It is, perhaps, significant that the four important forest trees, yellow pine, lodgepole, Douglas fir, and spruce, appear in the same order as in the preceding table, while limber pine and bristlecone pine have moved to positions just below lodgepole. Taking the data at face value, let us consider for a moment what these qualities of limber and bristlecone pine must mean. In the first place, it has been seen that these species, which are admittedly very adaptive "weed" trees, use considerable water without making much growth. In the second place, we see that relative to their leaf area or whole mass they use very little. In other words, they are in some way adapted to protect themselves from water loss, but along with that adaptation, perhaps as a result of it, they have very meager ability for making use of light in photosynthesis. It is readily seen how this may happen. If either of these trees, accustomed to growing on bare sites where there is no competition, has adapted itself through reduction in the number and size of its stomata, moisture loss is reduced and the ingress of carbon dioxid is likewise reduced. If, again, as the writer has sometimes noted, the needles are closely appressed for the purpose of mutual protection, then moisture is saved at the expense of the full insolation of each leaf. The same might be the result of thickened epidermis or heavy palisade tissue. It thus appears that almost any adaptation for the conservation

of water must result in inefficiency in photosynthesis, and it might possibly be stated as an axiom that a weed is a plant which through protective adaptations is facultative for a variety of environmental conditions but which for the same reasons is incapable of making a standard rate of growth.

The impression is, then, obtained that yellow pine, lodgepole, Douglas fir, and spruce are resistant to transpiration in almost the same degree as they are capable of making use of water for their development, and that none of them possess any special adaptations for preventing water losses which interfere with growth. The cause for the differences between the several species should, then, be sought in those internal conditions which may determine their photosynthetic capacities and the behaviors of their cell contents as solutions, as was done by Salmon and Fleming (*19*) in the study of the winter hardiness of grains.

Before turning to that subject, however, which will be considered under the heading "Sap density," the transpiration rates should be further analyzed.

Periodic Transpiration

In Table II the transpiration of the trees by months has been shown, with the amount for each tree expressed as a percentage of that for all the trees. From these data it may be observed that only a few of the trees maintained stable positions with respect to the whole. The greatest significance of this is to indicate that, if the growth could have been measured for shorter periods, the relative water requirements might not have been the same as those for the whole season.

Comparing the transpiration at the beginning and end of the season (April to June against October to November), it is found that the species may be arranged in the following order, those which show the greatest relative increases being placed first: Yellow pine, limber pine, spruce, bristlecone, Douglas fir, and lodgepole. Lodgepole and Douglas fir show actual decreases.

If we should eliminate trees 3 and 10, which were apparently affected by some unknown factor, it would scarcely change the relations of the species.

These data at least indicate that the internal conditions which control transpiration are variable and probably are affected by the building of new tissue, accumulation and distribution of carbohydrates, and other changes which may occur in a season's growth.

Response of Transpiration to Light and Air Movement

The variations of each tree during the season, as shown above, almost preclude the possibility of determining the responses of the species to the different environmental conditions which were produced from day to day, since such comparisons, to use the available data, must include days during all parts of the season.

Taking as a standard for each species the days during the season when there was no ventilation in the greenhouse and when the total recorded sunlight was in excess of 400 minutes per day, it is found, as shown by Table V.

1. That for days having from almost none to 400 minutes of sunlight, with other conditions equal, all the species show about 60 per cent of the transpiration for a standard day.

2. With bright diffuse light, such as might penetrate the canvas curtains on a sunny day, the transpiration varies from 21 per cent of standard for lodgepole to 32 per cent for yellow pine and Douglas fir.

3. With dull diffuse light, as on cloudy days, the percentages vary from 17 per cent to 23 per cent of standard. Though lodgepole shows at all stages the greatest depression from the lack of sunlight, it is hardly safe to say that this is a specific character.

TABLE V.—*Response of various species to different conditions of light and air movement*

Conditions.	Number of days.	Species.					
		Yellow pine.	Douglas fir.	Lodgepole.	Engelmann.	Limber pine.	Bristlecone pine.
Average transpiration (cubic centimeters per day, standard), no ventilation, over 400 minutes sunshine	12	21.82	[a] 13.59	11.86	19.84	9.62	13.22
Proportionate transpiration:							
No ventilation, less than 400 minutes sunshine	11	.62	.62	.54	.62	.62	.59
No ventilation, bright diffuse light	19	.32	.32	.21	.26	.31	.25
No ventilation, dull diffuse light	7	.23	.20	.17	.22	.18	.18
Some ventilation, over 600 minutes sunshine, temperature over 75° F	4	1.05	1.08	1.35	.98	1.18	1.18
Some ventilation, over 600 minutes sunshine, temperature under 75° F	2	.91	1.02	1.12	.86	1.11	1.10
Some ventilation, 400 to 600 minutes sunshine, temperature over 75° F	7	1.27	1.12	1.40	1.08	1.41	1.23
Some ventilation, 400 to 600 minutes sunshine, temperature under 75° F	13	.99	1.06	1.19	.99	.94	1.02
Some ventilation, less than 400 minutes sunshine, temperature over 70° F	10	1.00	1.05	1.06	.90	1.00	.99
Some ventilation, less than 400 minutes sunshine, temperature under 70° F	8	.59	.58	.64	.61	.63	.57
Some ventilation, diffuse light	5	.21	.28	.28	.31	.28	.29

[a] Only tree No. 4 used, account seasonal change in No. 3.

4. With ventilation, the transpiration of all species is increased over that without ventilation, other conditions being about the same. The amount of ventilation in the greenhouse was not sufficient to produce striking changes. The exposure of the trees outdoors for one day did not materially increase the transpiration rate, temperatures being considered.

Finally, since the specific responses are subject to the seasonal changes already noted, we may examine the results expressed by the total transpiration of the 12 trees during each day or longer period.

An attempt has been made to relate this to the vapor deficit, or differential between the atmospheric vapor pressure and the saturation pressure conceived to exist within the leaf, as determined by mean temperatures. For the latter there is available either the air thermograph record or the "sun" thermograph (blackened bulb) record. The latter seems preferable in theory, but the record is not very trustworthy because of very large corrections in the instrument as used during most of the season. This, together with the fact that atmospheric vapor pressures were determined only in the early part of each period (9 a. m.) and at its end (7 a. m.), makes the computation of vapor deficits the roughest approximation. In addition, it becomes evident that the leaf temperatures (and saturation pressures) should not be considered as equal to the black-bulb temperatures, but more nearly equal to what the wet bulb of the psychrometer would show synchronously. As the psychrometric data are not sufficient even to approximate the mean wet-bulb temperatures, the results of "sun temperature" computations alone will be shown. Recognizing that transpiration at night, because of the lack of sunlight, is in a different category from that during the day and

should be given much less weight in the total, the general scale of temperatures has been raised by computing the daily means as follows: The 12 hourly temperatures from 7 a. m. to 6 p. m., inclusive, are added to the temperatures at 8 p. m., 12 m. and 4 a. m., and the total is divided by 15.

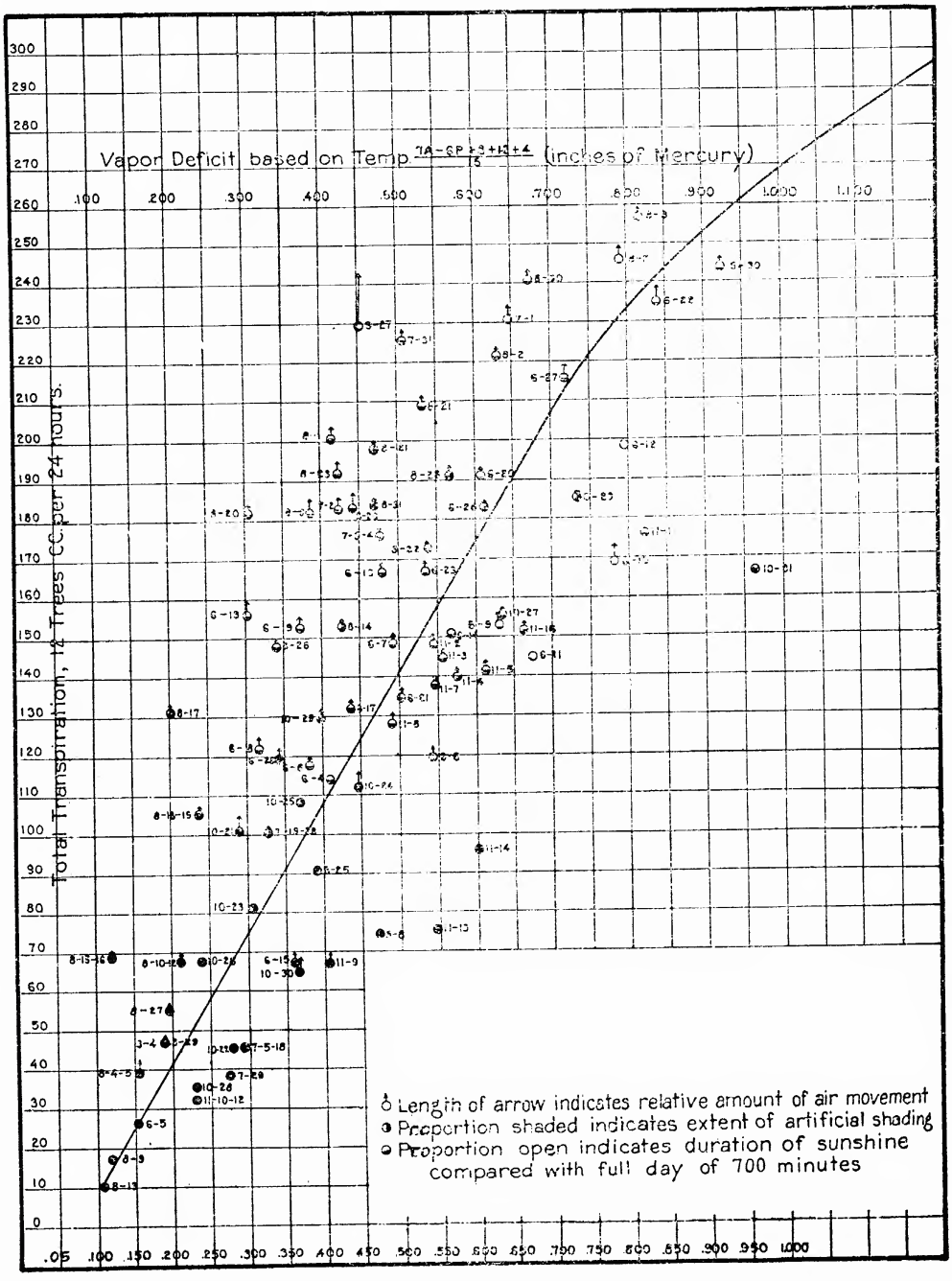

FIG. 1.—Transpiration in relation to saturation deficits in 1917. Numbers opposite circles give month and day of observation.

In figure 1 the results of such computations of vapor deficit for 84 periods during the season of operations are plotted with the daily transpiration sums. The basic data will not be tabulated, since it is voluminous and has no precise value. Instead, the general character of each period is indicated by symbols on the diagram. The mean curve which may be obtained from the 84 points is only suggestive in showing—

1. That for a given saturation deficit a wide variation is possible in the amount of transpiration induced. It is believed, however, that this is largely the result of insufficient data for computing both saturation pressure in the leaf and atmospheric vapor pressure.

2. Air movement increases transpiration somewhat more than has been allowed by the computations, which, in fact, made no allowance for this factor except as it might influence the depression of the psychrometer wet bulb.

3. The amount of sunlight is not the cause of much variation in the results. If anything, the use of black-bulb temperatures has made a little too much allowance for sunlight effects; or, in other words, the plants are not quite so strongly affected by light as is the black-bulb temperature.

4. The transpiration rate (average) is quite proportionate to vapor deficits until the former approaches a daily amount equal to the total weight of the plants, when transpiration, apparently, does not quite keep up with saturation deficits.

5. The transpiration at the end of the season is less than in the earlier months, days of like valuations being compared. The size of the plants, of course, increased during the season, but the increase in leaf area was almost wholly during the first month. It seems safe to say that old leaf tissues do not permit as much transpiration as young tissues.

6. The physical control of transpiration and the lack of plant control is fairly evident, though by no means proved.

TRANSPIRATION IN 1920

The striking differences between the species, indicated by the transpiration rates in 1917, both on the basis of growth accretion and relative leaf areas or leaf exposures, called, first, for a reasonable explanation of the temporary circumstances which produced such results, and, secondly, for a repetition to determine whether similar relations of the species might hold with new and different material and whether either the absolute or relative water requirements might be different under more natural environmental conditions.

The first need had been definitely pursued in the interim, and the second was fulfilled during the summer of 1920. In repeating the transpiration tests, it was especially sought to have as large an assortment of plant material as could be adequately dealt with.

MATERIAL STUDIED

Twenty-three pots of plants were used, compared with the 12 in 1917. Of these one containing a large yellow pine failed early in the season. An additional pot without trees served to measure the possible direct water loss from the soil. In a number of cases, two or three specimens were handled as one in a single pot, in order to give a better average result. (See Plates 4 to 6.)

The limber and bristlecone pines and a single large spruce specimen were from the same lots of stock which were drawn upon in 1917, having been in the Fremont Station nursery during the interval. The remaining trees were all younger stock grown in the same nursery, and mainly of fairly definite origin as to seed. Where possible a variety of seed sources was represented in the selections for each species, in order to determine the possibilities of variation between geographic forms of the same species.

The data on the trees used are given in Table VI.

TABLE VI.—*Source, size, growth, and condition of trees in 1920 transpiration test*

Species and source of seed.	Age.	Number potted.	Green weight.	Similar specimens dried.		Calculated dry weight of potted trees.	Weight at end of season.				Seasonal weight gain.		Leaf exposure from photograph.	Pot No.	Remarks.
							Green.			Total dry.					
				Number.	Dry-green ratio.		Living plant.	Air-dry salvage.	Total.		Green.	Dry.			
	Years.		*Gm.*			*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Sq. cm.*		
Yellow pine:															
Harney, S. Dak.	4	2	21.46	2	0.394	8.46	21.29	0.95	22.24	9.45	0.78	0.99	86	22	Slow in beginning vernation.
Do.	4	2	18.84	2	.394	7.43	22.19	.37	22.56	8.88	3.72	1.45	85	1	
Bitterroot, Mont.	4	2	15.34	2	.400	6.14	20.64	.22	20.86	7.80	5.52	1.66	107	18	Do.
Tusayan, Ariz.	4	2	17.78	2	.377	6.70	27.40	.42	27.82	9.66	10.04	2.96	114	15	
Douglas fir:															
Hayden, Wyo.	8	1	28.20	2	.419	11.81	28.44	.84	29.28	13.60	1.08	1.79	144	17	Almost no small fibrous roots, but some new growth made.
Leadville, Colo.	8	1	34.65	1	.408	14.13	31.86	2.22	34.08	15.55	−.57	1.42	140	10	Large proportion of new foliage.
Pike, Colo.	8	1	21.30	2	.426	9.08	23.17	.20	23.37	9.80	2.07	.72	142	7	
Lodgepole pine:															
Colorado, Colo.	5	2	19.81	2	.409	8.10	28.16	.20	28.36	9.91	8.55	1.81	111	3	
Washakie, Wyo.	5	2	22.27	2	.405	9.02	32.90	.46	33.36	11.84	11.09	2.82	119	23	
Gunnison, Colo.	5	2	32.61	2	.390	12.72	45.69	.32	46.01	16.53	13.40	3.81	170	24	
Engelmann spruce:															
San Isabel, Colo.	4	3	11.89	7	.392	4.66	20.28	.32	20.60	7.33	8.71	2.67	103	9	
Montezuma, Colo.	4	2	5.38	6	.391	2.10	10.30	.10	10.40	3.99	5.02	1.89	68	19	
San Isabel, Colo.	4	3	7.27	2	.388	2.82	13.02	.12	13.14	4.90	5.87	2.08	84	12	
Unknown.	8	1	55.69	1	.434	24.60	76.15	.77	76.92	30.64	21.23	6.04	295	11	Much slower than small spruces in beginning vernation.
Limber pine:															
Unknown.	9	1	64.18	2	.448	28.76	73.36	.68	74.04	30.93	9.86	2.17	239	16	
Do.	9	1	32.47	2	.448	14.55	31.13	.28	31.41	14.64	−1.06	.09	119	21	Buds barely extended during season; roots grew reasonably well.
Bristlecone pine:															
Unknown.	9	1	29.80	1	.444	13.23	31.59	.22	31.81	14.69	2.01	1.46	124	8	
Do.	9	1	12.55	1	.444	5.58	15.12	.06	15.18	6.60	2.63	1.02	70	4	
Scotch pine:															
Riga variety.	4	1	29.70	1	.394	11.70	31.30	.26	31.56	13.62	1.86	1.91	155	13	
Do.	4	1	21.40	1	.394	8.44	27.50	.63	28.13	11.21	6.73	2.77	108	2	Slow in beginning vernation.
Siberian larch:															
Russia.	6	1	9.87	2	.298	2.94	12.90	.98	13.88	5.87	4.01	2.93	98	6	Nearly all of foliage fallen and dry when final weight was determined; No. 6 dried earlier than No. 20.
Do.	6	1	15.38	2	.298	4.58	16.51	1.42	17.93	7.66	2.55	3.08	133	20	
All.	.....	34	527.84	47	400	.....	640.90	12.04	.....	.....	125.10	47.54	.....	....	

SOIL

The soil in which the trees were potted was nearly the same as that used in 1917. There had been added to the original soil a somewhat more clayey sand of granitic origin, so that in 1920 we find a greater water-holding capacity, a slightly lower moisture equivalent, and a third higher wilting coefficient. The data on the 1920 soil are:

	Per cent.
Saturation	36.46
Capillarity	31.02
Moisture equivalent	10.42
Wilting coefficient (average for spruce and yellow pine)	4.88

It was planned to carry about 12 per cent moisture, and to prevent the saturation of the lowest stratum of the soil by injecting the daily supply of water near the surface. The feed tube, then, instead of opening into the inverted clay pot at the bottom of the can, was bent about 2 inches below the surface and opened into the soil near the center of the can. Aside from this the potting arrangement was the same as in 1917.

The 12 per cent moisture would constitute, in the average of the 22 cases, 284.4 gm. of water. At the outset, however, the average pot was given 528.8 gm. of water, in order to create a very favorable condition. In the average case this supply lasted much longer than had been anticipated and was not brought down to standard until after July 1, so that during the first two months the moisture conditions were by no means uniform.

Finally, beginning September 3, the water content was gradually reduced, until on September 27 it was 70 gm. below the standard, or in the average case amounted to 9.05 per cent. This change was designed to simulate the usual autumn drying of the soil.

We thus have the following average conditions in the several pots, comparing with a mean availability of about 0.850 through the entire season of 1917:

May 11, 528.8 gm. = 22.31 per cent = availability 0.780; decreasing to 284.4 gm., or 12 per cent, about July 1 = availability 0.594; reaching September 27, 214.4 gm., or 9.05 per cent = availability 0.461.

It is, therefore, seen that in the osmotic sense the conditions for ready absorption of the water were far less favorable in 1920 than in 1917.

PROCEDURE

The procedure in handling the pots was in minor details almost the same as in 1917. On the other hand, as has been mentioned, they were not under glass but under a canvas cover which was raised during the entire day except when showers occurred, permitting unmodified sunlight to reach the trees and also allowing much freer air circulation, the primary effect of which was, undoubtedly, to prevent the occurrence of excessive temperatures. While the extreme sun temperatures recorded were not materially lower than in 1917, the air temperatures were scarcely above those outside the shelter and were very much lower than in 1917, when they averaged 6° F. higher than the outside air.

The revolving table on which the pots were exposed was nearly 5 feet in diameter and made a revolution every two hours.

RESULTS

Amount of Transpiration Compared with 1917

The first point to be noted in Table VII is that the amount of transpiration in 1920 was very much less than in 1917.

On the basis of mean green weight it was 42.4 gm. in 1920, as compared with 123.0 in 1917. On the basis of leaf exposure it was 8.81 gm. in 1920, as compared with 20.6 in 1917. For leaf area we have no data in 1920. It is evident, however, that, considering the amount of water used in relation to size of trees, the transpiration was only one-third to two-fifths as great in 1920 as in 1917. This may be accounted for—

1. By a season of only about 147 days for the average tree in 1920, as compared with 203 days in 1917.

2. By reason of much lower air temperatures in 1920.

3. By reason of considerably less sunshine in 1920, but especially the lack in June, when the driest atmosphere usually prevails. The following data for whole months give the sunshine in recorded minutes:

	1917.	1920.
May	8,622	9,598
June	15,807	8,903
July	12,932	11,310
August	10,496	11,704
September	10,442	11,695
Total	58,299	53,210

It is also probable that on account of the arrangement of the room the trees received a smaller percentage of the total sunshine in 1920.

4. The water of the soil was less readily obtainable in 1920.

TABLE VII.—*Actual water losses and transpiration in relation to size and growth in 1920*

Species.	Yellow pine.				Douglas fir.		
Pot No.	22	1	18	15	17	10	7
Transpiration:							
May 11 to 31	51.5	52.8	35.6	66.2	83.0	108.9	112.0
June	109.0	112.0	102.7	144.6	111.4	122 9	134.0
July	132.4	163.1	236.4	280.0	117.6	162.6	149.0
August	161.2	236.8	338.2	492.7	130.3	180.0	177.0
September	151.4	292.8	313.0	509.4	130.9	190.3	181.8
October (to day indicated)	(7) 31.6	(8) 80.5	(8) 73.7	(7) 105.9	(8) 47.8	(5) 32.1	(6) 39.7
Total for season	637.1	939.0	1,099.6	1,598.8	621.0	796.8	793.5
Correction, direct evaporation	79.4	79.6	79.6	79.4	79.6	77.7	79.0
Net transpiration	557.7	859.4	1,020.0	1,519.4	541.4	719.1	714.5
Transpiration:							
Grams per gram weight accretion—							
Green	715	231	185	151	501	.....	345
Dry	564	592	615	514	302	506	992
Grams per gram mean green weight	26.1	41.8	56.8	67.3	19.1	21.6	32.1
Grams per square centimeter leaf exposure	6.5	10.1	9.5	14.2	3.8	5.1	5.0

TABLE VII.—*Actual water losses and transpiration in relation to size and growth in 1920*—Continued

Species.	Lodgepole pine.			Engelmann spruce.			
Pot No.	3	23	24	9	19	12	11
Transpiration:							
May 11 to 31	64.5	83.7	94.5	125.9	93.5	99.6	181.8
June	154.8	246.6	243.2	177.3	143.3	127.9	327.7
July	196.3	548.3	607.1	212.1	149.4	194.1	607.8
August	394.0	726.0	923.6	341.3	240.5	272.5	894.5
September	435.1	627.5	708.9	363.6	224.8	227.8	926.0
October (to day indicated)	(8) 103.1	(6) 106.8	(6) 118.7	(5) 56.2	(7) 42.3	(5) 29.8	(5) 123.8
Total for season	1,347.8	2,338.9	2,696.0	1,276.4	893.8	951.7	306.6
Correction, direct evaporation	79.6	79.0	79.0	77.7	79.4	77.7	77.7
Net transpiration	1,268.2	2,259.9	2,617.0	1,198.7	814.4	874.0	2,983.9
Transpiration:							
Grams per gram weight accretion—							
Green	148	204	195	138	162	149	140
Dry	701	802	688	450	431	420	494
Grams per gram mean green weight	52.9	82.0	66.9	74.6	103.8	86.1	45.3
Grams per square centimeter leaf exposure	11.4	19.0	15.4	11.6	12.0	10.4	10.1

Species.	Limber pine.		Bristlecone pine.		Scotch pine.		Siberian larch.		All.
Pot No.	16	21	8	4	13	2	6	20	.....
Transpiration:									
May 11 to 31	131.2	79.2	57.3	74.3	81.7	39.1	71.9	69.2	.....
June	151.2	77.2	105.3	128.1	149.1	121.6	158.1	152.8	.....
July	321.3	84.1	128.8	114.5	183.4	231.0	236.2	165.6	.....
August	484.4	112.9	132.2	107.5	194.7	416.0	457.0	322.4	.....
September	.....	.....	.....	.....	.....	.....	(27)	(27)	.....
October (to day indicated)	534.4 (8) 132.5	125.2 (7) 31.4	108.4 (3) 7.1	87.6 (3) 6.7	212.7 (6) 38.2	454.7 (8) 102.1	123.5	152.8	....
Total for season	1,755.0	510.0	539.1	518.7	859.8	1,364.5	1,046.7	862.8	.....
Correction direct evaporation	79.6	79.4	76.0	76.0	79.0	79.6	70.3	70.3	.....
Net transpiration	1,675.4	430.6	463.1	442.7	780.8	1,284.9	976.4	792.5	247,940
Transpiration:									
Grams per gram weight accretion—									
Green	170	.....	230	168	420	191	243	311	198
Dry	773	4,785	317	434	409	464	333	257	522
Grams per gram mean green weight	24.4	13.5	15.1	32.0	25.6	52.6	94.0	49.7	42.4
Grams per square centimeter leaf exposure	7.0	3.6	3.7	6.3	5.0	11.9	10.0	6.0	8.81

On the basis of the growth made, the transpiration is also less in 1920 than in 1917, though not so strikingly so. For green-weight accretion the figures are 198 and 263, and for dry-weight accretion 522 and 880, respectively, for 1920 and 1917. In other words, for green-weight accretion it required 75 per cent as much water in 1920 as in 1917, and for dry-weight accretion 59 per cent as much. The difference between these two percentages and between the two years may be due largely to the fact that very little foliage falling in 1917 was salvaged and accounted for, while in 1920 this was carefully done. However, it is believed the amount dropped by the trees in 1917 was relatively very small and insufficient materially to affect the results.

It seems fairly evident that the transpiration per unit of growth is a more stable quantity than that per unit of leaf exposure or whole mass, in spite of the fact that the former is very much dependent on the whole leaf area functioning.

Water Requirements

Comparing now the species, as was done for the data of 1917, we have them in 1920 aligned as in Table VIII.

TABLE VIII.—*Comparison of different species as to water requirement*

Species.	Transpiration per unit of dry-weight accretion.	Probable error in average.
	Gm.	*Gm.*
Limber pine	2,779	850
Lodgepole pine	730	18
Douglas fir	600	160
Yellow pine	571	5
Engelmann spruce	441	6
Scotch pine	436	23
Bristlecone pine	376	49
Siberian larch	295	32

Resistance to Transpiration

On the basis of leaf exposures we have a very different arrangement (Table IX).

TABLE IX.—*Comparison of species as to resistance to transpiration*

Species.	Transpiration per square centimeter leaf exposure.	Probable error in average.
	Gm.	*Gm.*
Lodgepole pine	15.27	0.49
Engellmann spruce	11.12	.21
Yellow pine	10.08	.66
Scotch pine	8.45	2.91
Siberian larch	8.00	1.69
Limber pine	5.30	1.44
Bristlecone pine	5.00	1.10
Douglas fir	4.63	.39

EXPLANATION OF RESULTS

On thorough consideration of the meaning of the results which have been given above for both 1917 and 1920 tests, we come to the conclusion that neither method of comparing the species is very satisfactory when the number of individuals involved is insufficient to cover all possible variations. In these tests considerable variation in growth rate is to be expected as the result of more or less incomplete recovery from transplanting. The small spruces, for example, in 1920 showed no delay in starting new growth; the single large spruce came on satisfactorily after considerable delay; one large limber pine grew vigorously while the smaller one did not extend its terminal or branch buds over one-fourth

inch and put on no new foliage. None of the Douglas firs grew vigorously in 1920 while all of them dropped a good deal of their old foliage.

The water requirements and the rate of transpiration per unit of mass or leaf exposure are closely interrelated, it will be seen, for the following obvious reasons:

1. New shoots undoubtedly transpire more freely than old foliage.
2. When a plant is not growing it seems to transpire relatively little, either because it can not obtain the water or, possibly, because it has closed its stomata.
3. It therefore follows that the amount of transpiration per unit of mass or leaf exposure may be very much affected by the amount of growth made.
4. And it is equally apparent that the transpiration per unit of growth may be somewhat dependent on the total amount of foliage functioning, though it must be conceded that so long as the old foliage transpires, it probably is also capable of some photosynthesis, and therefore contributes to growth.

The important point is to recognize that an extreme case of poor growth may throw the specimen very high in one list and very low in the other list (for example, Pot 21 in 1920). It seems, therefore, only reasonable to eliminate from both records the individuals which have apparently not performed normally in the matter of growth. As the basis for normalcy is so meager, we can not bring ourselves to the elimination of any trees except one limber pine in 1917 and another in 1920.

On the other hand, what is true of individual trees affects the relations of the species. Apparently, small spruces are capable of a generally larger accretion percentage than similar trees of our other native species. As has been pointed out, this would be a very important factor in competition. Its bearing on absolute water requirements and drought resistance is not so plain, and we have had serious misgivings as to the desirability of comparing the species, in their moisture relations, on this basis. Nevertheless, it is fairly apparent that a high growth percentage in itself denotes something of superiority in the relation of the tree to its environment. It indicates either that the tree has some peculiar ability to make use of the available light or that it is more capable than others of supplying the water, or the carbon dioxid, in just the right amount to make photosynthesis effective. If either of the latter is a factor in the result, we may say either that the plant has superior ability to obtain water or that it has superior ability to retain it while keeping the stomata open for the ingress of carbon dioxid. There is left, therefore, little doubt in our minds that the tree of low "water requirement" as related to growth is in fact the tree which has the superior control over its water supply.

It is, therefore, important to compare the species on the basis of the growth made, in order to understand the marked differences between the absolute transpiration rates in 1917 and 1920, which leave the relations of the species so confused.

On comparing further Tables III and VIII, it is seen that with one or two exceptions, namely, yellow pine and bristlecone pine, the water requirements as determined in 1917 and 1920 are not so divergent that we need hesitate to combine them to obtain more effective averages, and it seems best to use the value for each tree in obtaining the mean. There are also given the mean growth percentages for each species. In figure 2 the general relation between growth rates and water requirements is plainly shown—a relation that seems logically unavoidable.

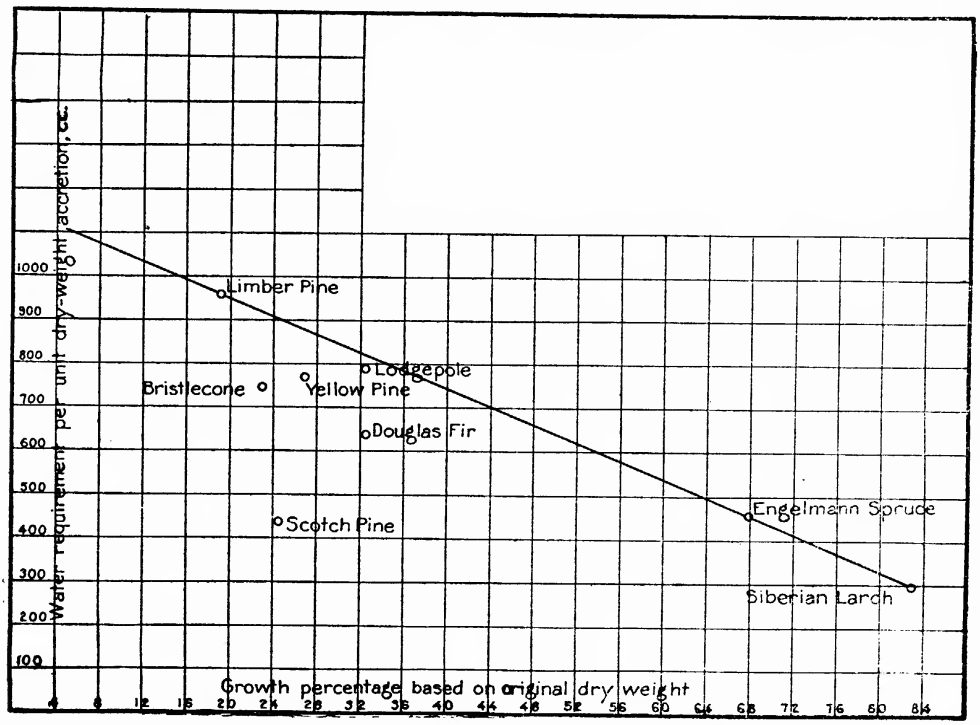

FIG. 2.—General relationship between water requirements and growth rates for all species in 1917 and 1920.

TABLE X.—*Water requirements and growth in 1917 and 1920*

Species.	1917			1920			Average water requirement.	Average growth percentage.[a]
	Number of trees.	Water requirement.	Growth percentage.	Number of trees.	Water requirement.	Growth percentage.		
		Gm.			*Gm.*		*Gm.*	
Limber pine	1	1,144	31.0	1	773	7.5	958	19.2
Lodgepole pine	2	954	46.6	6	730	27.8	786	32.5
Yellow pine	2	1,555	32.4	8	571	25.6	768	27.0
Bristlecone pine	2	1,110	31.3	2	376	14.7	743	23.0
Douglas fir	2	684	64.9	3	600	11.0	634	32.6
Engelmann spruce	2	525	75.2	9	441	66.4	456	68.0
Scotch pine	.....	.....	.....	2	436	24.6	436	24.6
Siberian larch	.....	.....	.....	2	295	83.5	295	83.5

[a] Dry accretion related to dry weight at beginning of season.

At least two important points are gained by the combination of the 1920 with the 1917 data. The position of limber pine, as the least effective user of water, is more nearly established, and lodgepole pine is brought into this class, where our empiric estimates would place it, as we shall see later.

But the main reason for presenting the growth data in Table X is to explain the positions of the species as given in Table IX. It is seen that Engelmann spruce maintained in 1920 almost as high a growth rate as in 1917, and this explains its appearance as a relatively extravagant user of water on the leaf-exposure basis. Conversely, limber pine and Douglas fir both appear as conservative users of water in 1920, evidently because they were relatively inactive.

In combining, then, the data for the two years on transpiration per unit of leaf exposure, we shall not lose sight of the fact that the positions of Douglas fir and limber pine, at least, and to a lesser extent that of bristlecone pine, are determined by relative inactivity in 1920, and that they really belong higher in the scale than here shown. There are possible means of making allowance for this influence of growth activity on the total transpiration, but they are so purely arbitrary that we hesitate to use them.

TABLE XI.—*Mean resistance to transpiration, 1917 and 1920 (growth data repeated to explain relative positions)*

Species.	Mean transpiration per square centimeter leaf exposure.	Mean growth percentages.
	Gm.	
Lodgepole pine	17.35	32.5
Yellow pine	14.96	27.0
Limber pine	14.15	19.2
Bristlecone pine	13.10	23.0
Engelmann spruce	11.97	68.0
Douglas fir	9.50	32.6

That these relations of the species are not fixed and might easily be modified by additional data or consideration of different climatic varieties is fairly apparent from the divergence of the individual results. On the same basis as Table XI, the three lots of yellow pine studied in 1920, each of which maintained a healthy condition and performed, relatively, much as it had been performing in the nursery, gave the following results:

Variety.	Transpiration per square centimeter leaf exposure.	Growth percentage.
	Gm.	
Harney, S. Dak.	8.3	11.7
Bitterroot, Mont.	9.5	19.5
Tusayan, Ariz.	14.2	27.0

There is every reason to believe these results were normal and express something of varietal difference. It is seen that the high growth rate of the Arizona form was accomplished with the extravagant use of water. Whether this is generally true of the more southern forms our data are inadequate to determine. Rather similar differences occur with lodgepole pine, but here the Wyoming form is the most extravagant and rapid growing.

ABILITY OF THE SPECIES TO OBTAIN WATER

A very considerable amount of light is thrown on the meaning of Table XI and our other discussions by considering the water requirements of each species in 1920, with the considerably less abundant water supply, as a measure of the ability of each to extract water from the soil. Thus, in comparing Tables III and VIII it was particularly noted that bristlecone and yellow pines, which in 1917 were more generous users of water, in 1920 took very low positions in the scale. The relative changes are indicated in the following table, where the use in 1920 is expressed as a percentage of the use in 1917. Only the better growing limber pines are considered. Comparison is made on the basis of Tables III and VIII and Tables IV and IX.

Species.	Relative transpiration in 1920.	Species.	Relative transpiration in 1920.
	Per cent.		*Per cent.*
Engelmann spruce	77.2	Limber pine	48.5
Lodgepole pine	66.2	Yellow pine	33.0
Douglas fir	57.6	Bristlecone pine	28.8

In some degree these observations are corroborated by the data in Table XII in which is shown the change in relative transpiration rates through a period in which the last pot listed for each species was given additional water. For the periods represented by September 28, 29, and 30, the water in each pot was at 70 gm. below standard, and except for the one pot of each species, the same amount was maintained on succeeding days. On the morning of September 30 the amounts in the special pots were increased 50 gm., and on October 1 they were brought up to standard. After this, they were not again watered until dried out to the original basis. If, then, the availability of the water, which was thus increased from about 0.461 to 0.594, as described under the heading "soil," has a bearing on the amount transpired, its effect should in all cases be apparent in the transpiration recorded October 1 and 2. In the table is shown what was the prevailing relative amount for the special tree, compared with others of the same species for the three days when water contents were the same. The relative amount on October 2 is then shown as a percentage increase.

It is to be noted that after October 2 the relative rates of the specially watered trees steadily declined, and in some cases went below the previous rates, until the day indicated by "W," when the first fresh water would take effect. This is plainly due to exhaustion of water close to the roots and is a commentary on the importance of transport within the soil.

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TABLE XII.—*Effect on transpiration of increasing the available water* [a]

Species.	Pot No.	Transpiration measured on the morning of—									Average relation Sept. 28 to 30.	Percentage increase Oct. 2.
		Sept. 28.	Sept. 29.	Sept. 30.	Oct. 1.	Oct. 2.	Oct. 3.	Oct. 4.	Oct. 5.	Oct. 6.		
Yellow pine	1	11.0	10.8	10.3	10.5	12.9	12.0	13.4	9.5	11.6		
	18	10.6	9.8	12.0	10.8	11.1	10.8	13.1	9.7	10.1		
	22	5.4	4.5	5.1	4.9	5.9	5.5	5.6	4.5	5.0W		
	15	18.7	17.4	19.5	21.2	28.1	20.3	19.4	10.5	13.6		
Relative amount in No. 15 [b]	.....	.693	.694	.712	.810	.940	.718	.605	.443	.510	0.700	34.4
Douglas fir	7	6.3	6.4	6.3	7.0	8.0	8.7	8.6	6.5	7.9		
	10	6.8	5.7	7.5	6.2	8.0	8.6	8.9	6.6	.....		
	17	4.3	3.7	5.1	6.4	7.0	8.0	7.4	5.8	.....		
Relative amount in No. 17 [b]	.....	.328	.306	.370	.485	.437	.462	.423	.443	.....	.335	44.8 30.5
Lodgepole pine	3	15.6	14.2	16.4	13.7	17.2	14.7	19.3	12.2	14.0		
	23	21.9	22.9	23.5	20.1	24.6	21.1	24.5	17.7W	18.9		
	24	23.1	19.9	23.8	24.4	32.6	27.3	23.4	16.5	18.9		
Relative amount in No. 24 [b]	.....	.616	.537	.597	.722	.780	.763	.535	.552	.593	.583	33.9
Engelmann spruce	9	12.1	12.6	13.8	12.3	14.7	13.3	15.3	12.9	.....		
	12	7.2	6.9	7.3	6.4	8.1	6.5	8.3	6.9	.....		
	19	6.9	6.8	7.6	7.2	8.1	6.8	8.7	6.5	.....		
	11	32.4	30.5	35.5	34.5	40.7	32.9	27.9	22.3	.....		
Relative amount in No. 11 [b]	.....	1.237	1.160	1.237	1.332	1.318	1.237	1.158	1.180	.....	1.211	8.8
Limber pine	21	4.5	3.6	4.2	4.5	6.0	5.3	5.5	4.0W	5.5		
	16	16.9	16.4	17.8	20.2	28.8	24.0	21.3	13.1	16.3		
Relative amount in No. 16 [b]	.....	3.76	4.56	4.24	4.49	4.80	4.53	3.88	3.28	2.96	4.19	14.5

[a] Last pot for each species increased 50 gm. effective October 1 and 20 gm. more effective on morning of October 2.
[b] The amount in the pot receiving extra water is expressed as a ratio to the total in other pots of same species.

From the data in Table XII we see that the limber pine was little stimulated, probably because its transpiration is always moderate. The spruce was still less affected, apparently because it is always able to satisfy its needs. Lodgepole, Douglas fir, and yellow pine were about equally helped and seemed greatly invigorated. The relatively high transpiration of the fir on October 1 and 3 can be accounted for only by an error in weighing.

To a certain extent, these performances may be accounted for by the root habits of the trees. It is possible that the finely divided and numerous roots of the spruce give it immediate control over so much more soil that it exhausts the available water much less quickly than those species which usually develop only a few coarse roots. To some extent this would also explain the ability of limber pine to obtain its water more steadily. On the other hand, either lodgepole or Douglas fir ordinarily has much better roots than yellow pine, yet these three were about equally stimulated by a heavy addition to the water supply.

On the whole, this matter is only suggestive and does not, we believe, explain the relative behavior of the species. That Engelmann spruce possesses a remarkably great ability to supply itself with all the water that is needed under the most trying circumstances, and that this ability is exceeded, among the species studied, only, possibly, by that of Siberian larch, seems proved beyond a shadow of doubt. This is plainly shown in the day-to-day records where, if there is a marked contrast in the amount of sunlight on two succeeding days, or in other conditions conducive to high transpiration, it is the spruce which is invariably able to live up to these conditions most fully. Thus we are enabled to say quite confidently that the relatively high rate of transpiration of spruce on cloudy days, as shown by Table V, truly expresses an ability to make use of all available light and does not signify that this species is unable to meet the conditions which cause high transpiration from all the species.

SUMMARY

It has seemed desirable to go into this matter fully on account of the complicating features introduced by the radically different results secured in 1917 and 1920, and in order that we might not deceive ourselves as to the true meaning of the results. It has been necessary for us to go through with this analysis in order to reach a conclusion, and it is hopeless to expect the reader to reach a conclusion by any other process.

It now becomes fairly apparent that transpiration is very much dependent on water supply and that the relatively low water use of some of the species in 1920, when the water supply was maintained at a low level, is not to be considered as a virtue but rather as evidence of a lack of ability to supply needs. And, even though in some cases growth may not have been seriously impaired by the inability of certain species to keep the leaves well stocked with water, yet it is perfectly evident that the species which show this inability in the most marked degree would soonest succumb in time of real drought or in the usual autumnal drought that occurs where there is strong competition.

There are, apparently, two slightly different problems to be considered in comparing the species. The one has to do with the relative requirements of different tree species of a unit size. The other has to do with

the amount of water required during the production of a unit of growth. Both relations are important in ecology. But, except with the weed trees, limber and bristlecone pines, we have found no essential difference in comparing the species on the two bases. To what extent a low water requirement means great drought resistance we shall see later. Apparently there is not a great deal of difference between the species at the minimum water point. There is evidently a great difference in their activity or vigor under better conditions, and this, perhaps, is the most important point we have brought out.

The important consideration is that the additional data secured in 1920 have not materially altered our conception of the physiological qualities of the species, which are best indicated relatively by Table III. It is true that in 1920 lodgepole pine used relatively more water and made less vigorous growth, so that now, by either Table X or XI, it appears as a more extravagant demander than yellow pine. Likewise Engelmann spruce kept up its rate in 1920 more nearly than Douglas fir and hence appears more extravagant. These facts, however, merely confirm the belief that the species which under favorable moisture conditions is most conservative is best able under all conditions to satisfy its needs. The reason for this will be more apparent after considering sap density and its osmotic bearings.

To summarize, briefly, for the species, what it is believed is shown by the preceding data and discussions:

1. Limber pine: Very slow growing, but also very conservative in the use of water. Represents highest development in structural protection against atmospheric conditions, but probably poor development in relation to the soil. Not adapted for competition.

2. Bristlecone pine: Not quite so far developed as limber pine in any respect mentioned.

3. Yellow pine: Relatively slow grower and has little protection against losses; consequently from either standpoint its water use is very high. Shows little ability to cope with drought conditions. Arizona form more vigorous and equally extravagant of water.

4. Lodgepole pine; More rapid grower than yellow pine.

5. Douglas fir: Apparently adapted to conserve water but growth rate not nearly equal to spruce, possibly being in these tests more adversely affected by transplanting because of the relatively long roots, which are characteristic, and their small numbers.

6. Engelmann spruce: Most highly developed of our native species to make use of all conditions of environment in vigorous growth. Is conservative of water and low in water requirements for growth. These characteristics may partly explain its shade tolerance and its success in competition.

7. Siberian larch: Although little studied, seems to be developed even beyond spruce in all particulars.

8. Scotch pine: Stands about midway between our pines in transpiration rate and lower than any of them when growth is considered. Seems to be developed along lines of spruce and fir for alpine conditions. It should be remembered we are speaking only of the Riga form.

SAP DENSITY AND THE VARIATION IN TRANSPIRATION RATES

Sometime before the transpiration tests which have just been described were made in 1917, carefully conducted drying tests on green and partly

dried lodgepole cones, in a calorimetric kiln, had clearly demonstrated that the amount of heat required to extract a gram of water from cones was not 536 calories but an amount always in excess of that, which increased rapidly as the amount of sap in the cones decreased through preliminary drying. This apparent increase in the latent heat of vaporization, it was thought after a study of the physical chemistry of solutions, might be related to the phenomena of rising boiling points and decreased vapor pressure with increases in the concentration. Unfortunately, no direct experimental work on this problem had been done, so far as I have been able to learn to date, and a number of physicists consulted agreed that in their interpretation of the theory of solutions a solute could not increase the latent heat of vaporization of the solvent.

The writer, in the most dependable tests it has been possible to make, has found that at the respective boiling points of various concentrations of common salt in water, the latent heat of vaporization decreased slightly with increased concentration, up to the point of saturation. Making allowance for the higher boiling point of the concentrated solution, it would appear that for a given temperature the latent heat of vaporization was practically a constant. In these tests an electric immersion heater was employed for the heat supply, the wattage being precisely measured; the evaporation was directly measured by weighing the solution; and allowance was made for direct radiation from the solution and vessel.

The greatest objection to these tests, or to any that we have so far been able to devise, lies in the difficulty of maintaining a constant temperature with a constant and measurable heat supply, at a relatively low temperature such as plant tissues may experience, and also at a relatively low temperature such that the radiation factor is not a great possible source of error. Until these difficulties are overcome we can hardly say that the problem of the latent heat of vaporization as it relates to plant evaporation has been satisfactorily treated.

On the other hand, it is a fairly simple matter, at either high or low temperatures, to determine that the rate of evaporation is very materially reduced with increase in concentration when the source of heat is outside the solution. This would make it appear that there may be a problem in conductivity quite as important as, if not more important than, that relating to latent heats. The resistance to drying, shown by rather concentrated solutions such as sirup, is quite well known. As an illustration of what we mean by heating from the outside, let us take the case of two identical evaporating dishes placed over a steam bath. The steam is constantly in contact with the bottoms of the dishes. It may not, however, give up its heat unless the surface of the dish is being cooled by radiation above or evaporation of the liquid in the dish. Such an exposure can not possibly give any indication of the quantity of heat utilized in evaporating from the dish.

Under such circumstances as these the writer found the evaporation from a saturated salt solution to be less than one-twentieth as rapid as that from pure water.

Similarly, exposing a number of test tubes mainly to the heat of the air in contact with them, the rates of evaporation were found to be depressed by somewhat dilute saline and sugar solutions.

Vessels and bottles in which the contents have been heated primarily by the rays of the sun have not shown any consistent depression of the evaporation rates due to solutes.

The available facts, then, which have a bearing on the possible influence of sap concentration on the rates of evaporation from similar bodies are:

1. At any given temperature the vapor pressure over a solution decreases as the concentration increases, indicating that the solution does possess a stronger hold on the molecules than does the pure solvent and that therefore the solute may at least decrease the rate of evaporation. There is nothing in the quantities involved, however, to indicate that this might be an important factor within the limits of cell-sap concentrations.

2. Calorimetric tests on the heat required for drying cones indicate an increase in the latent heat of vaporization as the concentration of solutions in the cone cells increases. Even admitting that the large number of tests puts the facts practically beyond question, there may be here a case of adsorbed water rather than a case of solutions, and with the molecular affinities and possible latent heats of the former we are not, just at present, concerned.

3. Carefully conducted tests on free saline solutions indicate that the latent heat of vaporization is not appreciably affected by concentration or at least not more so than might be deduced from paragraph 1.

4. Observations on the heating and evaporation of solutions by low-temperature, exterior sources indicate an inability, increasing with concentration, to absorb and transmit such heat in a manner conducive to evaporation. We shall not attempt to go into the theory of this. The important fact is that the heat of the air, and sunlight so far as it is absorbed by the exterior walls of the leaf or the interior cell walls, may be relatively ineffective in producing evaporation from a concentrated as compared with a dilute solution, while, apparently, such rays as were directly absorbed within the solution would be about equally effective in all cases. It goes almost without saying that, if such absorbed heat does not produce evaporation, it must increase the temperature of the leaf until a point is reached where absorption and radiation balance.

In view of these facts, when, at the close of the transpiration tests, it was discovered that the several species showed such unaccountable and surprising differences in transpiration rate, with respect to growth or mass or leaf area, the first thought was that they must exhibit differences which could be expressed in the qualities of the solutions from which the evaporation of water takes place.[5] This thought was too hastily transformed into action by igniting the specimens which had served for the transpiration tests, in the expectation that the ash weights would be an index to the solutes in the plants and the densities of their cell solutions. This supposition was, of course, erroneous in taking no account of the soluble carbohydrates as well as some of the mineral oxids which would be lost in ignition and which comprise the greater mass of the solutes. As indicated in Table I, the ash percentages are irregularly variable and are found to bear no relation to transpiration rates.

Having destroyed the best source of information on the physical qualities of the original specimens, the next step was to obtain specimens as nearly as possible like those used in the transpiration tests. This was done by securing trees of the same classes as those taken from the nursery in the spring of 1917 which had spent the growing season in the nursery.

[5] The writer wishes to acknowledge the very helpful suggestions of the article by Barrington Moore (*13*), which was received in galley proof at such a time as to aid very materially in solving the current problem, and which reviews a number of the more recent researches on this problem.

The specimens were collected on December 3, 1917, before the ground was frozen, and when there had as yet been no drying winds. From three to eight trees of each species were taken so as to secure a considerable mass of material. The tops were cut off at the root collar and those parts only were used.

PROCEDURE IN DETERMINING SAP DENSITY

The necessary data for determining the momentary density of the sap in a plant appeared to be—

(1) The weight of the green material, determined as quickly as possible after the material was collected.

(2) The weight of the soluble matter leached out with an abundance of water and evaporated.

(3) The weight of the insoluble pulp, oven-dried.

By adding together (2) and (3) and deducting from (1), the original amount of water (and other solvents) in the plant is obtained, and this, when divided into (2), gives the sap density, usually expressed as a percentage.

In these original tests the plant material for each species was ground to a pulp, and these pulps were allowed to stand in cold water of about 10 times the pulp volume, 3 waters being used for each. Finally the pulp was all accumulated on a filter and dried with the filter paper. The aqueous solutions were evaporated at temperatures not exceeding that of boiling water. In all cases the so-called "sugars" thus secured, after becoming dry, were not wholly resoluble, indicating that colloidal matter had been included and had passed through the filters. This matter was a small proportion of the total solids, however, and may be assumed to have equally affected all samples.

RESULTS

The sap density percentages obtained for the nursery seedlings, in the first tests made, were as shown in Table XIII, in which the water requirements are again given. Figure 3 shows that the sap densities and water requirements plot a curve which is remarkably perfect, considering the changes that have been noted in relative transpiration rates during the season, and the somewhat questionable value of the water requirement for limber pine, which must be based on the performance of only one specimen.

TABLE XIII.—*Sap densities and water requirements in 1917*

Species.	Sap density in material of Dec. 3, 1917 (tops only).	Water requirements of trees in transpiration tests.
	Per cent.	*Gm.*
Limber pine	19.6	1,144
Yellow pine	21.8	1,555
Bristlecone pine	22.4	1,110
Lodgepole pine	23.2	954
Douglas fir	27.9	684
Engelmann spruce	29.5	525

At first thought it does not appear probable that the density of the spruce sap, which is only one-half greater than that of yellow pine, is sufficient to reduce the relative water loss of spruce to one-third or one-fourth of that of the pine. It is not necessary to assume that the small water loss of spruce, as related to its photosynthetic activity and amount of growth, is entirely the direct result of the physical properties of the dense sap of this species. To determine how the result may be brought about, all the following factors must be given consideration:

1. Higher sap density means less evaporation per unit of available heat.
2. Higher sap density means higher leaf temperatures before evaporation can take place at a given rate, with the possibility that in sunlight the leaf may become warmer than the air and therefore lose heat by radiation and conduction.

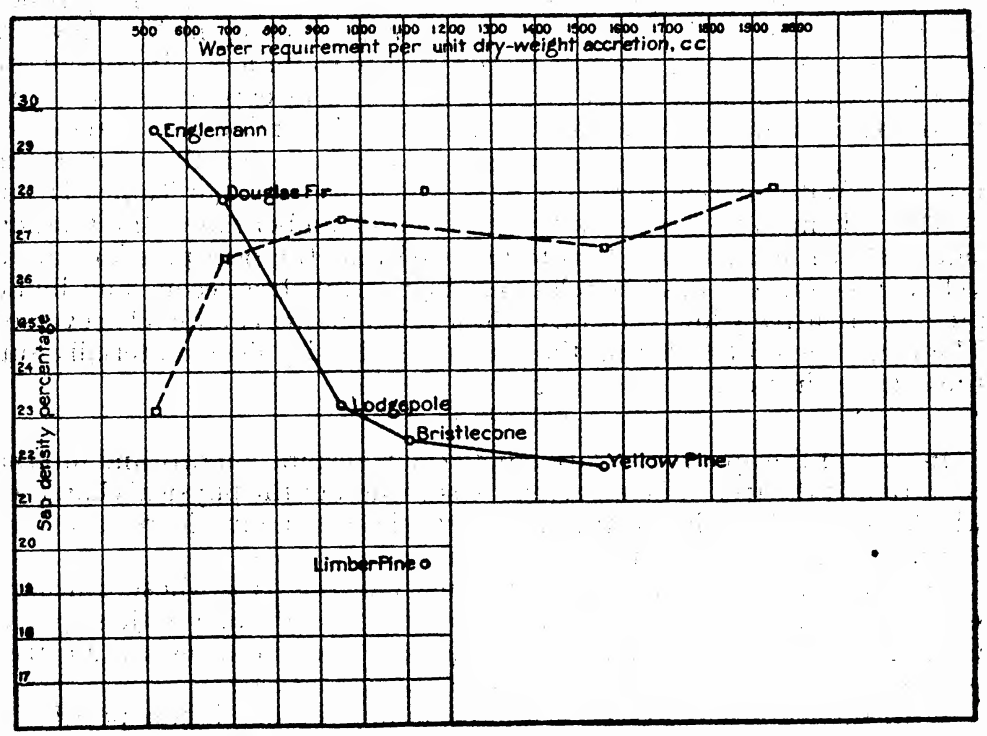

FIG. 3.—Water requirements in 1917 transpiration tests compared with sap densities on nursery specimens, December 5, 1917 (solid line) and sap densities in natural habitats, December 30, 1917 (broken line).

3. Higher sap density, by permitting higher leaf temperatures, should facilitate the photosynthetic process, thus relatively reducing the water requirement.
4. It must be equally true that greater photosynthetic activity or capacity will tend toward higher sap density as well as increased growth.

When, therefore, the question is asked, "Is the high sap density of spruce a direct cause of its low transpiration rate, or is the former merely a concomitant of greater photosynthetic activity, and is this last the really important physiological characteristic?" we are compelled to reply that the three things are so interdependent that all are equally causes and effects. It is left almost beyond question, however, that of the species we have considered the spruce represents the highest development and that this development is expressed in the highest growth rate (first columns of Table X) in the greatest current accumulation of soluble carbohydrates at the time of the December examination and in the most

effective use of water throughout the season. On the other hand, when we consider the low sap density of limber pine and its low water requirement in relation to either mass or leaf area, we obtain a suggestion that transpiration may be controlled by mechanical means rather than through the physical properties of the sap and that such control indicates a low state of development because it inevitably means the sacrifice of the absolute growth rate. Yellow and lodgepole pines, with relatively low sap densities, appear not to exert the mechanical control over transpiration and are, as a result, perhaps more fastidious as to growing conditions than limber and bristlecone pines.

While it seems important to have demonstrated that among the species of approximately equal development from the forester's standpoint, growth, photosynthetic activity, sap density, and the relative extravagance in water use are thus interrelated through simple physical control, yet the really important question is whether high or low sap density exerts a control over the more absolute water loss. In considering this it seems unquestionably best to use the leaf-exposure basis, since the maximum area exposed to the sun must determine very largely the total amount of energy which might be available for the evaporating process. Without repeating the data which are given in Tables II and XIII (omitting the slow-growing specimen of limber pine), the relationship is shown in figure 4. It is to be noted that the transpiration rate of yellow pine on this basis is relatively high, while bristlecone and limber pines are relatively low. In 1920, these relations are completely reversed. The facts leave little doubt that high sap density does materially suppress transpiration.

RESULTS IN 1920

In view of the apparent relation between transpiration rate and internal condition of the tree, it is important to see whether the physical characters which we might ascribe to the several species are in any degree constant. Let us first consider the transpiration material of 1920.

To obtain better data on the sap density of the trees whose transpiration was observed during 1920, sample trees corresponding to those potted were treated at the beginning of the season, and the transpiration trees were themselves treated at the close of the primary test.

With the freshly dug trees at the beginning of 1920 it was possible to grind and treat the whole plants in very much the same way as the tops were treated in December, 1917. Owing to the large number of lots involved, however, four sets of sample trees were merely dried, and it is necessary to deduce their approximate sap densities from other results for the same species.

In the fall examination it was considered of first importance to determine the dry weight of the trees, as a measure of growth, without the risk of losing any material or whole results through accidents. The trees were, therefore, first oven-dried. It was several months before opportunity presented itself to bring out this dried material, grind it in a mortar, make the extractions of sugars, and again dry the leached pulp. In this case no attempt was made to evaporate and weigh the sugars. This method is probably open to the criticism that a longer period is required to secure the same degree of leaching of solutes that may be expected with green material, and also that the drying of the material probably coagulates and holds some collodial matter that would, from green material, pass off with the solutes. This may account in part for the relatively low sap densities found at the end of 1920.

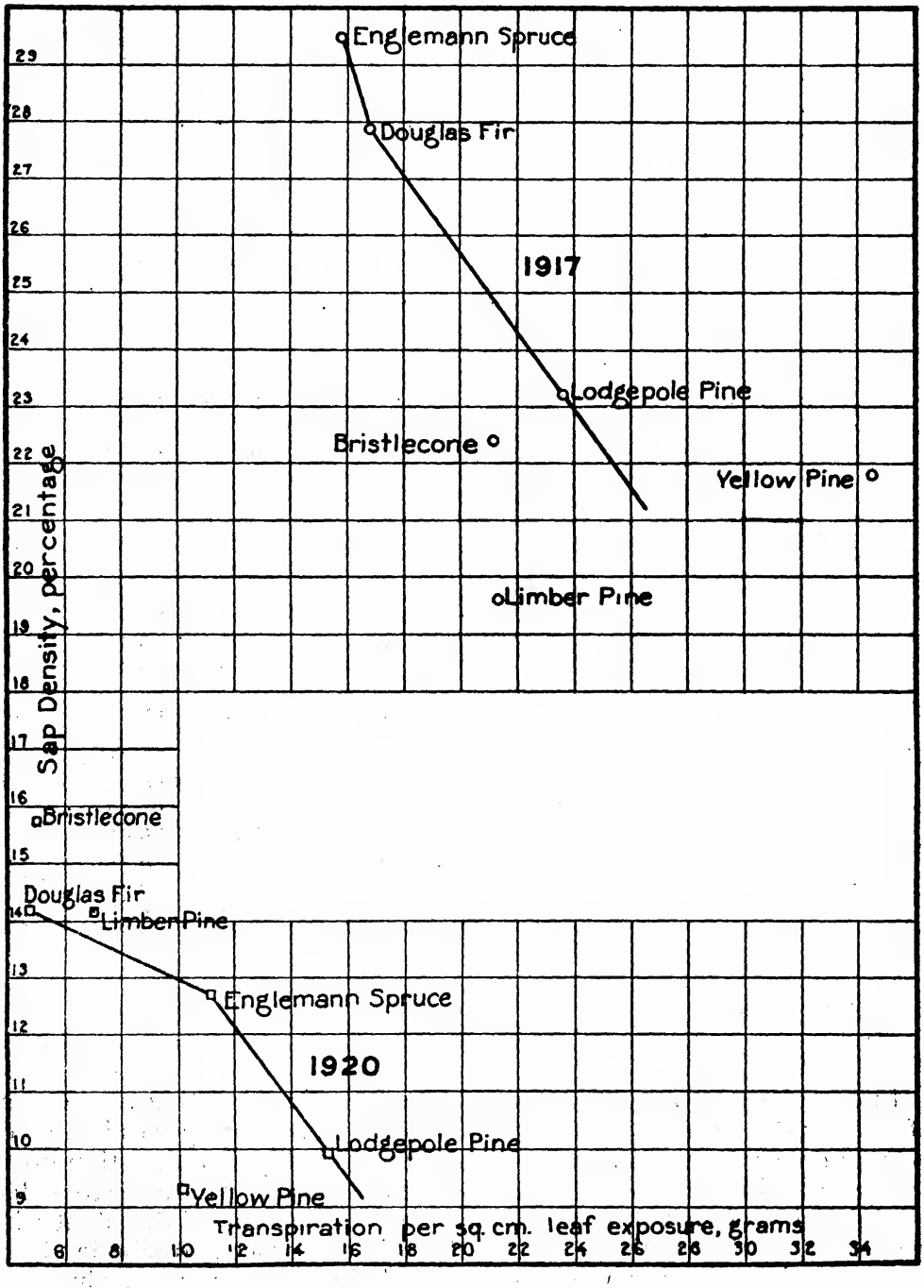

FIG. 4.—Relation between sap densities and transpiration rates on basis of leaf exposures.

The results of the two determinations are shown in Table XIV.

TABLE XIV.—*Sap density of trees in 1920 transpiration tests (whole plants)*

Species.	Pot No.	Source.	Sap density at—		Seasonal change.
			Beginning of season.	End of season.	
			Per cent.	*Per cent.*	
Yellow pine	22	Harney	13. 15	16. 18	
	1	do	[a] 13. 15	15. 59	
	18	Bitterroot	[b] 13. 50	13. 01	
	15	Tusayan	12. 71	9. 30	
Average			13. 13	13. 52	+0. 39
Douglas fir	17	Hayden	15. 24	15. 63	
	10	Leadville	14. 78	13. 25	
	7	Pike	[b] 15. 50	13. 71	
Average			15. 17	14. 20	−. 97
Lodgepole pine	3	Colorado	14. 72	10. 60	
	23	Washakie	[b] 14. 65	8. 80	
	24	Gunnison	14. 49	10. 38	
Average			14. 55	9. 93	−4. 62
Engelmann spruce	9	San Isabel	13. 70	Lost.	
	19	Montezuma	[b] 13. 65	12. 84	
	12	San Isabel	9. 91	12. 93	
	11	Unknown	13. 50	12. 32	
Average			12. 69	12. 70	+. 01
Limber pine	16	Unknown	16. 05	13. 02	
	21	do	[a] 16. 05	[c] 15. 36	−1. 86
Bristlecone pine	8	do	15. 75	16. 29	
	4	do	[a] 15. 75	15. 68	+. 23
Scotch pine	2	Russia	10. 43	10. 80	
	13	do	[a] 10. 43	12. 65	+1. 29
Siberian larch	6	do	8. 54	13. 75	
	20	do	[a] 8. 54	[d] 15. 05	+5. 86

[a] One test only for this class of material.
[b] Sugar not extracted. Sap density estimated from green-dry ratio.
[c] The specimen of high sap density is the one that made practically no growth.
[d] These sap densities determined after foliage was nearly all air-dry. Making allowance for this the sap densities should be about 2 per cent less.

In considering Table XIV it should be borne in mind that at the time of potting the trees for transpiration study some of the species had made very appreciable growth while others had probably felt the stimulus of spring very little. It is perfectly apparent from other data secured that the initiation of growth in the spring means a flooding of the plant with water. Thus the relatively low sap densities of spruce and Siberian larch are expressive of their response to relatively low temperatures, while that of yellow pine is more indicative of a low condition throughout the winter.

In view of the fact that the stage of the seasonal advance had affected the several species differently, it is questionable whether the spring data, or the changes throughout the season, have any great value in our present comparisons.

On the other hand, if the sap densities in the fall may be taken as indicative of conditions existing during most of the season, certain striking facts are in evidence. It has been mentioned that the abundant moisture supplied the trees in the spring, and the relatively dry condition later on, may have induced the production of a great deal of new tissue which the trees were not, later on, able to supply with adequate building material. This would seem most markedly the case with all the lodgepoles and with the Tusayan yellow pine whose growth was so vigorous. It is believed, therefore, that the evidence is fairly convincing that in 1920 either the moisture was not sufficiently available to permit effective photosynthesis in some of the species or that the sunlight and temperatures were below par in effectiveness. Possibly it is a combination of these things which left sap densities very low at the end of the season. Still, in comparing the absolute values with those of 1917, it should be remembered the earlier values refer only to the tree tops.

These sap densities are found to bear a broad relationship to the water requirements of the several species, though this is not so well defined as in 1917, probably because of the seasonal changes in water supply and the less favorable light conditions. Comparing the mean post-season sap densities with the transpiration per unit of leaf exposure, however, and again omitting the questionable limber pine, we have the data in Table XV, which have already been illustrated in figure 4.

TABLE XV.—*Transpiration and sap densities in 1920*

Species.	Transpiration per square centimeter leaf exposure.	Mean post-season sap density.
	Gm.	*Per cent.*
Lodgepole pine	15.27	9.93
Engelmann spruce	11.12	12.70
Yellow pine	10.08	9.30
Scotch pine	8.45	11.72
Siberian larch	8.00	14.40
Limber pine	7.00	14.19
Bristlecone pine	5.00	15.78
Douglas fir	4.63	14.20

It is again evident in the 1920 results that the effectiveness of sunlight in producing evaporation from leaves must be very considerably affected by the density of the sap involved. If we were to balance the variations in one season against those in the other, it is readily seen that the relationship would be almost perfect. To what extent these variations may be due to error in determining either sap density or leaf exposure must remain a question until a great deal more material has been examined. It does seem certain, however, that the relative positions of the species, in regard to either sap density or transpiration rate, are by no means constant. The best that can be done at present is to accept average values for each species, as has been already done in considering the transpiration independently. The explanations, already made, of variations in growth in the two seasons should be considered in connection with the variations in sap density.

STABILITY OF SAP DENSITIES

It has already been indicated that the sap density of a given species is by no means constant. In order that progress may be made in the use of the sap density measure, or the osmotic pressure determination, as an indicator of the relations between plant and environment, it is extremely important to realize (1) that the tree may pass annually through a definite cycle of changes and (2) that the current sap density may be quite largely influenced by current atmospheric conditions as well as water supply. If the tree were always able to supply as much water as was demanded by the losses at its leaves, then only a cyclic change would be apparent, dependent on cell division and photosynthesis, or primarily on temperature and light conditions. It might be said, therefore, that the species which shows the least fluctuation with current atmospheric conditions is the one best able to supply any demand for water, and it would seem that this species would best endure a long drought in the soil.

As to the cyclic change in sap density, it would seem that the following describe the general conditions of the seasons which bring it about:

1. In the spring we have rising temperatures and increasing duration and intensity of sunlight, which alone would increase the osmotic pressure in favor of the plant as against the soil. Coupled with this there is usually an abundant supply of moisture in the soil immediately after its thawing, often augmented by the melting of large masses of snow and by heavy rains. These conditions cause a heavy inrush of water and, because the atmospheric demands are at this season very moderate, an extreme turgescence of the tissues already formed becomes not only possible but unavoidable. It is believed this turgescence is the foundation for cell expansion and division, from which the new tissues arise.

2. With the advance of spring and advent of summer the moisture supply of the soil is usually much reduced, but even if this were not the case the formation of new tissues might be checked because of the very great increase in transpiration, due both to greater atmospheric demands and water losses from the new succulent tissues already formed. In the conifers, outward evidences of growth usually disappear abruptly early in the summer. The period of warmest weather, then, is not one for the formation of new tissues, but for the creation of the materials with which those already formed may be built up and solidified. Fruiting, of course, also demands some of these materials. The sap density should, therefore, increase from the moment that cell division becomes negligible.

3. The autumn season in temperate climates is almost universally the least favorable as regards current precipitation, and at this season the soil water is most likely to have been completely exhausted. For this reason the amount that can be supplied is often inadequate for all the transpiring members, and we witness the drying and falling of all deciduous leaves and of the oldest ones on the evergreens. Unless the water supply in the leaves becomes so low as to prevent the chemical processes, photosynthesis in the evergreens is not checked, and sap densities may be expected to reach their highest points, partly because the actual amount of water present is less than at any other season.

4. While under some circumstances the winter season may be one of almost complete dormancy, with photosynthesis stopped by low temper-

atures and little light and the movement of water stopped by freezing of the soil, such a condition does not describe the winters of the region in which we are particularly interested in this study. Here the winter days are often warm and bright enough to permit possibly some photosynthesis in the evergreens and certainly very considerable transpiration. Such days may be followed by severe cold of sufficient duration to freeze the tree and the soil to a depth of 1 or 2 feet. These cold waves are not uncommonly followed by warm winds which immediately thaw the foliage and may make great demands on its moisture before the tree stems and the soil thaw out enough to provide a new supply. Thus, in extreme cases great injury may be done, and in the usual weather cycles the tree is subjected to very marked changes in water supply and in the sap density of its foliage. At this season the sap density, it will be readily seen, may depend very greatly on the exposure of the tree, since the primary factor in drying is wind. A tree growing in a dense stand with a northern exposure passes through much more moderate changes than one in an open stand where both sunshine and wind may have full effect.

Sap Density in Period of Vernation

We have already pointed out, in connection with Table XIV, the possible bearing of different responses to temperature on the comparative sap densities in the spring of 1920. In Table XV are given additional data which, with the explanatory notes, make a very clear case. It is not surprising to find that a control test made with nursery material collected June 1, 1918, when growth of most species had begun, shows complete disarrangement of the December, 1917, values.

The material was collected as before, complete aerial portions being taken. These were placed immediately in stoppered bottles and were exposed each to the others' vapor until June 18, in order that from the losses or absorptions some idea as to relative osmotic pressures might be gained. The exposure was not long enough to give more than an indication. After this period the material was dried in the bottles, then leached and redried. Spruce specimens were inadvertently omitted from this collection.

TABLE XVI.—*Sap densities of nursery specimens at beginning of growing season, 1918 (tops only)*

Species.	Age.	Number of tests.	Average sap density.	Relative osmotic pressure indicated by vapor transfers.
	Years.		*Per cent.*	*Per cent.*
Limber pine	7	2	15. 54±1. 37	0. 978
Yellow pine	2–6	2	15. 10±0. 55	. 998
Bristlecone pine [a]	7	1	20. 37	. 980
Lodgepole pine	4	2	12. 22±0. 53	. 979
Douglas fir	6	2	16. 91±0. 41	. 969
Siberian larch [b]	4	2	10. 61±0. 98	. 972
Western white pine [a]	3	1	20. 33	1. 014
Scotch pine	3	1	13. 48	. 984
Aspen (new leaves)	0	1	14. 18	1. 012

[a] Buds not yet opened.
[b] One specimen with and the other without roots. The former showed the lower density.

The data indicate that the beginning of new growth had permitted the essential equalizing of sap densities, except with bristlecone pine and western white pine. It is difficult to see why the density for bristlecone pine should have remained high without giving it, apparently, a high osmotic pressure. The very thin sap of Siberian larch is accounted for by the advancement of its growth, which always begins earlier than that of any of the native species.

TABLE XVII.—*Sap density of trees in natural habitats after drying weather, December, 30, 31, 1918*

Species.	Site where collected.	Sap density.
		Per cent.
Limber pine	2 southwest slope	28. 67
	6 northwest slope	29. 86
	6 northwest slope	26. 42
	12 ridge	27. 08
	9 north slope	28. 39
	All	28. 08±0. 45
Yellow pine	2 southwest slope	[a]25. 64
	2 southwest slope	[a]29. 52
	6 northwest slope	27. 46
	4 east slope	25. 30
	12 ridge	25. 84
	All	26. 75±0. 59
Lodgepole pine	1 south slope	27. 24
	8 ridge	27. 61
	All	27. 42±0. 16
Douglas fir	2 southwest slope	27. 35
	4 east slope	26. 40
	12 ridge	28. 10
	9 north slope	24. 44
	All	26. 57±0. 56
Engelmann spruce	3 northeast slope	24. 38
	5 bottom	22. 78
	5 bottom	22. 16
	All	23. 11±0. 51

[a] On the morning of Feb. 23, 1918, corresponding specimens showed 21.05 per cent and 25.12 per cent, respectively, for these two trees. The specimen of lower sap density in each case was from a young, vigorous tree; that of higher density from a low limb of an old tree badly infested with mistletoe.

On the whole, while the determinations of osmotic pressure are not complete, it is indicated that they were essentially the same when the sap densities were nearly the same. We may, therefore, feel safe in assuming that for other conditions relative osmotic pressures will be about proportionate to sap densities, which might not be the case if the solutes of different species were materially different in composition and molecular weights. A boiling-point test made with accumulated solutes from all the species shows that an osmotic pressure of about 19 atmospheres may be expected when the sap density is 20 per cent. Freezing-point deter-

minations have also shown that with all of our conifers a sap density of 1 per cent is approximately equivalent to 1 atmosphere of osmotic pressure, this relation holding at least up to 20 per cent. Such tests have not, as yet, been sufficient to bring out any consistent differences in the saps of different species.

Winter Sap Densities in Natural Habitats

Attention may now be turned to determinations of the sap density of trees growing in their natural habitats, as made at the end of December, 1917. The foliage specimens were collected on the afternoons of December 30 and 31, both days being warm and the soil not yet frozen in any instance at a depth of a foot. The preceding week had been warm and dry, with a high evaporation rate for that season. Hence it may be expected that the results will show the influences of different exposures.

All material was from limbs at a height of about 4½ feet above the ground.

In this, as in all the following cases where only foliage is sampled, the outer half or two-thirds of the needles was clipped off with shears in sections about one-half inch long. This material was leached before drying and was otherwise treated as the ground pulps had been.

When these results are compared with those obtained from nursery stock on December 3 (fig. 3), it is seen that a very great but regular difference in the value exists. The average sap density of limber pine has increased 8 per cent, of yellow pine 5 per cent, of lodgepole 4 per cent; that of Douglas fir has decreased 1 per cent and that of spruce 6 per cent. These changes form almost a straight line when plotted with the original sap densities as abscissae.

This shows that sap densities in a given species are subject to great variations, but it does not mean that they have no significance. The differences between these field specimens and the nursery trees growing under uniform conditions reflect the fact that the pines had lately been subjected to the strongest drying influences, while the fir in part, and spruce wholly, had recently been protected from any severe drying. Also, owing to the protection afforded the latter species at all times, they had probably never had the benefit of full light and, therefore, may not have accumulated as large a supply of carbohydrates as the pines growing in the open.

To assume from this that spruce or fir is not subjected in the field to drying stresses equal to those experienced by the pines, or that the former would not tolerate great stresses as well as the latter, is altogether erroneous. These tests were made before the soil was frozen and before the winter exposure had had opportunity to bring about any degree of equilibrium between different sites. It is greatly to be regretted that this series of specimens could not have been duplicated late in the winter.

Winter Sap Densities Near Timber Line

On the other hand, specimens collected at high elevations, on January 1, 1918, only a day or two later, tell a very different story, for here the soil was already deeply frozen, and the exposure to evaporation had been very severe for the preceding six days.

These results and others which show changes with season, weather, and soil conditions are given in Table XVIII.

TABLE XVIII.—*Sap densities in exposed situations, at high elevations, 1918*

Species.	Location.	Sap densities.			
		Jan. 1.	Feb. 6.	May 7.	May 18.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Engelmann spruce..	Station F–16.....	[a]37. 30	31. 29	[b]33. 60	22. 16
Bristlecone pine.....	.....do.....	30. 75	28. 68	[c]19. 71	20. 84
Limber pine.....	Station F–13.....	31. 00	24. 00	.....	.....
Bristlecone pine.....	Cabin Creek.....	30. 56	30. 37	[d]21. 70	21. 50
Engelmann spruce..	Gulch near F–13.....	.....	23. 66	.....	19. 02
Alpine fir.....	.....do.....	.....	21. 45	.....	20. 62
Limber pine.....	Cabin Creek.....	.....	.....	.....	21. 07
Engelmann spruce..	Cabin Creek, edge of water.	.....	.....	.....	24. 51

[a] Similar specimen obtained Jan. 15, 1921, from a very exposed site showed 33.5 atmospheres of osmotic pressure by freezing-point method with 27.7 per cent sap density.
[b] Twigs from which needles were clipped showed at same time 19.95 per cent.
[c] Twigs from which needles were clipped showed at same time 15.84 per cent.
[d] Twigs from which needles were clipped showed at same time 15.19 per cent.

At Station F–16 there is at all times a contrast between the spruce and bristlecone pine specimens, until May 18, when thawing had become general. This is probably not altogether a specific difference but is due in part to the fact that the spruce was located in a hollow which collected snow and did not permit even temporary thawing of the soil until very late, while the bristlecone was on high ground only a few feet distant, from which the snow is usually swept away, and which might, therefore, thaw in a brief period.

It may be a very significant fact that although the soil temperatures at Station F–16 on February 6 were 21.5° F. at 1 foot, and 23.6° at 4 feet (as against 27.0° and 30.5° on January 1), and although there had probably been no thawing whatever during January, the sap densities of both spruce and bristlecone pine decreased during the month. The evaporation rate from February 1 to 6 was only about one-fifth as great as from December 26 to January 1, meaning, of course, much less current desiccation in the later period. But it is also indicated that at some time between January 1 and February 6 the leaves must have obtained moisture from some source. This might have been by transfer from the stems, if thawing of aerial parts occurred; but there is no apparent reason why the leaves should not have taken up vapor from the atmosphere during periods when the vapor of the atmosphere was practically saturated. The possibilities of such absorption, as a relief for winter drought conditions, are too important to be overlooked.

The material collected May 7 showed in all cases much lower sap density in the twigs than in the more exposed half of the needles, the latter being selected because logically subject to the greatest drying. This indicates that small variations in the results may be expected from clipping slightly more or less than half of the needles.

All specimens showed a decided drop in densities on May 7, by which time there was a great deal of surface thawing, except, as explained, around the roots of the spruce at Station F–16. This tree responded in the next period.

The spruce and alpine fir obtained from a protected stand in a gulch near F–13 both on February 6 and May 18 show plainly the advantages

of protection. The high density of the last spruce in the test, on May 18, is difficult to explain, in view of the exposure on a southwesterly bank and the apparent abundance of moisture. This may be due, however, both to the full exposure of the tree to light and to a possible high degree of nonavailability of the moisture as a result of acidity and lack of aeration.

Summarizing, it is evident that the sap density of any species or individual is not a stable quality but varies according to the amount of photosynthesis which is permitted and according to current conditions affecting water supply and transpiration. Nevertheless, there is found in these data no reason for changing the original conclusion that, given equal opportunities and exposures, the sap density of spruce will be higher than that of any of the other species; that spruce will tolerate a very great loss of moisture, and a resultant high sap density in the needles, without injury; and that it can, therefore, be said that spruce is not only better equipped to resist transpiration, other things being equal, but that the ability to resist transpiration and its possibly injurious effects makes spruce in reality the least moisture-demanding of all of the Central Rocky Mountain trees.

These conclusions, however tentative, must at least develop a wariness to accept average or temporary moisture conditions of the site as prima facie evidence of the relative moisture requirements of the species occupying it.

On the other hand, we have as yet no evidence that spruce is more drought-resistant than other species or that the sap density and the specific qualities that affect it react upon distribution through the water requirements. All that we have so far been able to show is that low sap density permits a species to occupy warm sites where the exposure is very great most of the time, while high sap density appears to hold the species to cool sites, where the winter drought may be severe, especially at high altitudes.

WILTING COEFFICIENTS FOR DIFFERENT SOILS AND SPECIES

If it could be shown that one species is capable of extracting the moisture of the soil to a lower point than other species before wilting or other injury to the plant was apparent, this would constitute direct evidence that the first species not only was less likely to experience fatal drought conditions but also was capable of sustaining higher internal osmotic pressures without injury to the protoplasm. When wilting occurs, if the condition has been approached gradually, it may be assumed that the osmotic pressure in the plant is essentially the same as in the soil, and the latter, of course, increases as the moisture content decreases and the concentration of the soil solution increases.

It has already been indicated in connection with transpiration in 1920 that spruce appeared to be able, under all conditions, to obtain the water required for free transpiration more nearly than any of the pines or Douglas fir. A similar test with the moisture gradually reduced to complete nonavailability would, perhaps, be preferable to wilting tests, which must be conducted with seedlings in order that the end-point may be observed ocularly. It is fairly evident that the seedlings may not show development of the internal characters which are important in this connection to the same extent as older trees. However, it can not be gainsaid that it is the seedlings which are subjected to the greatest dan-

gers, and it is their performance, rather than that of older trees, which determines the composition of forest types.

Although only a few wilting tests have been made in which the several species have been observed growing in the same soil, a considerable amount of information has been obtained on each species in a variety of soils; and by reference to the physical properties of these soils we may obtain fairly satisfactory comparisons. In each case, the species used was that one which occupied the given soil or predominated in the type in the field.

PROCEDURE

In general the intention has been to secure the wilting coefficient for the soil as found in the field—that is, with the normal admixture of rocks and gravel, since the saturation and capillary capacities and other physical measurements were on this basis. In this respect mountain soils present difficulties ordinarily not met with in agricultural work.

To attain this end it is not sufficient to sample the soil for moisture content after the seedlings have wilted. The mositure content must be determined for a mass of soil large enough to represent normal proportions of rock and finer material.

Pans about 10 inches square were used in the earliest tests, the soil being in a layer from 1 to 1½ inches deep. These were sometimes found to be too shallow to accommodate the rocks which should be included; consequently a standard round pan was specially made, having a diameter of 7 inches, a depth of 3 inches, a soil depth of 2½ inches, and an ordinary soil weight of about 4 pounds. A few holes were punched in the bottom of each pan to prevent excessive wetness and to aid aeration, it being the belief that with the pans on a bench the evaporation rate through these small holes could never be an important factor. In fact, though the soil surfaces have usually been paraffined, it has never been attempted to make the coatings air-tight, since the object is not to prevent water loss from the soils but to insure that when wilting occurs the moisture distribution throughout the soil shall be fairly uniform.

The vegetation has been secured by sowing seeds of the desired species in the pan of soil, watering these moderately, and permitting the seedlings to develop for about a month before coating the pans and cutting off the moisture supply. Beyond the age of a month the seedlings may rapidly lignify, so that the wilting does not occur promptly or is very difficult to detect. This is true of Douglas fir seedlings at any age. It is, of course, realized that seedlings of this age may not exercise the same control over moisture as would older trees.

The soil sample is placed in the pan in an air-dry condition and is oven-dried to determine its net weight. This practice may have had some effect on the colloids but is fully justified by the assurance it gives that micro-organisms will be eliminated and will not cause the untimely death of the seedlings. However, as mountain soils are rarely strong in clay, as the samples have always been air-dried first, and as the oven temperature has been only 92° C., it is thought any change in soil qualities may virtually be ignored.

After drying, a cupful of soil is taken from the pan, a weighed lot of seeds is strewn over the smooth surface of the remaining soil, and the cupful is then used to cover the seeds.

The moisture applied to induce germination and development of the seedlings has usually been left wholly to judgment, the intention being to give all that can be used and never to permit the surface to become dry.

The seedlings have been developed with abundant sunlight, in the greenhouse, avoiding excessive temperatures as far as possible. To make a satisfactory test each pan should develop at least 100 seedlings.

When the final weight of a pan is secured, with the seedlings wilted, deductions are made for the known weight of paraffin applied, as also for the weight of the seed used, which is assumed to be the same as that of the wilted seedlings and the loose hulls. This weight could generally be ignored without affecting the result appreciably, for the moisture content is usually 60 or 80 gm., as against 1 to 5 gm. for the seed of any species except yellow pine.

DIRECT COMPARISONS OF THE SPECIES

Not until 1920 was it possible to conduct special tests with two or more species in the same soil. The most comprehensive test, and therefore the least likely to be misleading, was conducted from April to September, 1920. In this case the soils were not sterilized by oven-drying, and considerable damping off of the seedlings occurred, which may be confused with legitimate wilting in the early stages. The pans were watered and weighed daily, and the losses of seedlings were recorded, so that it is possible to consider the losses at any stage. Because of the damping off, and also to make these results more comparable with those in which but one wilting period was recorded—that is, the time when practically the entire number collapsed—it seems best to consider in all these more recent tests the mean wilting point for the last 25 per cent of the total number of seedlings observed. Not infrequently the weakest seedlings die with twice as much water available as is required to sustain the strongest.

In this particular test the moisture equivalents of the five soils were determined first, under a force of 100 gravity; and, assuming that these quantities were indicative of the same degree of availability in each soil, the watering in each case was so gauged as to maintain this moisture equivalent. It may be remarked that this quantity was very favorable for germination and establishment. Later the water content of each pan was reduced to two-thirds of the moisture equivalent, finally to one-third, and from that point downward by 5 gm. stages. This is important because other tests indicate that the drought which a seedling will tolerate depends much on the moisture to which it has become accustomed.

The soils in this case were not paraffined, but some water was given almost every day in order to eliminate, so far as possible, extreme drying-out of the surface. It is significant that, perhaps on this account, the wilting coefficients are relatively lower than usual.

The results of this test, which have already been given in the research manual (*4, pt. 1*) to illustrate the relation between wilting coefficient and moisture equivalent, are given in slightly different form in Table XIX.

We shall not discuss the rather variable relations of these wilting coefficients to the physical measures of moisture-holding properties of the soils. Suffice it to say that other evidence points to the fact that the several wilting coefficients represent an osmotic constant in the different soils, while either the capillary moisture or moisture equivalents fall considerably short of this. This relationship will be discussed in connection with the field-moisture problem. The physical measures of soil moisture bear only a general relation to wilting coefficients and must be used with this understanding.[6]

[6] The reader is urged to note the discussion of this by Bates and Zon (*4*) where it is made plain that a constant ratio between wilting coefficient and moisture equivalent is impossible if a wide variety of soil types is considered.

The important thing shown by Table XIX is that the wilting coefficients of yellow pine, Douglas fir, and Engelmann spruce are essentially the same in all the soils, while that for lodgepole pine is much higher. There is, moreover, no evident reason for the fact that in some of the soils (sandstone and prairie shale) the wilting coefficient for spruce is lower than for either pine or Douglas fir. We must, at least at this stage, regard these variations as accidental.[7]

It will now be well worth while to determine whether, as between any two of these apparently equal species, a greater number of results brings out any difference. A very considerable amount of data has been secured on Douglas fir and spruce growing in the same soils. In introducing these data it is desirable to point out:

1. That ocular observations on wilting, especially when the moisture supply is steadily declining, tend to favor Douglas fir, because that species has a much stronger and more fibrous stem and rarely collapses. The evidence of wilting is, therefore, much less plain than in the frail spruce seedling, and, it seems likely, may not be obtained until a day or two after the fatal condition has first existed.

TABLE XIX.—*Wilting coefficients of the four important species in 5 types of soil*

Kind of soil.	Capillary moisture.	Moisture equivalent 100 gravity.	Mean wilting coefficients, best 25 per cent of the seedlings.				
			Yellow pine.	Lodgepole pine.	Douglas fir.	Engelmann spruce.	Average of four.
Granitic gravel sandy loam	*Per cent.* 26.58	*Per cent.* 10.55	*Per cent.* 2.28	*Per cent.* 2.42	*Per cent.* 2.16	*Per cent.* 2.26	*Per cent.* 2.28
Composite limestone loam	31.85	22.00	3.23	4.12	3.56	3.50	3.60
Composite sandstone loam	35.34	21.77	4.10	5.29	4.30	3.93	4.40
Prairie silt loam from shale	37.77	28.79	7.80	8.69	7.79	7.43	7.93
Composite lava silt loam	43.16	27.80	4.52	6.00	4.97	4.87	5.09
Average	.....	.....	4.39	5.30	4.56	4.40	4.66

2. That in the early stages of development Douglas fir roots more strongly than spruce and its roots reach a greater soil area, but particularly in these pan tests they reach the deepest layer of soil which may not be drawn on at all by the spruce if the wilting is accomplished at an early age. (See Plate 7, B.)

3. Therefore, in these tests it is evident that if spruce seedlings tolerate as great a degree of drought as fir, the moisture being known only through the whole pan weight, it must be through greater ability to extract water from the soil.

From Table XX it will be evident that the wilting coefficient for spruce is, on the whole, higher than that for Douglas fir. The difference is only about 3 per cent of the value for fir. Of the 23 cases cited, only 6 give spruce a lower value than fir, and 5 of these 6 are among the loose gravels

[7] Since the foregoing statement was written very convincing results have become available showing the different effects of each of these soils in stimulating the growth of each species, both according to chemical composition of the soil and the concentration of its solution. It can hardly be questioned that this has a direct bearing on the behavior of the seedlings as the wilting point is approached, and in fact that this entire problem is quite as much one of chemical relations as of the physical relations which have been discussed in this paper. It is hoped that something may be published on this chemical phase in the near future.

or sands of granitic origin, while only 1 is found in the more loamy soils. This is at least suggestive that in the soils of freer capillary movement the very meager root system of spruce is not so great a disadvantage. From these facts we certainly can not draw the conclusion that in the osmotic sense spruce has any less control over soil moisture than fir.

INDIRECT COMPARISONS OF THE SPECIES

A very considerable amount of information has been secured in the somewhat routine process of determining the wilting coefficients for a large number of soils of almost every possible origin in connection with nearly every study in which soil quality or soil moisture has been an important factor. For the most part the wilting coefficients have been determined for each soil only with respect to one species, that one being the species which characterized the soil or site in the field. It is obviously necessary, before these results may be used for a comparison of the species, that each result should be related to some other measure of the moisture-holding properties of the soils, and the best measure at present available for any considerable number of the soils is the moisture equivalent at 100 gravity. As we have seen in Table XX, however, even this does not bear a constant relation to wilting coefficients, when radically different types of soil are considered. Particularly does it seem that the coarse-grained granitic soils of the Pikes Peak region, which we have studied more than any other, have an unusually weak hold on the water until the amount is brought close to the wilting coefficient, so that the moisture equivalents of these soils are relatively low.

TABLE XX.—*Comparative wilting coefficients of spruce and Douglas fir in the same soils*

Kind of soil.	Moisture equivalent.	Wilting coefficient.		Ratio of wilting coefficient to moisture equivalent.	
		Fir.	Spruce.	Fir.	Spruce.
	Per cent.	*Per cent.*	*Per cent.*		
Granitic gravels over 50 per cent rocks and coarse gravel, less than 20 per cent silt and clay.	3.53	1.30	1.39	0.368	0.394
	4.35	1.73	1.94	.398	.446
	4.86	2.05	2.03	.422	.418
	5.03	1.77	1.80	.352	.358
	5.04	2.14	2.23	.424	.442
	5.06	2.54	2.34	.502	.462
	5.19	2.01	1.87	.387	.360
	5.57	1.94	1.71	.348	.307
	5.62	2.13	2.61	.379	.464
Granitic sandy loam	11.05	2.94	2.91	.266	.263
Granitic spruce soils, varying from rocky coarse sand to silt loam.	11.68	2.72	2.88	.233	.246
	11.72	2.53	2.79	.216	.238
	14.45	3.42	4.09	.239	.283
	19.95	6.41	6.44	.321	.323
	20.32	5.60	5.78	.276	.284
	22.02	5.25	5.61	.238	.255
	26.30	8.34	8.17	.317	.311
	29.84	7.44	7.98	.249	.268
	42.72	17.09	17.40	.400	.407
	73.50	17.53	18.56	.238	.253
Quartz latite rocky sandy loams	11.96	5.13	5.20	.429	.435
	13.98	6.96	7.14	.498	.510
	14.85	5.72	6.04	.385	.407
Average of all	.....	.....	.....	.3427	.3537
Mean difference	.....	.....	.....	.....	.0110
Probable error in mean difference	.....	.....	.....	.....	.0029

TABLE XXI.—*Miscellaneous wilting coefficients.*

YELLOW PINE

Sample No.	Station or forest.	Origin and character of soil.	Moisture equivalent.	Wilting coefficient.	Ratio of wilting coefficient to moisture equivalent.	Conditions of test.
			Per cent.	*Per cent.*		
326	Nebraska	Aeolian ridge sand	3.72	0.54	0.145	Without paraffin.
325	do	Aeolian bottom very fine sand	12.36	2.51	.203	Do.
70	Fremont F–2	Granite gravel	5.41	1.16	.214	
71	do	do	5.91	2.63	.445	
72	do	do	5.86	2.67	.456	
73	do	Granits sand	9.29	3.67	.395	
78	Fremont F–4	Granite gravel	5.20	1.79	.344	
79	do	do	4.69	1.28	.273	
80	do	do	3.70	1.43	.387	
26	Fremont F–6	do	6.73	2.44	.363	
29	do	do	5.24	2.65	.506	
25	do	do	4.48	1.85	.413	
121	Fremont F–12	do	5.87	1.99	.339	
132	do	do	4.62	1.97	.427	
130	Pike M–1	Granite coarse sand	8.91	2.36	.265	Do.
124	do	do	6.61	1.53	.232	Do.
117	do	do	6.76	1.68	.249	Do.
53	Black Hills	Limestone silt loam	30.55	15.33	.502	Do.
55	do	Sandstone loam	16.02	2.58	.161	Do.
62	do	Sandstone silt loam	22.30	6.41	.287	Do.
63	do	Schist loam	19.06	6.26	.328	Do.
122	Colorado	Sandstone fine sandy loam	16.96	9.97	.587	
127	Wagon wheel Gap.	Quartz latite loam	21.95	3.77	.172	
101	Cache	Volcanic ash, etc	28.23	12.57	.445	Do.
102	do	Silt loam	28.05	9.89	.342	Do.
103	do	do	29.66	10.14	.342	Do.
104	do	do	27.27	10.40	.382	Do.
632	Wagon wheel Gap.	Quartz latite sandy loam	18.43	7.50	.407	
	Fremont	Granitic sandy loam	10.42	4.09	.392	

LODGEPOLE PINE

1	Arapaho	Granitic loam	17.03	7.34	0.431	
2	do	Granitic sandy loam	11.32	3.02	.267	
3	do	Granitic fine sandy loam	16.75	3.50	.209	
4	do	do	7.63	2.75	.360	
5	do	Granitic coarse sand	7.75	5.70	.736	
6	do	Transported fine sandy loam	13.65	2.96	.217	
262	Medicine Bow	Gneiss coarse sand	13.45	4.72	.351	Without paraffin.
263	do	do	16.25	3.65	.224	Do.
264	do	do	11.62	2.99	.257	Do.
265	do	do	7.62	.76	.100	Do.
533	do	Gneiss fine sandy loam	12.90	4.69	.364	
540	do	do	13.78	5.22	.379	
541	do	do	14.75	4.75	.322	
558	do	do	13.76	5.61	.408	
559	do	Gneiss fine sand	10.22	4.00	.399	
127	Wagon wheel Gap.	Quartz latite loam	21.95	4.41	.201	
71	Leadville	Sandstone silt loam	21.34	1.90	.089	Do.
12	do	Sandstone fine sandy loam	12.35	2.52	.204	
57	Fremont F–10	Granitic sandy loam	9.27	3.55	.383	
48	Colorado	do	10.39	3.34	.322	

DOUGLAS FIR

8	Arapaho	Igneous fine sandy loam	13.31	4.12	0.309	
66	Fremont F–1	Granite gravel	4.29	2.31	.538	
67	do	do	3.60	.93	.258	
68	do	do	4.34	.76	.175	
69	do	Granite sand	8.25	2.13	.258	
17	Fremont F–7	Granite gravel	4.71	1.26	.268	
19	do	do	4.94	1.24	.251	
18	Fremont F–9	do	5.03	.96	.191	
127	Wagon Wheel Gap.	Quartz latite loam	21.95	5.11	.233	Without paraffin.
533	Medicine Bow	Gneiss fine sandy loam	12.90	4.04	.313	
540	do	do	13.78	4.88	.354	
541	do	do	14.75	4.44	.301	
558	do	do	13.76	5.50	.400	
559	do	Gneiss fine sand	10.22	3.76	.368	

TABLE XXI.—*Miscellaneous wilting coefficients*—Continued

ENGELMANN SPRUCE

Sample No.	Station or forest.	Origin and character of soil.	Moisture equivalent.	Wilting coefficient.	Ratio of wilting coefficient to moisture equivalent.	Conditions of test.
74	Fremont F-3...	Granitic gravel.....	7.87	1.54	0.196	Without paraffin.
75	.....do.....	.....do.....	6.53	1.51	.231	Do.
76	.....do.....	.....do.....	3.99	1.50	.364	Do.
76	.....do.....	.....do.....	3.99	1.40	.351	With paraffin.
35	Fremont F-5...	.....do.....	6.74	2.12	.315	Without paraffin.
33	.....do.....	.....do.....	7.62	2.64	.347	Do.
34	.....do.....	.....do.....	6.68	1.98	.297	Do.
110	Wagon Wheel Gap D.	Quartz latite sandy loam.....	20.50	7.56	.369	
111	.....do.....	.....do.....	17.38	3.59	.207	
112	.....do.....	.....do.....	17.72	4.14	.234	
632	Wagon Wheel Gap A-1.	.....do.....	18.43	6.93	.376	
	Fremont.....	Granitic sandy loam.....	10.42	3.73	.358	
6	Arapaho.....	Transported fine sandy loam...	13.65	3.22	.236	
243	Leadville.....	Granitic loam.....	45.40	7.60	.168	Do.
231	Battlement....	Lava loam.....	30.16	4.93	.163	Do.
232	.....do.....	.....do.....	21.83	3.73	.171	Do.
205	Bighorn.....	Granitic sandy loam.....	17.40	2.88	.166	Do.
206	.....do.....	.....do.....	13.63	2.17	.159	Do.
222	Gunnison.....	Limestone silt loam.....	28.16	3.31	.118	Do.
221	.....do.....	Limestone loam.....	22.78	2.69	.118	Do.

In Table XXI there are presented all the wilting coefficients which have not been given in the two preceding tables, and for which the corresponding moisture equivalents are available. On examining the data, however, it is readily seen that in each group those tests which have been made without coating the soil with paraffin give much lower coefficients than those in which the soil was coated. This is as might be expected and, since the proportion of such tests is variable in the different groups, it seems best to discard all data obtained in this way. We then obtain the following averages:

Species.	Number of tests.	Mean moisture equivalent.	Mean ratio wilting coefficient to moisture equivalent.
		Per cent.	
Yellow pine	16	8.42	0.3825
Lodgepole pine	15	12.90	.3468
Douglas fir	13	8.76	.3065
Engelmann spruce	7	14.58	.3044

When allowance has been made for the fact, which is evidenced by the mean moisture equivalents, that three-fourths of the usable data for yellow pine and half of that for Douglas fir were obtained with granitic gravel, or sand soils, although these soils do not much effect the other two groups, it is definitely decided that wilting coefficients are much lower for Douglas fir than for yellow pine, probably somewhat lower for yellow pine than for lodgepole, and certainly lower for Douglas fir than for spruce.

With this confirmatory evidence, no hesitancy need be felt in placing these four species in the following approximate relationships:

Species.	Approximate mean ratio of wilting coefficients to moisture equivalents on common basis of soil qualities.	Species.	Approximate mean ratio of wilting coefficients to moisture equivalents on common basis of soil qualities.
Lodgepole pine	0.35	Engelmann spruce	0.32
Yellow pine	.33	Douglas fir	.31

Certainly, from all the evidence available, the differences between the species are not any greater than here indicated. From all that we have found, it would probably be fair to say that in actual ability to stand drought, at least for the conditions existing in these pan tests, there is no essential difference between yellow pine, Engelmann spruce, and Douglas fir, the greater frailty and slow rooting of the spruce as compared with fir or pine being balanced by an actually stronger affinity of the spruce for any water within reach of its roots. On the other hand, lodgepole seems to stand out both as frail and slow-rooting, and with no compensating development of high sap density or osmotic pressure, so that it does succumb to drought much sooner than the others. In one test only, limber pine and bristlecone pine have shown themselves in practically the same class as yellow pine.

RESISTANCE TO EXCESSIVE HEAT

The sap densities observed in seedlings, and the relative rates of transpiration as apparently affected thereby, gave rise to the suggestion that there might be a specific difference in heat requirements based on this same set of internal conditions. While, on the one hand, the freely transpiring species of low sap density would seem to require a warm environment to counteract the cooling effect of this transpiration, on the other hand, the species of high sap density, which also seem to function more fully than others without full direct sunlight, appear to be always in danger of becoming overheated because, for some physical reason not fully explained, the heat absorbed is not so fully utilized in evaporation.

One thing which the close observation of seedlings in the wilting tests has made very plain is that at an early age all seedlings are very susceptible to injury just where the stems are in contact with the surface soil. At times it has seemed as though moisture absorbed by the roots might be extracted from the stems at this point, so blanched and shrunken do they become as soon as the surface of the soil becomes dry. On the other hand, it is perfectly evident that, as soon as the surface soil ceases to possess moisture to keep its temperature down, it may in sunlight easily become by far the warmest part of the environment. The measurement of the temperature at the warmest point is exceedingly difficult, but the showing of thermometers more or less submerged indicates that the soil surface not infrequently attains a temperature

of 160° F. It is, therefore, readily seen that in soils exposed to sunlight the injury resulting from drought at the surface may be indistinguishable from that due to superheating. Under ordinary circumstances the two injurious conditions will be inseparable.

The difficulty of determining the heat tolerance of seedlings at the point where they are commonly injured by heat is, because of the influence of moisture, very great. We have not been able to conceive a test of heat tolerance in the normal sense, except through the employment of sunlight or some other more powerful radiant energy. Every other possible plan of exposure to heat seems to have the objectionable result of injuring the foliage first, which rarely happens in nature, or of preventing normal evaporation with whatever protection that may afford.

Therefore, the only test [8] that has been made to determine the relative tolerance of heat by forest-tree seedlings has been on this basis of obtaining as high temperatures as possible in sunlight, with the air to some extent artificially warmed. The actual temperatures attained were measured only so far as was possible by placing mercurial thermometers directly above the soil surface. The seedlings of each species were developed in several pans, each of which represented a different moisture content. Because of the fact that the largest amounts of soil moisture permitted almost no injury, the moisture contents were in several cases lowered before the test was completed, so that the record is considerably confused. From the data secured, however, the following conclusions, admittedly tentative, may be drawn:

1. Injury to seedlings from excessive heat is plainly greatest when the seedlings are youngest. This introduces a complicating factor in the test, because exposures to high temperatures were begun before germination was entirely completed and when, therefore, there was the most marked difference in ages. Engelmann spruce ordinarily germinates most promptly and spontaneously. Consequently, while there was marked early damage to this species, the fact that there were few later germinations left the species then immune for some time. Lodgepole pine exhibits just the opposite characteristics and effects.

2. Seedlings which survive a certain degree of exposure are not likely to be injured until the conditions become considerably more severe.

3. The ease with which any species may be injured increases very markedly as the moisture content of the soil decreases. With the lowest content, 3 per cent, which in this soil was appreciably above the wilting coefficient, it may be questioned whether the injury was not due to drought almost wholly, since between waterings the wilting coefficient of the soil was reached.

4. At all times the nature of the wilting was indistinguishable from that which occurs with simliar seedlings when no excessive heat is involved. Consequently, it appears that wilting may be due as much to inability to supply transpiration losses as to the direct effects of the temperatures. The fact that no wilting was secured with 14 per cent moisture appears to bear out this idea, yet it must be remembered that this free moisture may have greatly reduced the temperature extremes of the surface soil. The fact that temperatures recorded just above the soil were not in excess of 135° F. further suggests that wilting was the result of transpiration losses rather than a direct temperature effect on the protoplasm.

[8] Credit for the conduct of this test should be given to Forest Asisstant J. Roeser, jr.

5. With this understanding of the situation we may say that in this test Engelmann spruce and lodgepole pine were most susceptible, while Douglas fir and yellow pine were about equally resistant. The factor which seems to control susceptibility is mainly structural rather than physiological—that is, it is the small mass of the spruce and lodgepole, and possibly their weak rooting, which causes them to stand out in contrast to yellow pine and Douglas fir under extreme drying conditions of relatively short duration.

This pairing of spruce with lodgepole suggests as strongly as do the high wilting coefficients for lodgepole the very poor ability of the latter to supply itself with water; but, in the light of the other facts secured, the same cause will not fully explain the behavior of spruce. It is believed it would be fairer in the case of spruce to say that high temperatures in direct sunlight create high internal temperatures and some direct heat injury. This hair-splitting distinction is necessary for the proper physiological interpretation which will agree with the other facts at hand. It may be added that the susceptibility of spruce to injury in sunlight has been very evident in many of the wilting coefficient tests.

EVIDENCE OF WINTERKILLING

Winterkilling of trees is generally recognized as the direct result of evaporation from the leaves or twigs at times when moisture can not be supplied to replace the loss, owing to a frozen condition of the soil. It is, of course, not confined to evergreen trees but may affect fruit trees, or even such hardy forest trees as honey locust, when devoid of foliage.

The conditions for winterkilling are usually provided by a very rapid rise in air temperatures and by wind which facilitates evaporation. The soil, of course, warming more slowly than the air, may not free its moisture for many hours after the beginning of the unseasonable air conditions. Likewise, if the tree stems have been thoroughly frozen, they may not be able to transport water until a great loss from the leaves and small twigs has occurred.

The conditions conducive to winterkilling are especially likely to be produced near the base of the Rockies from northern Colorado northward. The coniferous forests which are subject to this form of injury are therefore the low-lying yellow pine forests of the Black Hills and eastern Montana. Here the Chinook, a warm wind occurring at a season when the normal temperatures are below freezing, attains its most typical development.

A typical Chinook has not been noted within the locality of the present study. It has been shown, however, that in the Pikes Peak region the winds from January to March possess the powers of a modified Chinook. While they do not often bring extremely large rises in air temperatures, they are of high velocity, the air is dry, and the soils at all elevations, unless strongly isolated, are likely to be deeply frozen and remain so throughout the duration of the wind, which is often two or three days.

The Pikes Peak region therefore presents a good opportunity for the study of the relative resistance of the several species to this form of drought, for the desiccating influence is not confined to the low zone where only yellow pine occurs.

The present writer (*2*) has described in some detail the cumulative effects of winds occurring at the Fremont Station in January and March, 1916. It was shown that on a south exposure where yellow pine, Douglas

fir, and limber pine grow in a mixed stand, Douglas fir at first showed a more pronounced discoloration, but later the injury to yellow pine was seen to be much more severe, as only this species was defoliated. This injury was always much more pronounced on the west (windward) side of a tree, but it varied with different specimens, partly because the ground is strewn with large boulders which deflect the wind and also reflect sunlight. While in no case fatal (and even the general injury in the Black Hills in 1909 caused a very small percentage of deaths), this defoliation obviously must have a retarding effect on the growth of the whole tree. That the same kind of injury occurs at intervals of a few years, and that it hits "twice in the same spots," seems to be indicated by the one-sided development of most of the trees which were injured in 1916 (see Pl. 7, A). Buds and branches were rarely injured in this case, and new foliage appeared almost as early as on unaffected trees or parts.

In the nursery, where there was no snow to furnish protection during most of the winter, a better comparison of the species was possible because of the uniform conditions of soil and exposure. Yellow pine stock was damaged more than Douglas fir; Douglas fir far more than spruce. In fact, in only a few cases was spruce even discolored. With lodgepole the injury was usually confined to an exposed branch or leader, suggesting incomplete ripening of the previous season's growth.

This indicates, as do all other data, that spruce can bear drying to a greater degree than the other species, or at least that it resists the drying better, which comes to the same result. It is perhaps significant of the moisture-conserving adaptation of limber pine, which has been indicated by the transpiration tests, that there was no apparent injury to this species on the south slope where yellow pine was most plainly injured. It resisted wind-drying of this kind as well as any species. On the other hand, during the summer drought of 1917, limber pine was the only species showing injury to trees of large size.

SUMMARY

The relative qualities of the important forest trees of the Central Rocky Mountains, primarily from the standpoint of moisture relations, have been approached from five different angles. No one of these efforts has been free from errors, and no one would alone carry conviction, but the several results are corroborative with only insignificant exceptions. These comparisons of the species have been made on the basis of—

1. Measurements of the water used in relation to growth and leaf exposure of 3- to 9-year-old trees, under uniform conditions for all species.
2. Comparisons of sap density under uniform and varying growth conditions.
3. Measurements of the moisture of soils not available to young seedlings by direct comparisons of the species and also under varying conditions as to soil quality and atmospheric stresses.
4. Observations on fatality among seedlings under high temperature conditions.
5. Observations on the resistance to winter drought of specimens growing side by side, and as measured by the extent of injury to foliage.

It will have become apparent that there are several aspects of the moisture relations, that the several species studied do not always stand in the same relation one to the other, and that it is not even possible to state

that of the Rocky Mountain species one is distinctly more drought-resistant than the others. The mositure relations apparently vary much with the other environmental conditions, and it is perhaps the most important feature of this paper that a somewhat logical relationship has been shown to exist between moisture requirements and other requirements of each species.

We may, then, briefly outline the theory and at the same time observe how closely it applies to the behavior of each species under each of the situations that has been presented. We may take as our starting point the relative "tolerance to shade" of the several species, because this is a character which always has been quite closely observed by foresters and in which, empirically, rather definite lines have been drawn.

Briefly the physiological requirements appear to be related on this basis:

1. The species of greatest shade tolerance or greatest ability to make effective use of sunlight in photosynthesis will possess, other conditions remaining equal, after a period permitting accumulation the greatest amount of soluble carbohydrates in the leaves. In this fundamental respect we shall adhere, at least tentatively, to the classification indicated by the December, 1917, sap densities, as shown in Table XIII, placing spruce at the head of the list of our indigenous species, followed by Douglas fir, lodgepole, bristlecone, yellow, and limber pines.

2. The presence of considerable quantities of carbohydrates augmenting other solutes creates a dense sap, or solution, which does not evaporate so readily as a dilute solution. Because of the osmotic pressure exerted by a dense solution, there should at the same time be greater ability to extract water from the soil, though there is no evidence that at the end of the struggle one species tolerates appreciably greater drought than the others.

3. The presence of these solutes in large quantities is also, naturally, conducive to a high growth rate.

4. By restricting evaporation, the soluble carbohydrates may increase the net amount of the light energy available for photosynthesis, so that, whatever the original quality which made the plant effective, this quality is augmented by its own results.

5. By restricting the use of heat in evaporation, however, the dense cell sap may not only reduce the relative heat or light requirement of the species but may subject it to the danger of superheating. Of all the possible influences of the specific differences which give rise to the cell-sap differences, it is believed this is the most important ecologically and the most potent in its effect on the distribution of the species. If we assume distribution to be controlled primarily by this physiological factor, it becomes fairly simple to see how adjustments have been made to meet other conditions of the environment, principally in the form of structural adaptations, which differentiate the species beyond that difference which may arise from photosynthetic efficiency, and which may to a certain extent compensate for the physiological deficiencies.

If we accept the heat hypothesis as fundamental, we mean that each species will be limited in its distribution rather sharply by the maximum temperatures which it can tolerate (probably in the early seedling stage) and also limited in its growth by its minimum requirements, so that at a certain low temperature it is unable to compete with more highly developed species and hence loses its dominance in the forest. In the mountain forests, therefore, we should expect to find the six species zonated according to temperatures, in the order named just above.

This zonation holds, definitely, however, only for Engelmann spruce, Douglas fir, and yellow pine, which we have shown to be so equally developed as forest dominants that the fundamental physiological differences control all their relations. With the three more or less weedy pines there are, plainly, adaptations which are equally effective or more effective in controlling distribution. It is significant of the importance of high temperatures as absolute limitations that these three species are all found in higher and cooler zones than their physiological conditions necessitate.

Supplementing physiological characters, we may have stomatal reduction, thickened epidermis, or clustered leaves, all tending to reduce the absolute transpiration, but, while doing so, inevitably reducing either the intake of carbon dioxid or the effectiveness of sunlight so that photosynthesis and growth are reduced perhaps even more than is water loss. This seems to be the general line of protective development in the "weed" trees, limber pine and bristlecone pine, and to a lesser extent in lodgepole pine.

Again, resulting from gradual adjustment to the moisture conditions which accompany certain heat conditions, the forest trees have different root habits, or (shall we say?) are unequally stimulated to root development. It is believed that temporary stimulus has much to do with it, but inherited habit still more. Be that as it may, yellow pine and Douglas fir root much more vigorously at an early age than lodgepole pine or spruce. Almost as divergent are the germinating rates of the seed, lodgepole pine standing out as the most sluggish of the six species studied.

In the strictly physiological sense, spruce is undoubtedly the most highly developed of the indigenous species we have considered. This is evidenced by the sap densities which the trees show after long seasons of photosynthesis and by the amount of growth made in relation to the total amount of the water consumed.

In actual water consumed by a tree exposing a unit area to light (and wind) spruce is again the most economical, followed by Douglas fir, bristlecone, limber, yellow, and lodgepole pines. In this consideration, it is, almost without question, the special adaptations of the weed pines which put them down as only moderately extravagant, making them especially suited to exposed windy sites but wholly incapable of holding a permanent place in the forest. On the contrary, spruce maintains a moderate rate of transpiration under the driest conditions (so far as measured) for two reasons, namely, because it does not mechanically restrict losses but forges ahead with growth, and because when the water supply is low it is still more able than any of its competitors to supply its needs and is not so soon restricted either in transpiration or growth. These facts stand out very clearly. In this comparison we have placed Douglas fir next to spruce, believing that the actual position shown by Table XI is misleading, because the trees involved did not develop normally.

In resistance to winter-drying, limber pine with its peculiar structural development and spruce with its high physiological resistance have shown themselves about equally effective. Douglas fir and yellow pine follow with increasing weakness. Lodgepole pine shows greater resistance than would be expected, a fact which we shall not attempt to explain at present.

Considering the drought resistance of seedlings, through the wilting coefficients of a number of soils in which they have been compared, we find the same physiological properties evidently at work, though much

obscured by the relative sizes of the seedlings and their root developments. The seedlings of spruce and lodgepole pine are small and frail and in the first two or three months develop scarcely more than half the root produced by Douglas fir and yellow pine. As a result, even when carefully protected from excessively rapid water loss, lodgepole pine seedlings show far less drought-resistance than the others. Spruce seedlings, on the other hand, show quite as great resistance as those of pine or fir when not excessively insolated, and possibly a little more if the drought condition is approached every slowly. Limber and bristlecone pine seedlings, as meagerly observed, resist drought with the best of the others, no doubt because of a low rate of transpiration. In this connection the soil conditions leading up to wilting of seedlings should be borne in mind. Rarely is it possible for the roots to reach and extract all of the moisture which it would be physically possible for them to absorb. The completeness of this absorption depends very much on capillary movement in the soil. If the amount required by the seedling is small, this movement may supply the needs. Therefore, the rate of transpiration by the seedling is very important in determining, to a fine point, the degree of drought which it will resist.

In nature all possible rates of soil-drying are represented, dependent very much on the amount of insolation on the site and to some extent on the nature of the soil cover. The open south exposure will usually dry at the immediate surface very rapidly. Because of the lack of humus, however, the layer just below the surface may remain moderately moist so long as the quantity of water below is sufficient to maintain capillary movement. When that end is reached, the soil undoubtedly dries out very rapidly to a considerable depth. Even with the respite furnished by capillary movement, the whole process of drying, in continuously bright and dry weather, seems likely to be accomplished here sooner than in the contrasting site. This may be on bottoms or north exposures where the total moisture supply is sufficient to produce a closed stand, heavy shade, and the accumulation of humus. In this soil the surface litter and humus are rarely thoroughly wetted except during and immediately after the melting of snow. The more decomposed humus below, however, due to a lack of insolation and being protected by the surface litter, is rarely dry except after prolonged drought. It dries out slowly and steadily, however, both through the demands of the roots below and by direct evaporation. It follows that, since these demands in the aggregate are very large, such a soil may at unusual times, or possibly in the usual autumn drought, become extremely dry, especially so in the physiological sense, because of its high wilting coefficient.

On the one hand, then, we have the rapidly fluctuating moisture conditions of the well-insolated site, which, for the establishment of seedlings would appear to demand prompt germination and prompt deep rooting. Yellow pine is preeminently adapted to these conditions by reason of its large seeds, which produce large sturdy seedlings with a habit of immediate deep rooting. There is nothing in the evidence on this species to suggest conservatism in the use of water. Probably the extravagant use of water assists in protecting from excessive heat. Success is dependent on the roots reaching a layer of the soil which does not dry out dangerously through insolation. It follows logically that yellow pine can not attain success in the face of competition, either with the roots of established trees or with grasses and herbs whose use of the water would materially augment the drying of the surface layer.

The large moisture demands of yellow pine, with the normal amount of precipitation, can only be supplied in open stands, which first permit the safe establishment of the roots at a depth and later their extension into a large area of soil. This is of fundamental importance in the management of the species and explains the ultimate failure of one crop of seedlings after another in stands which are already moderately crowded or apparently fairly open.

Next in order on such sites we might expect to find Douglas fir, because it, also, produces a deep-rooted seedling. However, we should bear in mind that this species transpires less freely than yellow pine and hence may not be able to tolerate so much insolation. Extended observation shows that it will grow almost anywhere that yellow pine will grow, provided only the seedlings may have shade until they have passed the stage when most susceptible to heat injury. The fact that seedlings start in the shade of and in the densest root area of yellow pine trees shows that this species requires less moisture than yellow pine or, at least, that the moisture is not a controlling factor, and it is apparently for this reason that Douglas fir forms the climax forest except on the warmest yellow pine sites.

Limber pine and bristlecone pine are also, by germination and rooting habit, adapted to well-insolated sites. The sap density of bristlecone pine, however, is apparently considerably higher than that of limber pine, and therefore it succeeds better on cooler sites and on heavier soils. The physiological development of both species and their growth rates are so low that neither can hold a place in the forest in competition with spruce or Douglas fir.

On the other hand we have the poorly insolated sites, commonly described as "cool and moist," which are subject to comparatively slow and wide seasonal changes in their moisture conditions.

Spruce seedlings on account of their growth habit are able early in the season to penetrate the layer of loose organic matter which is in many seasons thoroughly wet only after snow melting. The small seeds germinate at a lower temperature than those of other species. Thereafter the roots show little stimulation to further growth. Even the dryness of the fall period does not appreciably stimulate root growth in the new seedlings, and it is believed that this is clear evidence of the ability of the species to extract water from the soil at a low degree of availability. Possibly because of their generally higher organization, spruce seedlings even prefer a low moisture content which results in greater concentration of the soil nutrients. In such a situation Douglas fir has no theoretical advantage over spruce except in case of a drought so prolonged as completely to dry out the soil layer in which the spruce roots are found. Then the deeper rooting of the fir should, apparently, count in its favor. But in the established forest this can hardly be a material advantage, considering the evenness and depth of the drying where the soil is well occupied by older roots.

The situation with regard to lodgepole is very different from that of our other forest trees, and the writer takes from the evidence the liberty of suggesting either that it is just approaching the physiological status of a full-fledged tree or that it is such a recent migrant to the Rocky Mountain region as to have failed by far to adapt its mode of growth to the poor moisture conditions usually found where its heat requirements are best satisfied. Some of the evidence on the latter point has been presented by the writer (*3*). Clements (*9*) has classed lodgepole

as even more intolerant of shade than yellow pine, and a fairly low photosynthetic efficiency is clearly indicated in this paper. Yet, while evidently demanding a great deal of light as well as moisture during the growing period, it shows no such habit as that possessed by yellow pine, of prompt germination or deep rooting. It is evident, therefore, that it is adapted only to sites with a steady supply of moisture and demands more than can usually be supplied by either of the contrasting situations which have been described. It is probably for this reason that it reproduces readily only where competition is decidedly lacking, adheres to the higher elevations where moisture is more abundant but where its growth rate is surprisingly slow, and has not penetrated to the south where there is a very sharp contrast between the summer rainy period and the clear, dry weather of autumn. In its range there is, to be sure, a generally steady decrease in the precipitation from May or June onward, but this is usually so gradual as to permit a great degree of adjustment.

CONCLUSION

In concluding this paper, it may be said that certain physiological relationships between the species which are of great importance, especially for a proper understanding of forest growth, have been brought out and tentatively established by approach from several angles, but that, from the standpoint of natural reproduction and in relation to all questions of natural distribution of the species, these relative physiological qualities are not shown to be more controlling than some adaptations of form and characteristics of behavior which may be adequately described only by the word "habit." Technical forestry or silviculture might be said to be based on the venerable concept that the several species of the forest vary in their demands for light or their tolerance of shade. This concept is not only not altered by the present results but is confirmed, and the relation of the photosynthetic capacity of a given species to its heat and moisture requirements is made much more clear and definite than it has appeared heretofore. Spruce is shown to be the most efficient of the species considered, not only because of its high photosynthetic capacity, but also because when this capacity is exercised the species automatically becomes economical in its use (by transpiration) of water and at least in this sense has low moisture requirements. At the same time it may be rendered sensitive to excessive temperatures. On the contrary, yellow pine, commonly thought of as very drought resistant, is found to require much light and heat and, with these, to use comparatively large amounts of water per tree of given size. Of course, the facts fit more closely with preconceived ideas when spruce stands are compared with pine stands, the much smaller number of individuals in the pine stand not only compensating for the high individual water use but being a most vital concomitant of this individual requirement. In other words, with the low moisture supply commonly available in the low elevations and warm situations (which alone insure proper development of the pine), wide root spread and an open stand are vitally necessary to insure the water supply of the individual tree. The practical importance of this fact in forest management should be very clear.

It will be seen, then, that these physiological relations principally clarify our conceptions of growth. Spruce is a better grower, a more efficient mechanism for growth, than Douglas fir, and fir more efficient

than pine. In forest planting, where use is made of trees which have been carefully nurtured beyond the stage of greatest susceptibility to heat and drought injury, this difference in the efficiency of our species, particularly the more effective use of water by spruce and fir, may be advantageously employed, the ranges of these species being artificially extended downward, without any injurious effects, while by natural reproduction such extension would be completely prohibited or would be very slow. With even greater care the economic loss resulting from the planting of any species in a situation too high or too cool, or in stands too dense for its proper development, must be avoided.

In short, when it is considered that any cutting of a forest, by admitting more light and creating higher temperatures in the surface soil and more rapid fluctuations of the moisture on which young seedlings are dependent, tends to encourage a species more "hardy" but of a lower order of development than the one which dominates the stand (not necessarily of lower technical value), it is not difficult to see that all forest management hinges on these relative physiological properties for which we have been groping. Finally, it may be said that all of the physiological relations are embodied in the now rather general conclusion of foresters that the highest returns can be had from forestry only when cutting is followed by planting.

This brings us to the original object of the present study, which has been to explain the existing natural forest types, to explain the distribution of the species. As has been pointed out, natural distribution is plainly influenced at present by habits and adaptations which have developed in each species and which to a considerable extent compensate or balance the more deeply embedded physiological qualities. Without again going into the details that have been brought out, we may illustrate to show how these developments affect natural distribution. In figure 5 an attempt is made to show the influence of these developments on distribution, in a broad way. It has been indicated that spruce may be very sensitive to high temperatures, but especially so at a very early age when the seedling is small and tender. In consequence of this weakness, which is a result of its most fundamental organic character, probably for ages no spruce seedling has been able to develop at a low elevation or on a very well-insolated site at a middle elevation. Such sites as are suitable in respect to insolation and heat must show almost invariably quite even moisture conditions and usually soils characterized by a surface layer of litter and humus. Consequently spruce has developed a rooting habit suited to these moisture and soil conditions—a relatively slow and feeble rooting habit which does not suffice for quick establishment under any other conditions. As a further consequence, spruce has evolved very small seeds, there being no need for large, sturdy seedlings or for deep rooting before the seedling may itself manufacture food. It is seen, then, without enumerating any other similar developments, that the species would have great difficulty in extending naturally to any sites other than the cool and moist ones on which it is commonly found. The common conception has been in error only to the extent of assuming that the essential feature of such sites is a large moisture supply. It is now fairly evident that the individual spruce tree does not require a large moisture supply even though this may insure the fullest development of the stand and, in view of this fact, that spruce may be used in planting where the moisture supply is relatively low.

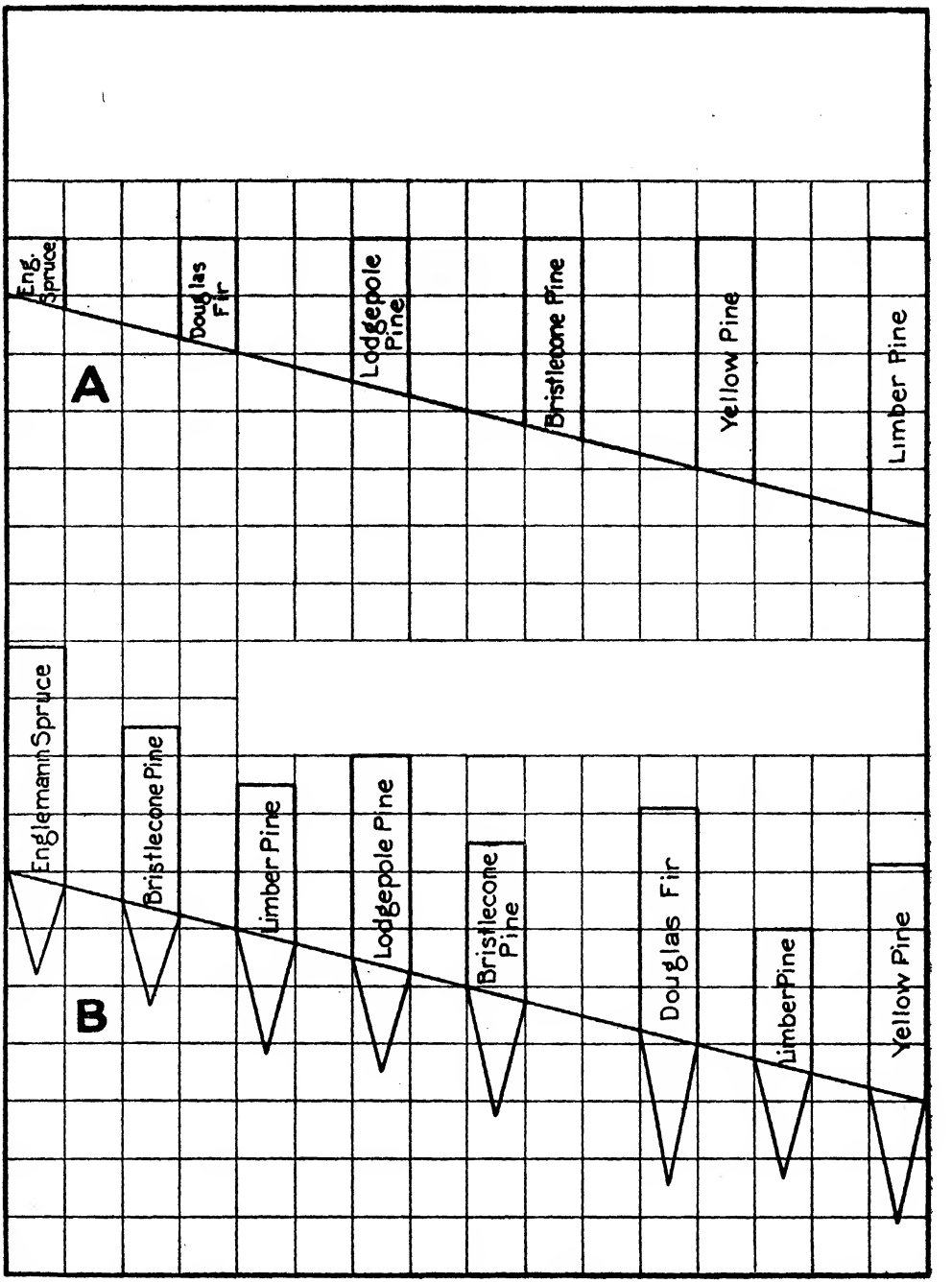

FIG. 5.—A, Theoretical zonation and relative heat requirements on the basis of photosynthetic capacity. B, Actual zonation and relative dominance as influenced by adaptations of roots and foliage.

It is to be hoped that this distinction between the temporary qualities of seedlings which acutely influence natural reproduction and extension of ranges and the more fundamental qualities of the species which later control growth reactions and economic values may be clearly held in mind, since it becomes increasingly apparent as time goes on that the factors controlling reproduction must be considered as almost independent of those controlling later growth.

In a succeeding paper on this subject it is hoped principally to show to what extent the environmental conditions of the different forest types differ and, in the light of what we have so far seen, to weigh carefully the importance of each condition so that those conditions which are really essential to the success of a given species may be clearly understood. The practical application of these facts in forest management may then be shown, it is hoped, in more definite terms.

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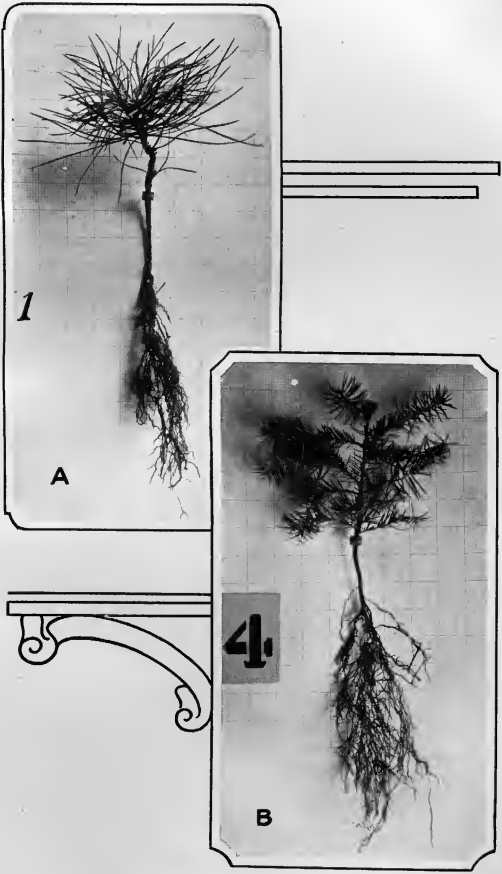
PLATE 1

A.—Tree No. 1, yellow pine, 1917.
B.—Tree No. 4, Douglas fir, 1917.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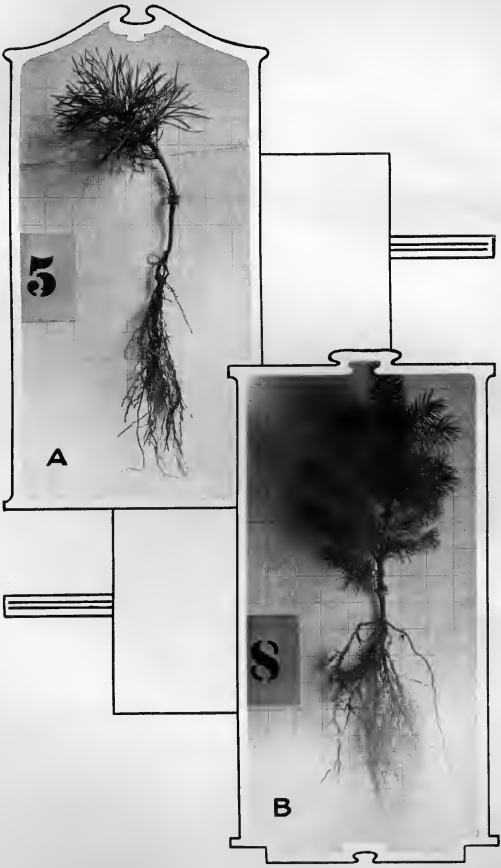
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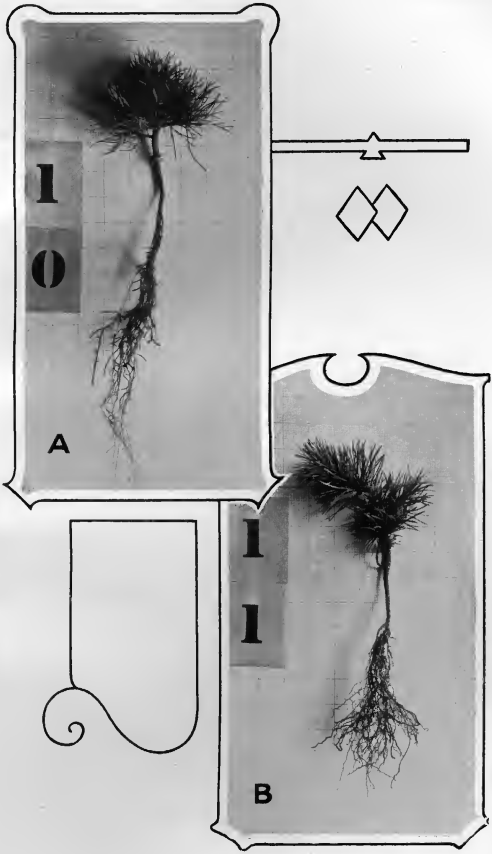
PLATE 2

A.—Tree No. 5, lodgepole pine, 1917.
B.—Tree No. 8, Engelmann spruce, 1917.

PLATE 3

A.—Tree No. 10, limber pine, 1917.
B.—Tree No. 11, bristlecone pine, 1917.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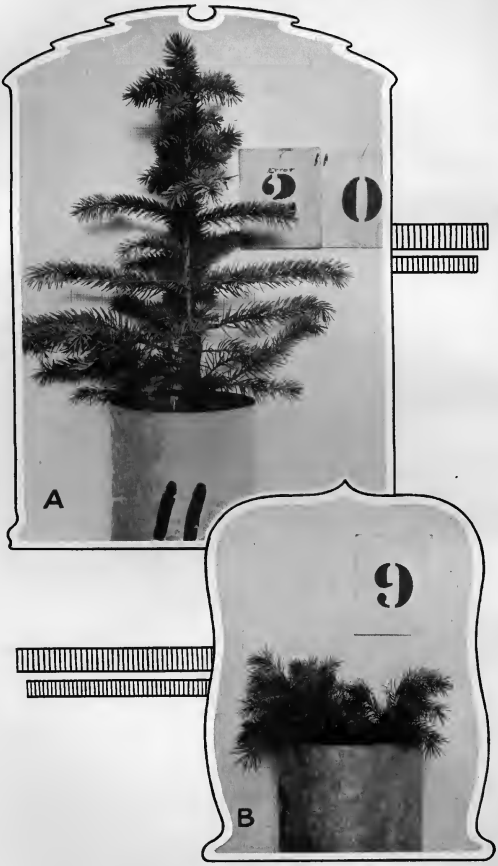
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PLATE 4

A.—Large spruce, No. 11, in 1920 transpiration test.
B.—Small spruces in Pot 9, 1920.

PLATE 5

A.—Arizona yellow pine, Pot 15, 192c.
B.—Montana yellow pine, Pot 18, 1920

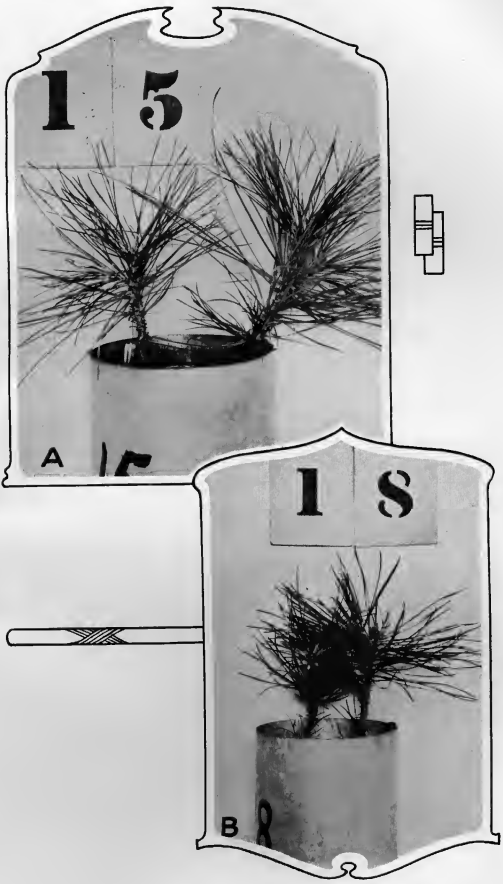
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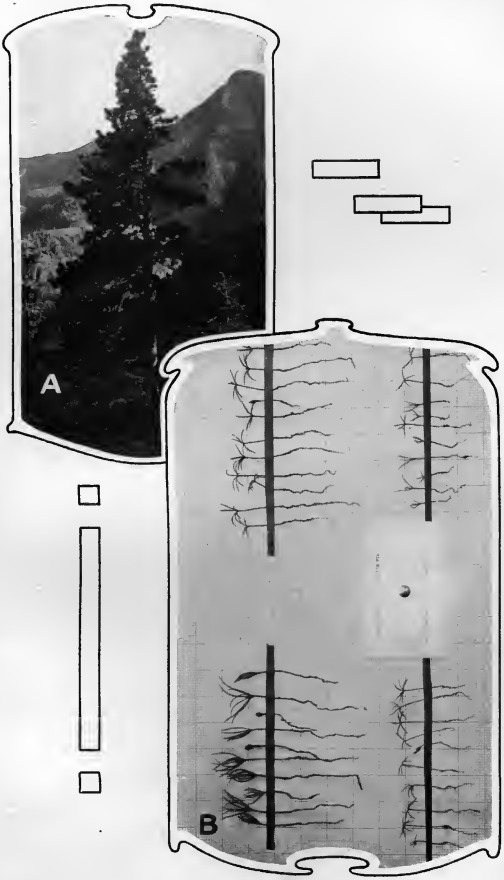
PLATE 6

A.—Vigorous limber pine, Pot 16, 1920. Water requirement 773 units.
B.—Sluggish limber pine, Pot 21, 1920. Water requirement 4,785 units.

PLATE 7

A.—Asymmetrical development of yellow pine, probably resulting from repeated winterkilling of limbs on the west side. (Looking south.) July 2, 1916.

B.—Relative root developments in moist sandy soil of seedlings 30 days after sowing.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A STUDY OF THE INTERNAL BROWNING OF THE YELLOW NEWTOWN APPLE[1]

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INTRODUCTION

As a result of the heavy losses of Pajaro Valley apples during the early development of cold storage practices in California, considerable effort by the Bureau of Plant Industry of the United States Department of Agriculture has been directed toward determining the cause of this trouble and toward devising methods of overcoming it. In 1910 Stubenrauch (*16*)[3] indicated a relation between the browning and the temperature of storage, lower temperatures favoring its development. In the report of this bureau for 1920 (*1*) it was stated that there was no relation between the acidity of the fruit and the trouble and that as yet no definite cause could be assigned for this disease.

OBJECTS OF THE INVESTIGATION

The present investigation is an attempt to determine the cause of internal browning, with special reference to (1) field conditions which are responsible for the susceptibility of the fruit and (2) the internal and external factors which are immediately responsible for its development in storage.

DESCRIPTION OF INTERNAL BROWNING

Internal browning as it occurs in the Yellow Newtown apple is a nonparasitic storage disease of the large isodiametric cells of the pulp. In apples stored immediately, regardless of the time of harvest, at −1.1° and 0° C. the browning generally becomes noticeable during the latter part of December, while in apples stored at higher temperatures its first appearance is proportionately later. The writer has not observed its occurrence in apples kept at temperatures of 8.3° or above.

In a cross-sectional view of the apple, the disease is first detectable in more or less elongated areas radiating outward from the central portion of the apple in the region opposite the basal end of the carpels. By cutting the apple in various planes it is apparent that the areas first browned lie adjacent to and radiating outward from the primary vascular bundles.

As the browning becomes more severe, it spreads most rapidly in the region of the secondary vascular bundles. In many specimens it

[1] Accepted for publication July 17, 1922. Submitted as a major thesis to the faculty of the Graduate Division of the University of California, May, 1921, in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to express his thanks to Dr. J. C. Whitten, Dr. J. P. Bennett, and Prof. E. L. Overholser for counsel and suggestions during the progress of this investigation. The writer is also indebted to Mr. H. E. Jacob, a graduate student, for assistance in harvesting the fruit, in preparing it for storage, and in making the observations.

[3] Reference is made by number (italics) to "Literature cited." p. 184.

advances far toward the calyx end of the apples in this region before it penetrates more than several millimeters into the pulp laterally near the initial point of browning. Although the browning seems to be confined to the region immediately adjacent to the vascular system in its most rapid penetration of the apple tissue, the bundles themselves are slow to show the browning. The large cells adjacent to the bundles are the first to become discolored. Later the small cells bordering on the bundles are affected, and finally in the advanced stages of the disease the bundles themselves become brown. In its advanced stages the disease may spread to all portions of the pulp, constituting a condition comparable to the usual type of storage breakdown. In the latter condition, however, the browning begins at the calyx end of the apple, involving all of the tissue in its spread, and is accompanied by softening of the affected region.

It often happens that in the very advanced stages the browning spreads into the small thick-walled cells of the epidermis, thus giving the fruit an appearance of being scalded. In its less advanced condition, the disease, however, is detectable only by cutting into the fruit, the skin retaining its natural color and luster and the flesh remaining firm.

METHODS AND PLAN

The storage phases of this problem were carried out in a cold-storage plant consisting of six rooms and two large insulated boxes, making available the following temperatures: 0°, 2.2°, 5°, 8.3°, and 13.9° C. The temperatures were maintained within ±1°, with the exception of the highest temperature, which varied from 12° to 14°. The humidity was practically constant in the different rooms, never varying more than 3 to 4 per cent.

The fruit was obtained from the Rogers Bros.[4] orchards, which are located about 1 mile east of Watsonville, Calif. The apples were packed and labeled under the respective trees from which they were picked. The fruit was not sorted, but represented tree-run apples, with the exception of the third picking in 1919, which was made up of grade "B" apples. The boxed apples were then expressed to Berkeley.

Three pickings were made each season. The earliest seasonal picking, 15 lots, was made just as the regular picking season was beginning. The second seasonal picking, a similar number of lots, came at about the middle of the normal picking season. The third picking was delayed until the close of the harvesting season. In each case a "lot" represented the fruit from a single tree.

The first two pickings for both seasons were stored the third or fourth day after picking. The storage of the last picking, however, was delayed approximately three weeks each season due to the slow shipment.

After arriving at the storage plant, the lots were divided into the required number of sublots for the several individual experiments. The sublots of approximately 80 specimens each, unless stated otherwise in connection with the separate tests, were stored in apple boxes. Sufficient space was always maintained between the boxes for normal ventilaton. Not only were apples of the same lot used in each experiment and in the control, but the apples of each test were placed under as nearly identical conditions as possible.

[4] The writer is greatly obliged to Messrs. C. J. Rogers and Marion Rogers for their interest and hearty cooperation in the work

As internal browning affects the flesh of the fruit, it was necessary to cut the apples in order to make observations upon their condition. The apples were cut perpendicularly to the axis of the core in a plane which passed approximately through the junction of the carpels with the stem. By cutting in this plane the browning was always detected, if present.

Observations upon the browning were made at monthly intervals beginning with February 2 for the 1919–20 season and with January 8 for the season of 1920–21. Twenty specimens from every sublot were cut at each of the four seasonal cuttings. By cutting several hundred apples on numerous occasions it was found that the error of observation in cutting only 20 specimens ranged from 2 to 5 per cent. When the results for the four seasonal cuttings were averaged, this error was reduced to 1 to 2 per cent. In recording the observations the following terms were used to designate the degree of browning:

1. NORMAL.—No browning apparent to the unaided eye; less than 0.1 per cent of cells affected. (Pl. 1, A.)

2. TRACE BROWNING.—Browning recognizable in the torus but not of sufficient intensity to lessen the market quality; 0.1 to 0.6 per cent of cells affected. (Pl. 1, B.)

3. SLIGHT BROWNING.—Browning in a sufficient degree of intensity to lessen the market quality of the fruit but not to such a degree as to make the apples objectionable for culinary purposes; 0.6 to 10 per cent of cells affected. (Pl. 1, C.)

4. MODERATE BROWNING.—Browning of such an intensity as to render the apples unsuitable for ordinary culinary purposes. At this stage of browning the tissue was more generally discolored throughout the torus, from 10 to 30 per cent of the cells being brown. (Pl. 1, D.)

5. SEVERE BROWNING.—This term refers to a degree of browning which upon cutting gave the apples an appearance of being rotten within. In these apples the structure of the tissue exhibited a marked degree of disintegration in all portions of the specimen; 30 per cent or more of the cells affected. (Pl. 1, E.)

PRESENTATION OF DATA

RELATIVE DEGREE OF INTERNAL BROWNING EXHIBITED BY YELLOW NEWTOWN APPLES GROWN IN THE PAJARO VALLEY AND ELSEWHERE

During this study apples were also obtained from other localities in California and from important districts in other States where this variety is successfully grown to determine whether or not the browning was confined solely to apples grown in this valley. All of the fruit was shipped to Berkeley by express and was then stored at 0° C. under the same conditions as the fruit from the Pajaro Valley.

The figures obtained in these tests indicate that Yellow Newtown apples generally are more or less susceptible to internal browning. The disease in apples from points other than the Pajaro Valley, however, has not been sufficiently severe to render it an economic problem.

The fact that all the apples showed browning would seem to indicate that either something peculiar to the variety makes it susceptible to browning or that the trouble lies in the regions in which it is at present most extensively grown. Both of these conditions appear to be more or less responsible for the browning. The fact that other varieties of apples grown in the same districts, with the exception of the Pajaro Valley, are immune to this disease would at least suggest that the Yellow New-

town exhibits a varietal characteristic of susceptibility to internal browning.

The effect of the region in which the fruit is grown upon its susceptibility to browning is indicated by the fact that several varieties of apples such as the Yellow Bellflower and the Red Pearmain, which normally show no tendency to brown, are susceptible to this disease when grown in the Pajaro Valley. These varieties do not brown when grown in any of the other Yellow Newtown districts. Furthermore, the Yellow Newtown, when grown in this valley is much more susceptible to internal browning than when grown elsewhere. The climatic conditions of the Pajaro Valley, therefore, seem to exert an influence upon the development of apples which has not been shown to occur elsewhere and which renders them susceptible to this disease.

RELATION OF TIME OF HARVESTING TO INTERNAL BROWNING

The importance of the time of harvesting of the fruit in the control of nonparasitic diseases of the apples has been stressed by Powell and Fulton (*13*), Brooks, Cooley, and Fisher (*4*, *5*, *6*), and others. Correspondence with cold-storage managers showed that some of them believe that internal browning is, at least in part, the result of picking the apples too green. The riper apples which have a higher sugar content, according to these men, are more resistant to browning.

An investigation of the effect of the time of harvesting upon internal browning was started in the season of 1919–20. Three pickings of fruit were made for both this and the 1920–21 seasons. The fruit of the first picking was "hard green" in maturity and of a solid green color; that of the second picking was "firm green" and signs of the yellow color were becoming evident; while the fruit of the last picking was "overripe" for harvesting and showed a considerable amount of yellow over the entire surface. The fruits for each lot were picked from all portions of the same trees at each of the pickings and were then stored under identical conditions at 0° and 2.2° C. The effect of the time of harvesting upon the severity and rate of browning are shown in Tables I and II.

The figures of Table I indicate that the later-picked fruit browned much more severely in every test. In the case of the apples stored at 2.2° C. during the season 1919–20, the actual figures in the table show little difference in the amount of browning. When the difference in the storage dates is taken into account, however, a considerable difference in favor of the earlier pickings becomes apparent. In the other cases the relation of time of harvest to the browning is obvious.

The figures of Table II indicate that the fruit of the last picking browned two and one-half times as rapidly as that of the second picking and the fruit of the second picking browned one and one-half times as rapidly as that picked at the beginning of the harvest season.

The sugar content of the fruit picked at the time of harvest September 26, October 16, and November 6 was 9.4, 10, and 11.4 per cent, respectively, which appears to indicate that a higher sugar content favors browning. By analyzing a large number of samples, however, it was shown that the sugar content does not influence the resistance or susceptibility of the fruit to browning. It might also be expected that the change in acidity of the fruit of the later picking, due to its more mature condition, and the subsequent prolonged storage would affect its resistance to browning. This was found not to be true. Although the titrable

acidity decreased with maturity and subsequent storage, the active acidity as indicated by the P_H value of the expressed juice remained practically constant. This possibly accounts for the fact that the decrease in total acidity does not influence the resistance of the fruit to browning.

TABLE I.—*Relation of time of harvesting of the fruit to internal browning*

Storage temperature.	Date of picking.	Date of storage.	Condition of fruit at end of storage period, approximately Apr. 1.				
			Normal.	Trace.	Slight.	Moderate.	Severe.
SEASON OF 1919–20							
° C.			*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
2.2	Sept. 28	Oct. 2	45	50	5	0	0
2.2	Oct. 18	22	50	45	5	0	0
2.2	Nov. 22	Dec. 8	45	45	10	0	0
0	Sept. 28	Oct. 2	40	20	15	15	10
0	Oct. 18	22	20	45	20	10	5
0	Nov. 22	Dec. 8	15	35	20	10	20
SEASON OF 1920–21							
2.2	Sept. 27	Sept. 30	75	10	5	10	0
2.2	Oct. 16	Oct. 19	60	35	5	0	0
2.2	Nov. 6	Nov. 26	5	45	30	20	0
0	Sept. 27	Sept. 30	30	40	20	10	0
0	Oct. 16	Oct. 19	20	45	25	10	0
0	Nov. 6	Nov. 26	0	30	45	25	0

TABLE II.—*The relation of time of harvesting of the fruit to the rate of development of browning*

Storage temperature.	Date of picking.	Condition of fruit.	Weeks in storage.	Marketable.[1]	Unmarketable.
° C.				*Per cent.*	*Per cent.*
0	Sept. 26	Hard green	20	60	40
0	Oct. 16	Firm green	15	60	40
0	Nov. 6	Overripe	6	60	40

[1] Marketable fruit includes both normal and trace browned specimens.

EFFECT OF TEMPERATURE UPON INTERNAL BROWNING

STORAGE TEMPERATURE

At the time apple storage was introduced in California, investigations by Powell and Fulton (*13*) had brought the cold-storage men to the general belief that all apples could be stored most successfully at −5/9° to 0° C. It was soon found, however, that great losses were incurred through the deterioration of Yellow Newtown apples of the Pajaro Valley at these temperatures. As a result of these losses investigations (*16*) were undertaken which brought about the storage of all the Pajaro Valley apples at 2.2°.

At the University of California, where apples of the Pajaro Valley have been stored at −1.1°, 0°, and 2.2° C. since 1916, it has been found that considerable browning occurs after the first of February, even in the fruit stored at 2.2°. Aside from the browning, however, these apples keep satisfactorily. It was, therefore, thought advisable to store apples at various temperatures above 2.2° in order to determine (1) the lowest temperature at which internal browning will not develop during the normal storage period and (2) whether or not the temperature which is sufficiently high to prevent internal browning is also sufficiently low for practical storage purposes.

During the season of 1919–20 the only other temperature available above 2.2° C. was that of room temperature. However, in 1920–21 apples were stored at 5°, 8.3°, 13.9°, and 21° in addition to the usual temperatures used for these apples. In the 1919–20 season 15 lots of the second picking were used in these tests, while in 1920–21 two lots of fruit from the same two trees for each of the first two pickings were used. Since all the apples browned in relatively the same proportions at each of the temperatures, only the averages for the second picking are given in Table III.

TABLE III.—*Effect of storage temperature upon internal browning*

Storage temperature.	Condition of fruit after 4 months' storage.				
	Normal.	Trace.	Slight.	Moderate.	Severe.
°C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
SEASON 1919–20					
−1.1[a]	15	60	20	5	0
0	15	35	30	15	5
2.2	40	55	5	0	0
21	100	0	0	0	0
SEASON OF 1920–21					
−1.1	15	35	25	20	5
2.2	40	35	20	5	0
5	95	5	0	0	0
8.3	100	0	0	0	0
13.9	100	0	0	0	0
21	100	0	0	0	0

[a] There was considerable freezing at −1.1°C. in the early part of the storage season, which retarded the browning.

The figures of Table III show a definite relation between the amount of browning and the temperature of storage. The effect on browning of only a few degrees change in temperature is very striking. At 0° C., for instance, in the 1920–21 season, only 15 per cent of the fruit remained normal, while at 2.2° 40 per cent was normal, and at 5° 95 per cent of the fruit was normal. Browning did not occur in any of the fruit stored at a temperature of 8.3° or above.

It becomes manifest, therefore, that internal browning does not occur at a temperature a few degrees above that used in the commercial storage

of apples. Furthermore, the Yellow Newtown is known to be one of the best keeping apples, and it may be held quite satisfactorily in basement storage until May if well-matured, sound fruit is used. Thus it appears that where prompt storage under uniform conditions is possible the fruit can safely be held at temperatures sufficiently high to prevent browning without other forms of deterioration developing. In commercial practice, however, it would probably not be expedient to store apples above 5° C. It would, nevertheless, be advisable to store these apples at or just below this temperature.

The browning was not only increased in severity as the temperature decreased below 5° C. (as shown by the figures in Table III) but its development was also more rapid. This relation of temperature of storage, with the time of initial appearance and the subsequent development of the browning, is illustrated by the graphs in figure 1.

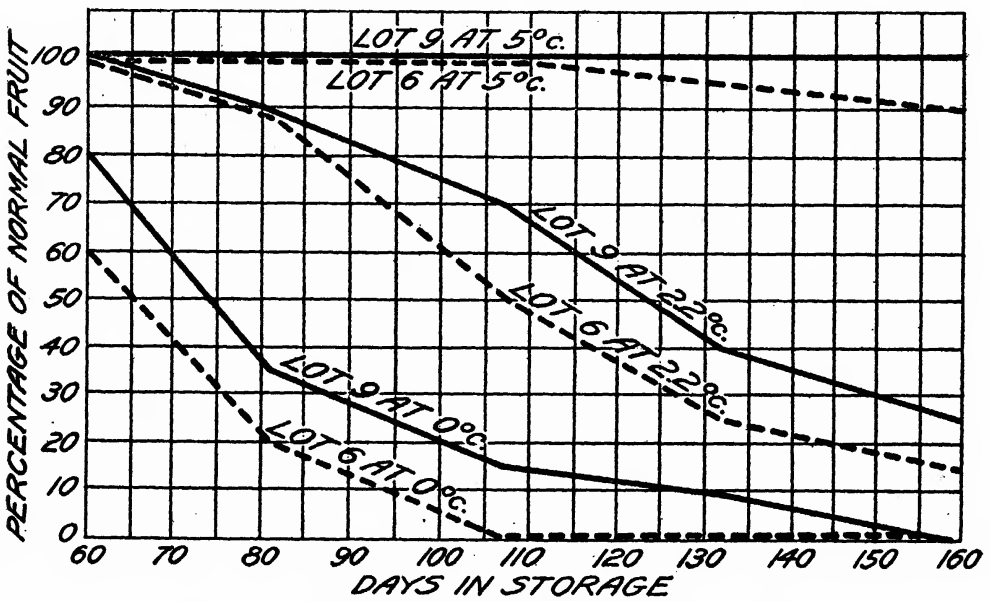

FIG. 1.—Effect of temperature upon the rate of development of internal browning.

ORCHARD TEMPERATURE

In the early investigations upon internal browning, letters, in the form of questionnaires, were sent to the leading fruit men of the Pajaro Valley. In reply to the question asking when the browning was most prevalent, many of the fruit growers attributed its occurrence to the cold, foggy weather which characterizes this valley during the latter part of July and the early part of August, just at the time the fruit is growing most rapidly.

With this observation as a basis, experiments were started in the spring of 1920 to determine the effect of orchard temperature and fog upon internal browning. During the first week of May, 1920, a tent of black cambric cloth was erected over a single average tree which bore a normal set of fruit. The sides of the tent came within about 8 feet of the ground. Thus, all the branches with fruit were shaded continuously. At the same time the tent was erected, 100 individual apples on an adjacent tree were placed in black cloth bags. A similar number of apples on adjoining trees were placed in black bags on the first of June and July, respectively.

Owing to the lack of necessary equipment, it was impossible to measure the exact effect of the tent and black bags upon the light intensity. Nevertheless, it is thought that the light exclusion as such is negligible, since it had little or no effect upon the amount of browning as shown by the figures of Table V. The number of foggy days might, of course, exert an indirect effect by influencing the temperature.

The effect of the tent and black bag upon the temperature, however, was very striking. The mean daily temperature at the core of the bagged fruit, as indicated by self-recording thermometers, was from 2.5° to 5.5° C. higher than that of apples normally exposed. In the case of the tented tree, a lower mean temperature was maintained by the shading and lack of free circulation of the air. Here the temperature, as recorded by accurately regulated thermograph instruments, was found to be from 2° to 4.5° lower than that for a similar position in an adjacent untented tree.

The fruit from the tented tree, the bagged and the normally exposed fruit from the same trees, and fruit from two adjacent trees for control were harvested at the first pickings of the 1920–21 season. All the lots and controls were stored under identical conditions at 0° C. The results of these experiments are given in Table IV. As the fruit of the two pickings behaved similarly, only the averages are given in the table.

TABLE IV.—*Effect of orchard temperature upon internal browning*

Treatment.	Condition of fruit at end of storage period, Apr. 1.				
	Normal.	Trace.	Slight.	Moderate.	Severe.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Fruit placed in black bags where temperature of apples was approximately 4° C. above that of fruit in open	95	5	0	0	0
Fruit normally exposed	30	35	25	10	0
Fruit from under tent where temperature was about 3.5° C. below that in open	5	15	40	35	5

The figures of Table IV show a very definite relationship between the orchard temperature and internal browning. A daily mean temperature of 4° C. above the normal temperature of the orchard practically prevented the browning, while a mean temperature of 3.5° below the normal mean orchard temperature greatly increased the amount of browning over that which occurred in the normally grown fruit.

This relation between orchard temperature and the amount of browning becomes more impressive when the temperature records of this valley are compared, for the years of severe and of moderate or no browning for this region, with the temperature records of other districts where this variety of apple grows satisfactorily and where the browning is not a problem. The graphs in figure 2 represent the mean temperature for June, July, August, and September for the Pajaro Valley, Calif.; Albemarle County, Va.; and Rogue River Valley, Oreg. If the record for the Pajaro Valley is considered, it will be seen that in 1908 and 1914, years in which very heavy losses through internal browning occurred, the mean temperature for these four months was very low. For 1909, 1910, 1915, and 1916, when the mean temperature for these

growing months was nearly normal, the severity of the browning was less. In 1912, 1913, 1917, and 1918, years of higher mean temperature for the months of rapid growth, there was no browning in the commercial storage plants. Comparing the temperature records of the Pajaro Val, ley with those of Rogue River Valley, Oreg., and Albemarle County, Va.- it is found that these regions have a mean temperature of 2.8° and 6.2° C. higher, respectively, than that of the Pajaro Valley.

Results which further confirm this relation of orchard temperature to the browning were obtained by collecting fruit from well-exposed and from shaded portions of the tree. Two lots of fruit were collected from the upper southwest periphery of two trees where the fruit received the maximum effect of the sun's rays, while two other lots were picked from the lower north, shaded portion of the same trees where the fruit was continuously in the shade. These lots were stored side by side at 0° C. The results of this experiment are given in Table V.

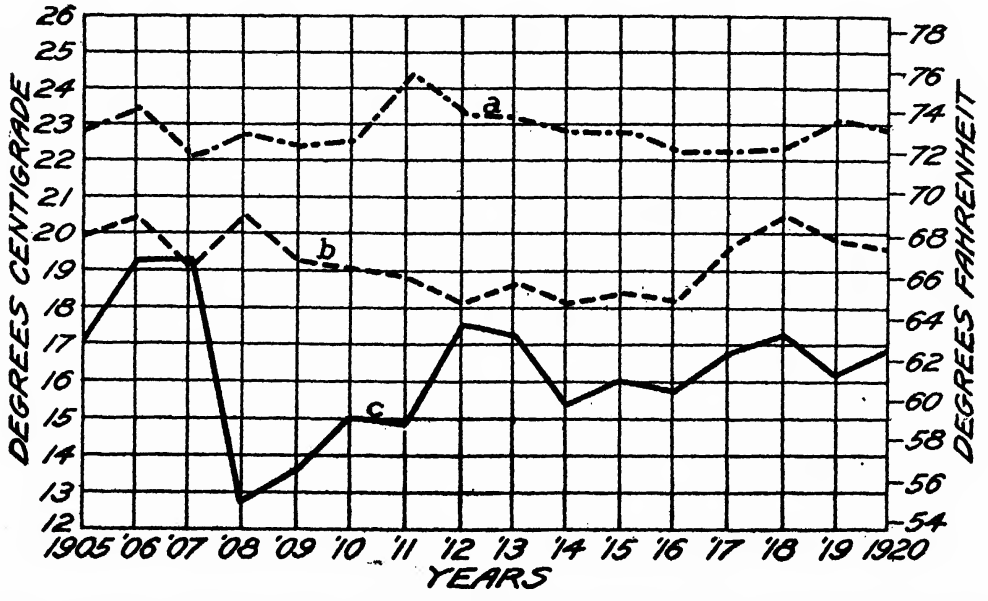

FIG. 2.—Mean daily temperature for June, July, August, and September from 1905 to 1920 for (*a*) Albemarle County, Va., (*b*) Rogue River Valley, Oreg., and (*c*) Pajaro Valley, Calif. (From climatological data reports, U. S. Dept. of Agr. Weather Bureau 1905 to 1920.)

TABLE V.—*Effect of exposure of the fruit on the tree upon internal browning*

Location of fruit on tree.	Condition of fruit at end of storage period.				
	Normal.	Trace.	Slight.	Moderate.	Severe.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Well exposed, on upper southwest periphery of the tree	35	55	10	0	0
Shaded, on lower north side of the tree	15	50	25	10	0

These figures show that the quantity of normal fruit from the well-exposed portions was 20 per cent greater than that obtained from the shaded portions of the same trees. While no records were taken with regard to the temperature of the fruit at the different exposures, it is safe

to assume that the mean daily temperature of the well-exposed fruit was somewhat higher than that from the shaded side of the tree.

Further evidence which seems to show the definite relation of low orchard temperature to the development of internal browning in this variety of apple is brought out in the results obtained from the fruit received from New York State. One lot of fruit was obtained from the Cornell Agricultural Experiment Station, where the mean summer temperature is only slightly more than 1° C. above that of the Pajaro Valley and where cloudy or rainy weather frequently prevails during the growing season. The other lot was obtained from northern part of Dutchess County, N. Y., where the mean summer temperature is approximately 3.2° higher than that of the Pajaro Valley and where, as a rule, there is an absence of cloudy or rainy weather during the growing season. After express shipment to Berkeley, Calif., these lots were stored under identical conditions at 0°. The figures in Table VI give the results of this test.

TABLE VI.—*Effect of orchard temperatures upon the susceptibility of New York Yellow Newtown apples to internal browning*

Source of the fruit.	Weeks in storage.	Condition of fruit at end of storage period.				
		Normal.	Trace.	Slight.	Moderate.	Severe.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Cornell Agricultural Experiment Station, N. Y.	14	5	50	15	0	0
Dutchess County, N. Y.	13	100	0	0	0	0

The quantity of normal fruit obtained from Dutchess County was 95 per cent greater than that from the Cornell Station. This points very strongly to the fact that orchard temperature during the season of rapid growth is an important factor in the development of fruit which is susceptible or resistant to internal browning.

The results obtained on the effect of orchard temperature upon the subsequent susceptibility of the fruit to browning point to the possibility that the mean temperature for the growing season in the Pajaro Valley hovers around the lower limit for the normal development of this variety of apple.

RELATION OF ESSENTIAL OILS TO INTERNAL BROWNING

Before taking up a discussion of the data with regard to the relation of essential oils to internal browning, a few references will be made to previous work on the effect of essential oils and allied substances upon the cell.

Dixon and Atkins (*8*) have shown that anaesthetics increase the permeability of the plasma membrane, for the cell sap is readily expressed after their application. When applied for this purpose, however, the anaesthetics were toxic and their effect irreversible. Since a distinctive mark of an anaesthetic is the reversibility of its action, Osterhout (*12*) made measurements upon tissues to determine whether the increase in permeability, usually observed to follow their application, is due to the anaesthetics or to toxins. He concludes that the anaesthetics produce

a decrease in the permeability which is reversible and the subsequent increase in permeability is due to the accumulation of toxic substances as a result of the action of the anaesthetics. In 1910 Armstrong (*2*) and his co-workers showed that, under the influence of anaesthetics and certain other substances which they called hormones, reactions occur in the cells which indicate that the enzyms and their substrates were brought into contact. Among the results of this mixing of the enzyms and substrates, as observed by these workers, was an oxidation which resulted in pigmentation. These workers also state that these phenomena are constantly taking place in the plant but that under normal conditions their products are passed off before they become injurious. Under abnormal conditions, however, they may accumulate in sufficient amount to greatly hinder the activities of the tissues and eventually to cause the death of the cells. Giglioli (*9*) found that essential oils markedly influence the movements of water, enzyms, and soluble substance through the cell membrane. Later, Giglioli (*10*) also demonstrated that the enzyms could be removed from yeast cells by rendering them permeable with essential oils.

During the past year Power and Chestnut (*14*) have isolated the essential oils of the apple. They have shown conclusively that essential oils are being produced continuously by the apple in sufficient quantities to be detected. In 1919 Brooks, Cooley, and Fisher (*5*, *6*, *7*) found that apple-scald, a nonparasitic storage disease which is generally confined to the surface of the fruit, was apparently due to volatile substances which are produced by the fruit when held for some time under the more or less abnormal conditions of storage. In substantiating this contention, they present data which show that the disease is reduced to a minimum by removing these volatile substances from the fruit by air circulation or by storing the fruit in wax or oil wrappers that are known to be good absorbents of essential oils.

After making observations upon the appearance of the fruit in internal browning and in advanced stages of apple-scald, the writer became convinced from the firmness of the tissue and the way in which these diseases spread into the flesh of the fruit that there is a similarity between these two storage diseases. Histological examinations of affected tissues further emphasized the analogy which exists between these diseases. For the histological studies, sections of the apple torus were cut by means of a freezing microtome. The sections were dropped directly from the razor into acidified absolute alcohol which fixed them and prevented any additional browning. They were then passed through xylol and mounted in Canada balsam without staining. The examination of a large number of cells brought out a very striking similarity between browning and scald in the progress of the discoloration in the tissues as well as in the individual cell. In the tissue there was no regularity in the spread of the disease from one cell to the other, since isolated cells showing browning were always found to be scattered among the normal cells near the regions of scald or browning. In the cells in which the progress of the browning could be followed it was found to be identical in the two diseases. The browning started at the periphery of the cell and from there spread to all parts of the cell along the more concentrated strands or areas of cytoplasm. The discoloration was, as a rule, more intense in the region of the nucleus which is near the surface in the apple cells. Plasmolysis accompanies the advanced stages in browning, until, in the very severe stages, the

protoplast occupies only a small fraction of the cell. The cell wall remains unchanged. As a result of this apparent similarity between these diseases the writer carried out experiments upon the control of internal browning which had proved effective in reducing the amount of apple-scald.

AIR MOVEMENT AS A PREVENTIVE OF INTERNAL BROWNING

Two sublots of apples of the 1919 and 1920 seasons were stored in slat boxes, one box being wrapped in the ordinary manner while the other was stored without wrapping. These apples were then ventilated by a fan for 10 to 20 minutes twice each week. In the season of 1920–21, apples were also placed in sealed containers which were fitted with tubes for pulling air through with a filter pump. Thirty-three apples of the same lot were placed in each container, while a similar number in ordinary storage served as a control. The results of these experiments are given in Table VII.

TABLE VII.—*Effect of air movement upon the development of internal browning*

Treatment.	Season.	Storage temperatures.	Condition of fruit at end of storage period.				
			Normal.	Trace.	Slight.	Moderate.	Severe.
		°C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Apples in common storage	1919–20	2.2	35	60	5	0	0
	1920–21	0	5	75	15	5	0
Apples in slat box, wrapped, ventilated 10 to 20 minutes twice each week	1919–20	2.2	45	50	5	0	0
	1920–21	0	60	40	0	0	0
Apples in slat box, not wrapped, ventilated 10 to 20 minutes twice each week	1919–20	2.2	100	0	0	0	0
	1920–21	0	75	25	0	0	0
Apples wrapped but not ventilated with filter pump [1]	1920–21	0	0	5	10	50	35
Apples wrapped, air drawn through slowly with filter pump [1]	1920–21	0	70	25	5	0	0
Apples not wrapped, air drawn through slowly with filter pump [1]	1920–21	0	70	30	0	0	0

[1] Apples sealed in cans with arrangement for slow renewal of air.

The figures of Table VII indicate that where ventilation was employed there was a great decrease in the amount of fruit that exhibited the disease. The figures also show a very definite relation between the effectiveness of the ventilation and the severity of browning, for in every case the wrapped fruit showed more browning than did that which was not wrapped. This definite reduction by ventilation in the amount of browning would seem to indicate that the trouble is favored by the accumulation of deleterious substances which were removed by both the intermittent and the slow but continuous air movement.

GAS ABSORBENTS AS AGENCIES IN THE PREVENTION OF INTERNAL BROWNING

Several sublots of the 1919 and 1920 crops of apples, picked October 18, were stored at 0°C. in oil wrappers which were known to be good absorbents of gases. For this purpose commercial 10 by 10 inch apple wrappers were saturated with the given wax or oil. The number of treatments included and the results of these tests are given in Table VIII.

TABLE VIII.—*Effect of gas absorbents upon the development of internal browning of apples stored at ° C.*

Treatment.	Condition of fruit at end of storage period.									
	Season of 1919–20.					Season of 1920–21.				
	Normal.	Trace.	Slight.	Moderate.	Severe.	Normal.	Trace.	Slight.	Moderate.	Severe.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Cocoa-butter wrapper	30	45	20	5	0	50	40	10	0	0
Olive-oil wrapper	40	60	0	0	0	55	45	0	0	0
Wesson-oil wrapper	50	50	0	0	0	.....	.....	.....	.....	.....
Mazola-oil wrapper	50	45	5	0	0	.....	.....	.....	.....	.....
Vaseline wrapper	70	25	5	0	0	45	55	0	0	0
Lard-tallow wrapper	.....	.....	.....	.....	.....	15	75	10	0	0
Paraffin wrapper	.....	.....	.....	.....	.....	5	80	15	0	0
Control, common wrapper	.....	.....	.....	.....	.....	5	85	10	0	0
Control, no wrapper	.....	.....	.....	.....	.....	5	65	20	10	0
Average for oil wrappers	48	46	6	0	0	34	57	7	0	0
Average for the controls	20	25	25	20	10	5	75	15	5	0

These data indicate that the amount of browning can be reduced by employing agents which absorb essential oils or emanating gases. Since all the tests as well as the controls were stored in identical boxes and under as nearly as possible the same conditions in the storage rooms, the beneficial effect of the oil wrappers must lie in their ability to prevent the accumulation of injurious substances. There was a reduction of about 30 per cent in the number of specimens showing browning in each case.

At the time the fruit picked October 18, 1920, was stored, 10 portions of 33 specimens each of lot "A" were placed in sealed containers with various wrappers as listed in the table. This was thought to be a more accurate method of determining the effectiveness with which certain absorbents control the disease. It would seem logical to assume that there was always a considerable supply of esters or other deleterious material in the storage room; hence the wrappers in the open boxes should absorb these as readily, if not more so, than the substances from the individual specimens which were wrapped, thereby dissipating their ability to function as active absorbents. In the sealed containers, however, the esters to be absorbed were more nearly confined to those produced by the inclosed fruit, and this should materially lengthen the period during which the oils in the wrappers would act as absorbing agents. The results of these tests are given in Table IX.

TABLE IX.—*Effect of gas absorbents upon the development of internal browning*

Treatment.[1]	Condition of fruit after 11 weeks at 0° C.				
	Normal.	Trace.	Slight.	Moderate.	Severe.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Ordinary commercial wrappers	0	5	0	60	35
Wrappers impregnated with cocoa butter	65	35	0	0	0
Wrappers dusted with animal charcoal	45	50	5	0	0
Wrappers dusted with silica powder	0	5	5	45	45
Wrappers impregnated with olive oil	55	45	0	0	0
Wrappers impregnated with paraffin	25	65	10	0	0
Wrappers impregnated with lard-tallow mixture	30	60	10	0	0
Wrappers impregnated with vaseline	65	35	0	0	0
Commercial wrappers, with 225 cc. concentrated potassium hydroxid in bottom of container	80	20	0	0	0
Commercial wrappers, with 225 gr. soda lime in bottom of container	0	5	5	50	40

[1] Apples sealed in cans with arrangement for slow renewal of air.

The data in Table IX show a very striking relation between the prevention of the browning and gas absorbents. In these tests 95 per cent of the treated fruit was marketable, as compared to only 5 per cent of that of the controls. The figures also indicate a definite relation between the capacity of the various absorbents for taking up esters and the prevention of the disease. Paraffin, which according to Gildemeister and Hoffman (*11*) has an absorbing power of approximately one-half that of the other substances, showed the least prevention of browning. The poor showing made by the tallow-lard mixture was possibly due to the fact that it became rancid before the experiment was little more than started.

All the tests with gas absorbents, as well as those with air circulation, seem to indicate that internal browning is caused by the accumulation of certain materials in the nature of essential oils or other volatile substances.

A very perplexing question which then arises is that of the appearance of scald on the surface while internal browning develops in the flesh of the fruit. In an attempt to answer this question, the writer placed fruit under optimum conditions for the development of both diseases. That is, apples were placed in stagnant air at a temperature which favors the development of the disease. The results of these tests were very interesting. In every case it was found that the scald and browning developed almost simultaneously. The scald rapidly developed into what is termed "deep scald," while the browning diffused outward at a similar rate from the points of initial appearance about the vascular bundles. In many specimens where the diseases were retarded more on one side of the fruit than on the other, a very interesting comparison of their spread could be made. The generally observed appearance of scald on the surface without the internal browning and the reverse condition would then seem to indicate that these two regions of the fruit are most susceptible to the essential oils, or that these substances accumulate more pronouncedly in these than in any other region of the apple. The disease seemingly appears first in that region which is most

susceptible. In the Yellow Newtown, apparently, the region of greatest susceptibility is in the flesh, while in those varieties that scald readily, it is at the surface.

If internal browning and apple-scald are caused by the accumulation of essential oils, which can be removed by ventilation or by absorption, the question arises as to why the preventive action of ventilation and absorbents is less marked in the control of internal browning than in apple-scald. This difference in the effectiveness of the prevention is undoubtedly due to the fact that apple-scald is the result of the accumulation of deleterious substances on the surface of the fruit where the absorbent can be brought close to them. Internal browning, on the other hand, is caused by an accumulation of the deleterious substances deep in the tissues, from whence they can be removed only by the very slow process of reducing their concentration at the surface, thereby inducing them to diffuse outward.

INCREASE IN PERMEABILITY PRIOR TO THE APPEARANCE OF INTERNAL BROWNING

If internal browning is due to the action of some deleterious substance which tends to accumulate in the flesh of the apple under storage conditions, there should be some evidence of its action before the browning actually occurs. By this it is meant certain alterations will occur in the cells which will permit the browning to take place. Possibly the most important, as well as the most probable, change which could take place is that of altering the permeability.

The changes in permeability were determined by measuring the resistance offered by the tissue to the passage of an electric current. Electrodes for this purpose were patterned after those used by Small (*15*). The electrodes were mounted so that they stood 2½ mm. apart and in such a manner that they would be pressed ½ cm. into the tissue. The measurements were made by the Kohlrausch method. The fruit was cut as in making the observations upon the browning and the electrodes were then pressed into the various regions in which measurements were to be made. The readings were made by bringing the minimum point to the same position on the bridge each time. These readings, therefore, indicate only the relative resistance of the different regions in the fruits. (For the information of some readers it may not be amiss to state that a decrease in the resistance offered to the passage of an electric current is interpreted to mean an increase in the permeability of the cells.)

Since it was impossible to obtain the above apparatus until late in the season, the results which are recorded in Table X give only one stage in the permeability changes that occur during the course of an entire storage season. All the measurements, with the exception of those given under tests No. 4, 5, and 11, were made in tissue which showed no browning.

TABLE X.—*Permeability at the surface and in the region of browning*

[Expressed in ohms resistance]

Test No.	Fruit tested.	Majority of readings.		Extremes of variation.	
		In region of browning.	At the surface.	In region of browning.	At the surface.
1	Apples of lots 6 and 9, stored at 8.3° C.	1,600 to 1,800..	1,600 to 1,800..	1,500 to 2,000..	1,500 to 2,000.
2	Same as (1), but stored at 5° C...	2,300 to 2,500..	2,600 to 2,800..	2,000 to 2,600..	2,300 to 2,800.
3	Same as (1), but stored at 0° C. (These lots browned at 0° C.)	1,100 to 1,300..	2,300 to 2,500..	1,100 to 1,800..	2,200 to 2,800.
4	Same as (3), but reading taken in trace brown tissue.	900 to 1,200....	2,300 to 2,400..	800 to 1,300....	2,000 to 2,800.
5	Same as (3), but reading made in severely brown tissue.	600 to 900.....	2,200 to 2,400..	500 to 1,100....	1,900 to 2,600.
6	Fruit from black bags, very resistant to browning at 0° C.	2,200 to 2,600..	2,600 to 2,900..	2,000 to 2,900..	2,300 to 2,900.
7	Apples from same tree as bagged fruit, very susceptible to browning at 0° C.	1,000 to 1,200..	2,300 to 2,500..	1,000 to 1,600..	2,000 to 2,700.
8	Virginia apples, very resistant to browning at 0° C.	2,500 to 2,700..	2,600 to 2,800..	2,300 to 2,800..	2,500 to 2,800.
9	Santa Cruz Mountain apples, very resistant to browning at 0° C.	2,200 to 2,500..	2,500 to 2,800..	1,700 to 2,500..	2,300 to 2,800.
10	Lots 3, 5, and 8, very susceptible to browning at 0° C.	1,000 to 1,500..	2,400 to 2,800..	1,000 to 2,000	1,700 to 2,800.
11	Same as (10), but readings made in moderately browned tissue.	700 to 900.....	2,300 to 2,600..	500 to 1,100....	1,600 to 2,800.

The figures of Table X show very definitely that there is a change in the permeability. At 8.3° C., where browning does not develop, there was an increase in permeability. These apples, however, were rapidly approaching storage breakdown, due to overripening at this relatively high temperature. The permeability had not increased in the apples stored at 5° where the ripening process was much slower and where the fruit remained free from browning. This was also true of the fruit at 0° which was resistant to the browning. In the fruit stored at 0° that which was susceptible to the browning, there was a greater increase in permeability in the interior of the specimens than occurred in the fruit stored at 8.3°. The fruit at 0°, nevertheless, was not approaching storage breakdown but incipient browning. In the specimens showing browning the permeability continued to increase with the advance in the severity of the disease. Therefore these data seem to indicate that, just prior to and accompanying the end of the storage life of these apples, there is a very marked increase in permeability, regardless of whether deterioration is brought about by storage breakdown or by internal browning.

INCREASE IN PERMEABILITY DUE TO ESSENTIAL OILS

If internal browning be due to the accumulation of essential oils or similar deleterious substances which change the permeability, these oils should also increase the permeability when applied to the surface of the cut fruit. In order to test this property of these substances, several essential oils in great dilution were applied to the fruit about the electrodes of the conductivity apparatus. After the initial resistance was taken a drop of the solution was applied around the electrodes. Then the reading of the resistance was made every 5 minutes for a period of 20 minutes. The results of these tests were recorded in Table XI.

TABLE XI.—*Effect of essential oils upon the permeability of apple tissue*

[Expressed in ohms resistance]

Treatment.	The initial resistance.	Resistance at various intervals after the application of the essential oils.			
		After 5 minutes.	After 10 minutes.	After 15 minutes.	After 20 minutes.
Control, no treatment (current on continuously)	2,900	2,800	2,800	2,400	2,200
One drop of 0.1 per cent solution of amyl acetate	2,800	1,600	800	550	350
One drop of saturated solution of amyl valeriate	2,900	1,600	1,000	800	600
One drop of 1 per cent solution of acetaldehyde	2,900	1,600	1,000	750	500
One drop of 0.001 per cent solution of amyl acetate	2,900	2,000	1,100	900	600
One drop of 0.001 per cent solution of acetaldehyde	2,700	2,100	1,200	900	800
Water about the electrodes	2,800	2,400	2,200	2,100	2,000

The figures in Table XI show conclusively that essential oils increase the permeability when brought in contact with fruit tissues. These data indicate also that only a very small accumulation of essential oils might be sufficient to increase the permeability of apple cells, allowing the oxidase and substrates to come in contact, thus resulting in the browning. This is especially true when the greatest dilutions of the substances used in these tests are compared with the normal essential oil content of some apples as given by Power and Chesnut (*14*).

GENERAL DISCUSSION

In view of the relation of the browning with lower mean temperatures, it seems possible that the more severe browning of the mature fruit was due to exposure to the lower temperature which prevailed during the latter part of the harvesting season. The fruit of the second picking was exposed to a mean temperature of about 2.5° C. below the mean temperature of the growing season for three weeks after the fruit of the first picking was harvested. The fruit picked November 22 was exposed for six to eight weeks to the influence of a mean daily temperature of 2.5° to 8° below that prevailing at the time and before the first picking was made. As a whole, these data point to the possibility that the low temperature favors those conditions within the fruit which are necessary for the development of browning. This weakness in the fruit, if it can be considered as such, may be due to an abnormal development of the protoplasmic structure of the apples or to an accumulation of some deleterious substance which brings about a more rapid cessation in the normal functioning of these structures in storage. This seems probable since there was no appreciable difference between the resistant fruit and the fruit very susceptible to browning in constituents such as sugars, acid, and the P_H value of the expressed juice, which, it is generally believed, might influence a reaction of this sort through their effect upon the equilibria within the cells.

The accumulation of essential oils or similar deleterious substances also seems to be rather closely linked with the weakness in these apples that shows up in storage. This is indicated by the great reduction in the amount of browning that is brought about through the employment of air circulation or the impregnation of the wrappers with good absorbents for these substances. It has been further demonstrated that the permeability of the cells, which is the most probable change that might precede this browning or similar reactions, is increased very rapidly by essential oils when applied even in great dilution to the apple tissue. It was also found that there was an increase in permeability prior to and accompanying the death of the cells in the apple regardless of whether death was due to the usual type of storage breakdown (which is the result of overripening) or to internal browning.

The data obtained upon the relationship of temperature and the accumulation of essential oils or similar volatile substances to the browning, although not conclusive, point to several possibilities concerning the cause of this disease. When these apples are grown at a mean temperature as low as that of the growing season of the Pajaro Valley, they may fail to develop normally, hence when they are placed in storage the flesh of the fruit exhibits a susceptibility to injury through the action of the volatile emanation of the apple. This is indicated by the behavior of the fruit from different regions as well as by that from under the tent and in the black bags. This lower temperature may not only affect the development of the fruit but also apparently influence the production or accumulation of the volatile substances which are immediately responsible for the browning. This becomes apparent when the great difference in the amount of browning which developed at the several storage temperatures is taken into account. Seemingly there is a greater production of these substances at the lower temperatures, or otherwise they must accumulate more rapidly in those regions of the torus that are first to show the browning.

The reduction in the development of the browning by the use of gas absorbents also indicates that these volatile substances are present in injurious amounts at the lower temperatures under the ordinary conditions of storage. The more rapid accumulations of the deleterious substances may seem the more probable way of accounting for the injurious amount of these substances when the decrease in their volatility and the decrease in the permeability of the tissue at the lower temperatures is considered. However it is likely that there is also a greater production of these substances under the somewhat abnormal conditions of the lower temperatures of storage.

The nature of the process which results in the browning becomes of interest in connection with the preceding possibilities as to the cause of this trouble. Plausible explanations of this process could possibly be ascribed to an increase in the permeability of the protoplasm which permits the enzyms and their substrates to mix, or to the inactivation of some inhibiting substance. These changes might be brought about by the accumulation of certain substances such as the essential oils which are produced by the apple in storage and which apparently have a toxic effect upon the protoplasm of the cells.

In the normal cells the enzyms are prevented from acting upon their substrates by inhibitors or through lack of contact due to the possibly impermeable nature of the phase surface of the protoplasm. When the phase arrangements in the protoplasm, however, are acted upon

by toxins, these substances are no longer prevented from coming into contact. Similarly, the toxins may act upon the inhibitors to inactivate them. As a result of this liberation, the tannins of the apple cells may be oxidized to a brown by the oxidase which is also present in the mature fruit. It has been indicated by Bartholomew (*3*) that changes similar to these precede the blackening of the tissue in blackheart of potatoes. This explanation of coloration based upon a change in the permeability is also supported by the fact that before browning occurs there is a great increase in permeability of the cells as indicated by the conductivity measurements.

SUMMARY

(1) Internal browning is a nonparasitic disease of the large isodiametric cells of the flesh of the fruit.

(2) All Yellow Newtown apples, regardless of where grown, may in some years be susceptible to internal browning. This variety when grown under conditions prevailing in the Pajaro Valley has proved to be much more susceptible to this disease than when grown in other fruit regions.

(3) The later the fruit was picked, the greater the amount of browning which occurred in storage.

(4) No browning occurred even after four to six months storage in any of the fruit stored at 8.3° C. or above.

(5) The browning at the end of six months' storage at 5° C. was very limited and mild, and did not detract from the commercial value of the fruit.

(6) At 2.2° C. approximately 70 per cent of the apples showed browning by April 1, figures for each of the three seasons show. During the season 1920–21 which was average with regard to browning, only 50 per cent of the fruit stored at this temperature was marketable on April 1, 1921.

(7) At 0° C. practically all the fruit showed browning before April 1, each season. Only 20 per cent of the fruit stored at this temperature was marketable on April 1, 1921.

(8) A lowering by about 3° C. of the mean orchard temperature during the growing season, by tenting or shading a tree, greatly increased the susceptibility of the fruit to browning. After 4½ months' storage at 0° the fruit of the tented trees showed 25 per cent less of normal specimens than that of adjacent trees which were naturally exposed.

(9) An increase of 4° C. in the mean orchard temperature, by bagging individual apples in black cloth during the growing months, markedly increased the resistance of the fruit to this disease. The bagged apples showed 66 per cent more of normal specimens after 4½ months' storage at 0° than the naturally exposed fruit of the same trees.

(10) The browning was greatly reduced by ventilating the fruit.

(11) The browning was also reduced by impregnating the wrappers with oils and waxes which were good absorbents of essential oils.

(12) By measuring the electrical resistance of the apple tissue, it was found that there was an increase in permeability prior to the end of the storage life of the apple, regardless of whether death was due to the usual storage breakdown or to internal browning.

(13) It was demonstrated that essential oils when applied to the apple tissue even in great dilution rapidly increase its permeability.

(14) The data indicate that internal browning is due to the accumulation of essential oils or similar deleterious substances which are produced by the apples in storage. This shows that internal browning and apple-scald are quite closely related with respect to cause.

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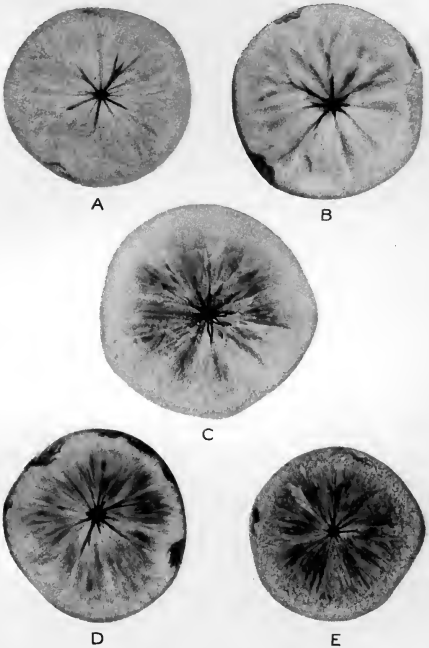
PLATE 1

A.—A normal apple.
B.—Average condition of trace browning.
C.—Average condition of slight browning.
D.—Average condition of moderate browning.
E.—Average condition of severe browning.

Internal Browning of the Yellow Newtown Apple

PLATE 1



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ON THE USE OF CALCIUM CARBONATE IN NITROGEN FIXATION EXPERIMENTS[1]

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In reviewing the literature on nitrogen fixation by soil bacteria one is impressed with the great variety of media that have been employed by different investigators. Many of these media, while generally satisfactory, have not proved entirely so when employed by other investigators with slightly different environmental conditions.

It is not the purpose of this paper to enter into any discussion of the relative merits of different media, but rather to call attention to a frequent fundamental difference and its possible bearing upon the success attending their use. This difference is the presence or absence of calcium carbonate.

Winogradsky (*9*)[2] in his original experiments on nitrogen fixation by anaerobic bacteria used a dilute solution of the various salts necessary to furnish the elements essential to growth. To this was added a simple sugar as a source of energy and an excess of calcium carbonate to neutralize the acids formed from the sugar. Winogradsky's medium has been almost universally adopted for anaerobic nitrogen-fixing experiments.

Beijerinck (*2*), studying the aerobic Azotobacter group of nitrogen-fixing bacteria, found that a 0.02 per cent solution of K_2HPO_4 in "Leitungswasser," to which was added a source of energy, furnished the necessary conditions for good growth of these organisms. Either the water or the inoculum must have furnished the other essential elements in sufficient quantity. The reaction of this medium was unaltered, the statement being made that—

> Die Nährlösung reagiert durch das K_2HPO_4 schwach alkalisch

and that—

> Die Alkalisch Reacktion ist für den versuch günstig.

Beijerinck preferred mannite or a salt of propionic acid as a source of energy because—

> Mannit kann nur schwierig und langsam, Propionate durchaus nicht der Buttersäuregärung anheimfallen.

Beijerinck further states that—

> Die Produkte die Oxydation sind Kohlensäure und Wasser.

However, he realized that in impure cultures from soil, organic acids might be formed. Beijerinck failed to secure appreciable fixation of nitrogen by pure cultures.

Lipman (*3*) began a study of the Azotobacter group of nitrogen-fixing organisms shortly after Beijerinck. He demonstrated that Beijerinck's failure to secure fixation in pure cultures was due to the unfavorable re-

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[2] Reference is made by number (italic) to "Literature cited," pp. 189–190.

action of his media. The media adopted by Lipman was composed of tap water 1,000 cc., mannite 15 gm., K_2HPO_4 0.5 gm., $Mg.SO_4$ 0.2 gm., a drop of 10 per cent solution of ferric chlorid, and enough sodium hydroxid to make the solution slightly alkaline to phenolphthalein. Lipman showed that within certain limits the quantity of nitrogen fixed was proportional to the quantity of sodium hydroxid added. He further demonstrated that the addition of $CaCO_3$, even to the medium made alkaline to phenolphthalein with sodium hydroxid, rendered it more favorable for nitrogen fixation. With regard to the influence of calcium carbonate Lipman (*4*) says:

> It is clear therefore, that the presence of calcium carbonate stimulated growth either directly by furnishing calcium, or indirectly by making available more phosphorus, sulphur, and magnesium.

However, Lipman apparently lost sight of the value of calcium carbonate, for he did not recommend its use in his laboratory guide (*5*).

Ashby (*1*), apparently following the lead of Lipman, proposed that the acidity arising from the phosphate be neutralized with sodium hydroxid and in addition an excess of calcium carbonate be added. The medium proposed by Ashby has been more widely used than any other. It has the following composition: Distilled water 1,000 cc., mannite 12 or 20 gm., $Mg.SO_4$ 0.2 gm., KH_2PO_4 0.2 gm., NaCl 0.2 gm., $CaSO_4$ 0.1 gm., and 0.5 gm. of $CaCO_3$ to each culture of 75 or 100 c. c. The phosphate is dissolved separately in a little water and made neutral to phenolphthalein with sodium hydroxid. Ashby found that the presence of calcium carbonate favored nitrogen fixation and that Azotobacter would sometimes develop in the presence of calcium carbonate but would not form a film if the carbonate were left out. Ashby also found that magnesium carbonate was even more efficacious than calcium carbonate, thus showing that calcium was not the essential constituent. Other investigators have since shown that other basic compounds can be substituted for calcium carbonate.

In addition to the three types of media just mentioned Löhnis and his students have made rather extensive use of soil extract to which was added K_2HPO_4 and mannite or some other simple organic source of energy. In comparing the fixation of nitrogen in a medium of this type with and without the addition of calcium carbonate Löhnis and Pillai (*7*) found, as a rule, slightly greater fixation where the carbonate was added. However, Löhnis failed to adopt the use of calcium carbonate generally in his work, or to recommend its use in his laboratory guide (*6*).

It remained for Stoklasa (*8*) to produce the necessary evidence for a correct understanding of the function of calcium carbonate in nitrogen-fixation experiments by demonstrating quantitatively the formation of organic acids in cultures of Azotobacter. A survey of the accumulated literature on the subject will show, however, that many investigators failed to realize the significance of Stoklasa's results.

Practically all investigators agree that a neutral or alkaline reaction is desirable, if not essential, for the best development of nitrogen-fixing organisms. In most work some effort is made to adjust the medium to an alkaline reaction before inoculating, but in many cases no effort is made to maintain such a reaction. Even the influence of the inoculum upon the initial reaction has usually not been taken into consideration.

So far as the writer is aware no one has ever reported a detrimental effect upon nitrogen fixation from the presence of calcium carbonate in

the medium, even when present in large excess. This is an important consideration, however, since if it has no toxic effect upon the organisms it may be added in excess of initial requirements and thereby tend to maintain a favorable reaction throughout the experiment.

There are a number of isolated experiments such as those cited above showing the effect of calcium carbonate upon the growth of nitrogen-fixing organisms. In the course of some experiments, conducted by the writer, in which the relative growth of Azotobacter from a large number of different soils was compared there was an opportunity to observe the effects of $CaCO_3$ on nitrogen fixation.

METHODS

The medium employed had the following composition: Mannite 20 gm., K_2HPO_4 0.2 gm., $MgSO_4$ 0.2 gm., NaCl 0.5 gm., $FeCl_3$ trace, and water 1,000 cc. Two-hundredths gm. of $CaCl_2$ was sometimes added, although in most cases because of the high calcium content of local soils the $CaCl_2$ is not essential and was without effect. In those tests to which no $CaCO_3$ was added the medium was always rendered slightly alkaline to phenolphthalein with sodium hydroxid. When $CaCO_3$ was to be added the medium was sometimes first rendered slightly alkaline to phenolphthalein and at other times the reaction was unaltered prior to the addition of the $CaCO_3$. Fifty cc. of the medium were placed in 300-cc. Erlenmeyer flasks, and the $CaCO_3$ was added in the form of sterile powder just prior to inoculation. No superiority is claimed for this medium over a score of others that might have been used. Obviously a medium with as variable composition as that containing tap water or soil extract would be unsuited for comparative work that must extend over a long period of time.

Samples were always set up in duplicate and total nitrogen determinations made on the whole sample. Total nitrogen determinations were also made in duplicate upon the inoculum. The inoculum consisted of 10 cc. of the supernatant suspension prepared by shaking one part of soil (50 to 100 gm.) with two parts of water and allowing to settle long enough for the larger particles to sink to the bottom. It is believed that such an inoculum is more representative of a mass of soil than 5 gm. of soil, and at the same time the quantity of solid material added is not sufficient to interfere in the least with total nitrogen determinations. Incubation was at room temperature for three weeks. In estimating the quantity of nitrogen fixed that present in the inoculum was deducted. Only the average of check determinations were recorded.

Frequent examinations of the cultures were made, both macroscopically and microscopically to ascertain whether Azotobacter were present. If Azotobacter make an appreciable growth it can usually be recognized by the appearance of the film. A microscopic examination of an unstained mount from such a film will reveal an unmistakable picture. A film is sometimes encountered which at certain stages in its development resembles quite closely an Azotobacter film, which, under the mircoscope, is found to be composed almost entirely of filimentous fungi, no organisms typical of Azotobacter being observed. In other instances nontypical films examined under the microscope would be found to be composed largely of fruiting fungi, the spores of which often closely resembled individual cells of Azotobacter.

If these examinations failed to reveal organisms morphologically similar to Azotobacter they were regarded as absent. Owing to the above-mentioned complex conditions it is quite possible that Azotobacter were sometines reported present when in reality they were absent and vice versa. The end to be gained did not seem to justify the large amount of time that would be necessary to isolate and identify Azotobacter from the various soils. It is believed that if Azotobacter are not present in a soil in sufficient numbers and vigor to develop unmistakable evidence of their presence by the methods just described, for practical purposes they may as well be absent.

RESULTS

Several hundred samples of soil from Kansas and other States have been examined by the methods described above. The following is a comparison of the average quantity of nitrogen fixed by 200 soils.

All samples	5.87 mgm.
Presence of $CaCO_3$	7.10 mgm.
Absence of $CaCO_3$	4.60 mgm.
Azotobacter film formed	7.70 mgm.
No Azotobacter film formed	4.10 mgm.

There were only two samples that failed to show some nitrogen fixation, and both of these were in media containing no $CaCO_3$.

When calcium carbonate was added to the medium an Azotobacter film was formed from 117 samples, or 58 per cent of the soils. The average quantity of nitrogen fixed in these was 8.1 mgm. The average quantity of nitrogen fixed in the 83 samples having no Azotobacter film was 5.7 mgm.

When no calcium carbonate was added to the medium an Azotobacter film was formed from 75 samples, or 38 per cent. These had fixed on the average 7.1 mgm. of nitrogen. One hundred and twenty-four samples, or 62 per cent, produced no Azotobacter film, and the average nitrogen fixed for these was 3.1 mgm.

Twenty-seven samples, or 14 per cent of all soils examined, fixed more nitrogen in the samples to which no $CaCO_3$ was added, while 173 samples, or 86 per cent, fixed larger quantities of nitrogen in those samples receiving an addition of $CaCO_3$. The microscope revealed Azotobacter in cultures from 130 samples, or 65 per cent of all. No Azotobacter were observed in cultures from 70 samples, or 35 per cent of all. Some nitrogen fixation took place in practically all samples inoculated regardless of the source of the soil.

There were 12 samples containing Azotobacter or organisms resembling Azotobacter that failed to form an Azotobacter film. The average nitrogen fixed by these 12 soils where $CaCO_3$ was added was 6.2 mgm. The average in the absence of $CaCO_3$ was 3.1 mgm. This is 0.5 mgm. higher than the average fixed by those giving no film when $CaCO_3$ was added and exactly the same as those giving no film in the absence of $CaCO_3$. It is highly probable, therefore, that some soils contain Azotobacter but are incapable of initiating the growth of an Azotabacter film in a mannite culture solution.

Practically all soils that failed to produce Azotobacter films formed more or less heavy films of fungi in the medium containing $CaCO_3$. As a rule, no such films were formed in the medium containing no $CaCO_3$. Whether or not these fungi are associated with the increased

nitrogen fixation under these conditions is not known. It is possible that the films of aerobic fungi were a factor in maintaining anaerobic conditions and thereby stimulated nitrogen fixation by anaerobic organisms. The fungi were usually slow to develop, indicating that their development depended upon some subsequent change, possibly the accumulation of nitrogen or of calcium salts of some organic acids. The number of samples that failed to develop fungi films were hardly sufficient to give a comparison of the quantity of nitrogen fixed in the presence and in the absence of a film. It is perhaps significant, however, that the average quantity of nitrogen fixed by the 7 samples which failed to grow films of fungi in the presence of $CaCO_3$ was only 2.6 mgm., compared with 6.0 mgm. for the 76 samples producing a film. This would indicate that the fungus growth is in some way associated with the fixation of nitrogen either as a factor or as a result.

It is evident from the preceding data that practically all soils will bring about the fixation of appreciable quantities of nitrogen under the conditions of these experiments. A large percentage of the soils examined however, failed to initiate the growth of Azotobacter. There are, therefore, other organisms which are capable of fixing appreciable quantities of nitrogen. Such organisms seem to be quite widely distributed in nature.

CONCLUSIONS

(1) The quantity of nitrogen fixed in the presence of Azotobacter is greater than when it fails to develop.

(2) The number of soils capable of initiating the growth of Azotobacter under the experimental conditions here described is greater by 20 per cent if $CaCO_3$ is added to the medium than if it is omitted.

(3) The quantity of nitrogen fixed in a medium containing $CaCO_3$ is, for practical purposes, always equal to and in most cases greater than when $CaCO_3$ is not present in the medium.

(4) The presence of $CaCO_3$ exerts a greater beneficial effect upon those organisms, other than Azotobacter, that bring about the fixation of nitrogen than upon Azotobacter itself.

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GUMMOSIS OF CITRUS[1]

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PART I.—GUMMOSIS DUE TO PYTHIACYSTIS CITROPHTHORA

INTRODUCTION

The purpose of Part I is to present the results of an investigation, begun in 1912, into the nature, causes, and manner of development of certain types of gummosis of Citrus trees. In the three parts of this paper the term gummosis will be employed in the broader sense, in which it applies not only to the process of gum formation but also to the diseases or pathological effects in which gum formation is one of the conspicuous features. When employed in connection with specific diseases, supplementary terms will serve to show its modified meaning.

Part I will deal mainly with the causal relation of *Pythiacystis citrophthora* Sm. and Sm. to one of the most widespread and destructive forms of Citrus gummosis in California. The relation of another fungus, *Phytophthora terrestria* Sherb., to a similar disease, mal di gomma, in Florida is also included. Part II will present the results of an investigation into the relation of *Botrytis cinerea* Lk. and other fungi to other types of gummosis in Citrus. The last section of this paper, Part III, will deal with gum formation as such, the conditions influencing its formation, and its relation to diseases. The results of investigations on the control of some of these diseases will be discussed in a bulletin to be issued from the California Agricultural Experiment Station and will therefore receive only a brief mention in this paper. A preliminary report regarding control has already been published by Fawcett, (*24*,*26*).

Previous investigators had come to the conclusion that all gum diseases of Citrus trees in California originated independently of microorganisms, according to Smith and Butler (*56*). It was held that these diseases were largely autogenous in their nature and were frequently induced through the effects of certain climatic or soil conditions alone. It now appears evident that these environmental conditions can not by themselves initiate all the severe forms of gummosis in Citrus earlier attributed to them, although many of these factors are found to play, as they do in most parasitic diseases, an important rôle as contributing

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[2] This investigation was started in the spring of 1912 under the auspices of the State commission of horticulture and was transferred to the University of California in the fall of 1913. The author wishes to acknowledge the encouragement given by Dr. A. J. Cook and Prof. R. E. Smith at the beginning of the work, the assistance rendered by J. D. Culbertson, J. A. Prizer, and many other Citrus growers. Acknowledgment is also due to Bruce Douglas for laboratory assistance during a portion of the time of the investigation and to those members of the staff of the College of Agriculture who aided by many suggestions and criticisms.

conditions which favor infection and invasion of the host by causal parasites.

Since, however, a nonparasitic explanation for these gum diseases in California had become so firmly established in the literature of the subject, it has been necessary to carry on a large series of carefully controlled experiments before concluding that fungi are necessary factors in the initiation and development of certain of these diseases. For the same reason the author deems it necessary to present his data in considerable detail.

The failure to recognize the parasitic factor in previous investigations, as regards Pythiacystis gummosis at least, is probably due in part to the following facts which will be brought out in more detail later: (1) The fungus initiating the disease is found in the bark in its vegetative stage only; (2) it dies out rapidly in tissue already invaded, leaving only a fringe or band of live mycelium at the advancing edges of the invaded tissues; (3) under adverse temperature and moisture conditions the organism frequently dies completely in the tissue, especially on resistant varieties, giving the appearance of a pure physiological effect; (4) in advanced stages the stimulus to gum formation spreads out far in advance of the band of invading mycelium, producing an outer gummous zone in which localized gum pockets are frequently formed. This outer gummous zone is usually free from the causal organism. Any attempt to find the organism in regions other than the narrow band at the outer rim of the invaded zone would result in failure.

HISTORICAL REVIEW OF CITRUS GUMMOSIS

In this historical review of the literature of Citrus gummosis it has been found impossible to separate with certainty the different forms of gummosis which recent investigations have shown to be distinct. It is believed, however, that most of the more destructive, rapidly developing forms mentioned by various writers were similar to those represented by Pythiacystis gummosis in California and mal di gomma in Florida.

Two of the earliest European writers on Citrus, Ferrari (*29, p. 156–158*) in 1646[3] and Sterbeek (*60, p. 177*) in 1682, briefly discuss certain forms of Citrus gummosis occurring in the orange plantings of Europe. Two other early writers on Citrus referred to by Savastano (*52*) as mentioning some minor form of gummosis were Clarici in 1726 and Corrado in 1787.

The first highly destructive type of gummosis of Citrus on record appeared in the Azores in 1834. Sweet-orange trees which had grown to the age of 200 to 300 years, and which were producing 6,000 to 20,000 oranges apiece, were found by Fouque (*32, p. 837*) to be affected with a very destructive form of gummosis (*1*). Yellow gum is mentioned as exuding on the trunks near and sometimes beneath the surface of the ground. The trees put on heavy crops of fruit, and the leaves turned yellow and fell off in great quantities. This brief description appears to indicate a form of disease resembling Pythiacystis gummosis. It was supposedly transferred from the Azores to the vicinity of Lisbon, Portugal, where a similar disease appeared in 1865.

A similar type of Citrus gummosis, according to Savastano (*52*), was extremely destructive in many Mediterranean localities. It was established in Messina, Italy, in 1863 and was reported near Reggio in 1864.

[3] Reference is made by number (italic) to "Literature cited," pp. 232–235.

The disease advanced rapidly from Messina, passing into the province of Catania to Acireale. In 1865 it was established at Palermo, raging severely there until 1870. In all the Sicilian Citrus orchards the gummosis was present in such intensity as to constitute a true epidemic which destroyed all the trees. The orchards were later replanted with sour-orange stocks. In 1870 the disease was established in the region of Genoa. It became scattered (not epidemic) in the orchards of the Naples, Amalfi, and Gargano regions. Briosi (*11*) estimates the damage from gummosis in Italy as $2,000,000 from 1862 to 1878.

In Greece, in Tunis, and in Spain gummosis became distributed in varying degrees of intensity. Briosi refers to its destructiveness in the Balearic Islands (near Spain) in 1871. In more recent years a similar disease has been reported in the Oasis of Tripoli by Leone (*44*) in 1918. Gummosis was attracting attention in Cape Colony, South Africa, in 1891.

In Australia a destructive gummosis is referred to by Alderton (*3*) as occurring in New South Wales between 1860 and 1870, by McAlpine (*46*) as occurring near Sydney in 1867 and in Queensland in 1876. It is reported by Kirk (*43*) in New Zealand in 1885.

In the United States, records place its appearance at about the year 1875 in California according to Mills (*48*) and 1876 in Florida by Curtiss (*17*). In Florida, as footrot or mal di gomma, it attracted serious attention in 1879, following a very wet year according to Hume (*41*). Moore (*49, p. 128–132*), in 1881, speaks of its recent appearance, and Swingle and Webber (*64*) in 1896 report it as still gradually spreading. In California, gummosis was a serious trouble in nearly every Citrus locality by 1878. It was spoken of by Garey (*34, p. 81–82*) as the only Citrus disease of importance at that time. The horticultural literature of this period indicates that the discontinuance of the use of the common lemon, lime, and citron as stocks and the general adoption of the sour orange and sweet orange as the principal stocks in California were due to this disease.

Some of the other localities in the American continents where gummosis has been an important disease are reported in Paraguay by Bertoni (*9*), in Brazil by Averna-Sacca (*5*), in Mexico by Gandara (*33*), in Cuba by Cook (*15*) and Cook and Horne (*16, p. 35*), and in Porto Rico by Stevenson (*62*).

Viewing the history of Citrus gummosis from the investigational standpoint, Briosi (*11*) studied a gummosis (mal di gomma) in Italy and described a fungus, *Fusarium limoni* Briosi, which he considered to be a factor in the development of the disease. McAlpine (*46*) regarded a similar type of severe gummosis in Australia as undoubtedly of an infectious nature and referred to the same fungus as the causal agent. His description of the disease indicates that it is similar in character to the form which Pythiacystis gummosis takes on large orange trees in California. It is quite possible that the "slender wandering filaments" which he found penetrating the tissue may have been those of a Pythiacystis-like fungus. In this connection it is of interest to note that in a letter to the writer in February, 1917, G. P. Darnell-Smith of the Department of Agriculture, New South Wales, reports finding *Pythiacystis citrophthora* on specimens of Citrus affected with a gum disease sent from the Norfolk Islands, east of Australia. Later, F. Stoward, in a letter of October 1917, reports having isolated *P. citrophthora* from lemon fruits in Western Australia and having confirmed its pathogenicity by inoculation.

Comes (*14*) produced gumming in Italy by inoculations with a bacterium which he called *Bacterium gummis* Comes. *B. gummis* is also mentioned by Averna-Sacca (*5*) in connection with gummosis of Citrus in Brazil and by Gandara (*33*) in connection with gummosis in Mexico.

A number of other investigators, among whom were Swingle and Webber (*64*), considered the severe gum diseases as probably infectious and due to some organism invading the bark, but little work of an experimental nature with Citrus appears to have been done until recent years. Fawcett (*22* and *23*) showed that a gumming of branches of Citrus in Florida was due to the presence of a fungus similar to *Diplodia natalensis* Evans. The same fungus was found by Burger (*28*) to be the causal agent in a twig disease of the peach.

Not alone in Citrus but in a number of other plants, especially Prunus, definite diseases accompanied by large gum exudations have been shown in recent years to be due to specific organisms. Among these may be mentioned forms of gummosis on cherries reported by Aderhold and Ruhland (*2*), Griffin (*37*), and Barss (*8*); on apricot and other deciduous fruits reported by Barrett (*6*); and on plum reported by Higgins (*39*).

There have been other investigators who concluded that the severe gum diseases in Citrus were due, not to the invasion of organisms, but to certain stimuli operating upon the affected parts. Savastano (*51*) made a comparative study of gummosis in both Prunus and Citrus and concluded, because the histology was the same in both genera, that gummosis in Citrus arose largely from wounds or traumatisms. This conclusion was in agreement with the views of many previous investigators as to gummosis in Prunus. Among these were Sorauer (*58*) in Germany, Prillieux (*50*) in France, and others. Savastano, in a number of papers in recent years on gummosis in Citrus, has modified this earlier view. He has distinguished clearly between mere gum formation as a general phenomenon and gummosis in connection with definite diseases. In one of these later publications (*52*) he accepts Comes's (*14*) conclusions as to the bacterial origin of the definite disease type and concludes that the aggravating conditions or causes influencing the occurrence of gummosis are lack of light, clayey, water-holding soils, level ground as compared to hillsides, excessive moisture about the roots, wounds from grafting or from digging about the roots and so forth. Most of these contributing conditions are those favorable for gummosis due to *Pythiacystis citrophthora* in California, or mal di gomma due to *Phytophthora terrestria* in Florida. In this connection it is of interest to note that R. E. Smith (*57*) found lemon fruits affected by typical brownrot like that due to *Pythiacystis citrophthora*, in a low-lying, poorly drained grove in Sicily, where footrot was very prevalent as mentioned by Fawcett (*25*).

Still other investigators have concluded that organisms are not at all involved in the initiation of gummosis but that certain conditions within or without the host are solely responsible for the diseases. Bertoni (*9*) in Paraguay appears to have considered Psorosis and a Pythiacystis-like form as phases of the same disease and concludes that poor condition of nourishment is the primary contributing cause. Later (*10*) he believed that shade was a corrective for gummosis. Grossenbacher (*38*) concluded that untimeliness of bark growth in connection with drought and low temperatures was related in some unknown way to gummosis of the mal di gomma type in Florida.

The previous views regarding Citrus gummosis in California, based in part on investigations on Pythiacystis gummosis, are fairly well indicated by the following quotation from Smith and Butler (*56*):

> The lesions, ulcers, or affected areas produced, are not primarily the seat of the trouble. They represent rather the effect of what may be called a general constitutional derangement showing itself by external outbreaks or symptoms at whatever points may chance to be most susceptible. ..... What may be called a primary weakness exists back of the visible symptoms, and in this weakness can be sought the fundamental cause and nature of the disease. It is, therefore, not necessary to identify a parasite, or strikingly evident climatic or soil conditions, to account for diseases of this class. ..... It may be said here without extended discussion, that in no case have we been able to recognize or demonstrate the presence of any fungus, bacterium, or other parasitic organism as the cause of any form of citrus gum disease.

Some of the paramount conditions mentioned by Smith and Butler as contributory to the occurrence of gum disease are the accumulation of soil against the trunk, excessive moisture where poor drainage or careless irrigation exists, the heaping of manure against the trunk, low budding, lack of loose aerated soil and sweet orange stock on heavy soils. It is now known that these conditions are favorable for infection by the fungus and the development of the disease.[4]

Previous investigations and observations on Citrus gum diseases had led, therefore, to three general hypotheses: (1) That gum diseases were brought about by organisms capable of infecting and invading the tissue under certain contributing conditions; (2) that gum diseases were due to wounds or other external stimuli other than microorganisms acting immediately on the parts affected; (3) that gum diseases arose autogenously being due to internal derangements of the host brought about by or without the influence of certain factors of the environment acting on the host as a whole.

PYTHIACYSTIS GUMMOSIS

SYMPTOMS

Pythiacystis gummosis, with its associated rot of the fruit, is probably the most widespread and destructive of the Citrus gum diseases. The most striking features of the disease on the common lemon,[5] which is the most susceptible form, are copious exudations of gum and large dead patches of bark on the trunk, followed by yellowing and dropping of leaves. On old sweet-orange trees and other partially resistant forms, the dead patches are usually smaller and show a greater tendency to become self-limited.

In the earlier stages of the disease (Pl. 1, A; 3, A; 5, A) the extent to which the bark is invaded by the fungus can only be determined by lightly scraping the thin surface of bark in the vicinity of the exuding gum until the green color is seen. The margin between the invaded tissue and the sound bark then shows only as a difference in color, the normal green shading off gradually into a drab. The bark is not softened but remains firm. After a considerable time it becomes sunken and begins to crack longitudinally.

[4] In this connection it should be mentioned that Smith (*55*) was also among the first to accept the conclusions to which the first experimental work by Fawcett (*24*) led, although these were opposed to the views which he had formerly held.

[5] The names of species and varieties of Citrus will be used in accordance with Swingle (*63*), as follows: common lemon, *Citrus limonia* Osbeck; rough lemon, a horticultural variety of *C. limonia* Osbeck; sweet orange, *C. sinensis* Osbeck; sour orange, *C. grandis* Osbeck; citron, *C. medica* Linn; trifoliate orange, *Poncirus trifoliata* Raf. The word lemon when used alone will refer to the common lemon and the word orange to the sweet orange.

On healthy, rapidly growing lemon trees the area of killed and darkened bark, elliptical to irregular in outline, is usually 15 to 30 cm. in vertical length and half that in width, when the gum first becomes apparent. By that time the fungus has been invading the tissue usually for a period of from two to four months. The removal of the bark at this time will show that the outer margin of the invaded zone is about coextensive with that seen on the surface (Pl. 3, B). Most often in young trees the death of the cambium and inner bark precedes slightly the death of the outer bark. The upward extension from the point of infection is usually many times its lateral and usually much greater than its downward extension.

In the cambium region surrounding an actively invaded area evidence of an influence extending from the margins of the dead bark will be found. There is simply a production of clear, watery gum which seems to originate in the region of the embryonic wood among the live cells without any apparent fermentation or decay. This region which is not yet darkened beyond the invaded portion, will be spoken of in this paper as the "outer gummous zone." It may in time extend considerable distances upward and downward and small distances laterally from the margin of the invaded zone. It has been traced for 60 and 90 cm. upward. The extent of this outer gummous zone varies especially with the age and rapidity of development of the disease lesion and the condition of the tree. In some cases it is much larger than the invaded zone and in others much smaller.

The inner surface of the bark in the invaded zone in a lesion of considerable size varies from mineral brown to burnt umber or fawn color [6] and the same discolorations will be found on the surface of the wood just at or beneath the cambium. (Pl. 3, A, B.) The discoloration does not extend far radially (usually only 2 to 5 mm.) into the woody layers. The cambium region in the outer gummous zone is chamois to yellow ochre in color, fading out gradually at the margins into the normal white color of the sound woody surface.

Frequently when the bark is irregular in contour, gum pockets will be formed, 2.5 to 5 cm. in longest axis, due to the rapid and unequal formation of gum. The gum accumulates near the cambium and by pressure separates the bark from the wood at certain places, forming definite pockets. The pressure is usually relieved by a break in the bark before the pockets become large. A few deeper gum pockets of considerable size have also been found, situated in the outer gummous zone, beneath layers of wood 3 to 6 mm. in thickness, showing accumulations of gum under pressure. The gum, which is watery and clear when first formed, hardens as it comes to the surface, apparently by loss of water, and finally becomes brittle. On the surface the hardened gum usually ranges from mahogany to chestnut in color.[6] The gum accumulates on the surface in long narrow ridges (Pl. 1, A, B; 4, A; 5, A, D; 7, A) or in oval masses, or runs down and collects in masses on the soil, depending upon the rapidity of formation and the dryness of the air. During periods of heavy dews and rains it gradually dissolves and disappears (Pl. 1, D; 5, E).

Only in rare cases where the surface of the bark is moist during the development of the disease is there any sign of fungus development to

[6] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

be detected without a microscope, and even then it is somewhat difficult to see because of the hyaline nature of the vegetative hyphae. The invading hyphae frequently die out rapidly behind the marginal fringe of advance, and quite often they die out completely over a part or all of this outer margin, so that progress of the disease is checked or entirely arrested. Such cases are often found in trees having some resistance, especially in orange and pomelo trees, or where the weather conditions subsequent to infection become unfavorable to the parasite.

In trees on which the disease has been present for a long time, the dead bark over the invaded portions dries, shrinks, and cracks. The larger cracks are mostly vertical, with smaller horizontal cracks (Pl. 1, D; 2, D, E; 5, E, F). A thin layer of the wood immediately under the invaded bark will usually be found to be infiltrated with hardened reddish brown gum which protects the under layers from rapid drying out and appears to protect the wood to a considerable extent against the entrance of wood-rotting fungi.

On old sweet-orange trees, the invaded areas are usually less extensive and more restricted laterally than on the common lemon. There is usually less gum than on the lemon. With the orange there is greater tendency than with the lemon for the invading fungus to die out—for the invaded area to become self-limited. Frequently the invaded areas on old sweet-orange trunks extend upward from the soil surface as narrow tongues of killed bark. On younger and frequently on older vigorous orange trees growing on heavy clay soils the disease may assume much the same characteristics as on the common lemon.

ISOLATION AND IDENTIFICATION OF PYTHIACYSTIS CITROPHTHORA

Culture tests were made from different parts of gummosis lesions, beginning at the center of a lesion and taking samples about 1 cm. apart upward, downward, and laterally from the center. A number of organisms were found, as would be expected, in the dead and discolored bark tissue. From recently killed and dying bark tissue at the advancing margin of the darker areas (invaded zone) a fungus was obtained which was afterwards found to be identical with that producing brownrot mentioned by Smith and others (57) of lemon fruits and which, even before its identity with *Pythiacystis citrophthora* was known, was shown to be capable of reproducing the same type of bark disease. Beyond the margin of the killed areas, however, all through the outer gummous zone the inner bark tissue was usually found to be entirely free from microorganisms, although internal gumming had set in 15 to 60 cm. above and somewhat less below the margin of the killed tissue.

Pythiacystis citrophthora was first isolated from the bark of diseased lemon trees in September, 1912, at Whittier, Calif., but its identity was not recognized at that time.

The method which the author finally found most useful for determining the fungus was to cut out with a sterile scalpel small bits of diseased tissue from the margin of the invaded portion and place them in tubes of corn meal agar. After two to four weeks' growth on this medium the characteristic sporangia were formed. By this method pure cultures were readily obtained, and when other organisms were present as contaminations *Pythiacystis citrophthora* could be identified by its characteristic sporangia. The Pythiacystis may readily be separated from contaminating fungi by transferring the mixed culture to a jar of water

containing lemon fruits. The Pythiacystis will enter the fruit and produce brownrot, leaving the associated organisms behind. It may then be isolated more easily as a pure culture from bits of the infected fruit.

After the discovery that *Pythiacystis citrophthora* was capable of inducing the disease it remained to be shown that this fungus was commonly present in connection with this type of gummosis. Accordingly, a survey was made of various Citrus localities in California. During the investigations (1912–1916) *P. citrophthora* was isolated 109 times from the diseased bark of 68 Citrus trees, representing about 30 different orchards in 10 counties of California, extending from San Diego on the south to Butte County on the north. It was also isolated from one locality in Arizona (Table I). In addition, the fungus was reisolated in 40 different cultures from 20 cases of gummosis produced by inoculation.

TABLE I.—*Isolation of Pythiacystis citrophthora from gummosis lesions from natural infections*

Variety.	Season.	Number of localities.	Number of trees.	Number of positive cultures.
Lemon	Spring	2	14	21
	Summer	8	19	32
	Fall	6	20	25
Sweet orange	Spring	5	5	12
	Summer	4	4	9
	Fall	2	4	4
Pomelo	Winter	1	1	4
	Spring	1	1	2

These 109 cultures were obtained from 53 lemon trees, 13 sweet-orange trees, and 2 pomelo trees. All of the lemon, 9 of the sweet-orange, and the 2 pomelo trees were affected with typical gummosis of the trunks with the invaded portions of bark extending some distance above the surface of the soil. Four of the orange trees had lesions low down, near or upon the main roots, as is typical of mal di gomma or footrot lesions.

Although many isolation culture tests were made from various portions of the affected bark, including the outer gummous zone, and even some of the unaffected tissue, all of the successful isolation cultures except one were obtained from the advancing margin or outer fringe of the invaded zone. Isolation tests were made every month in the year except during January and February, and cultures of the fungus were obtained during each of the 10 months. The secondary organisms most commonly obtained in cultural tests from the older portions of the invaded zone back of the margins were species of Fusarium, Alternaria, Cladosporium, and Colletotrichum, and a large number of bacterial species.

INOCULATIONS WITH DISEASED TISSUE

Even before the causal fungus was discovered the infectious nature of the disease was determined by means of a large number of inoculations with diseased bark, the results of which are represented by the following typical examples.

On February 27, 1912, at Chula Vista in San Diego County, the following inoculations were made on 16-year-old lemon trees in heavy soil. The inocula were inserted into vertical cuts about 2 cm. long, made

through the bark of healthy tree trunks after first washing the surface with water and with alcohol. Paraffined paper was then tied over the cuts, control cuts without inoculum being used for comparison. In these preliminary experiments, the inocula used were as follows: (1) Bits of diseased bark tissue from near the margin of an actively enlarging gummosis lesion; (2) a bit of gum-filled woody tissue taken just outside the area of killed bark, in the outer gummous zone; (3) a piece of exuded gum from the same lesion; (4) bits of bark from a sound, healthy tree (used as controls). A cut was also made without inserting inoculum of any kind.

Of these inoculations, the first (consisting of two trees) brought about a development of the disease beginning at the point of inoculation. The other cuts all healed without any exudation of gum or visible injury to the bark. It will be noticed that the inoculations resulting in disease were made from tissue cut from the advancing edges or margins of recently killed tissue, while the others were from tissue or gum beyond this killed area (outer gummous zone) or from healthy bark. The development of the disease in one tree will be described, since it proved to be typical of the severe form of Pythiacystis gummosis as it occurs on lemon trees in the coastal sections of California.

On April 24, 1912, about two months after inoculation, gum was exuding rapidly and flowing downward to the surface of the soil, 15 cm. below, and hardening in a 15-mm. ridge. On August 2, over five months after inoculation, the exterior area of discolored bark was 15 by 25 cm. with a copious exudation of gum, forming three ridges on the bark surface and with a large mass of gum on the surface of the soil (Pl. 1, A). The foliage on the inoculated side of the tree was beginning to turn yellow. On September 19, 1912, the area of killed bark had extended upward and laterally, until it occupied a space 30 by 30 cm. and covered one-third the circumference of the trunk (Pl. 1, B). On November 15, 1912, the bark was dead on half the circumference of the trunk and the gum had formed ridges on the surface. The leaves on the affected side of the tree were very yellow and were dropping. The foliage on the side not affected was still healthy and normal. This tree now presented the appearance typical of many trees in the same locality which were affected naturally with Pythiacystis gummosis. On March 11, 1913, a little over a year from the time of inoculation, only 10 cm. of the 90-cm. circumference of the tree contained live bark at the level of the greatest lateral extension of the disease. At the place of greatest extension upward (46 cm. from the surface of the soil), the disease was arrested and callus tissue had formed. On May 24, 1913, the invaded area had extended only slightly beyond that of the previous observation. Pieces cut from the margins of the drying bark (Pl. 1, D) yielded cultures of *Pythiacystis citrophthora*. Most of the gum first formed had been dissolved and carried away by the rains. The bark on the side first affected had dried and contracted, showing longitudinal fissures just like those seen in old naturally occurring cases. Gum was exuding on the margins of the killed bark. In every respect this was a typical case of the form of gum disease due to *P. citrophthora*. On September 3, 1913 (18 months after inoculation) only about 5 cm. of live bark remained on the circumference of the trunk (Pl. 1, C), and the tree was considered useless from a commercial standpoint.

Many other series of inoculations were made with tissue from Pythiacystis gummosis lesions in different localities, varying widely in climatic and soil conditions, with the same general results as to type and progress of the disease. Inoculated trees contracted the disease just as readily on light sandy soils in orchards in which Pythiacystis gummosis had never occurred before as in orchards on heavy soils in which the disease had previously prevailed (Pl. 1, E, F). Variations in the development of the disease in individual trees and at various seasons of the year are indicated in Table II, which summarizes some of the inoculations with tissue from diseased lesions.

TABLE II.—*Inoculations on trunks of 16-year-old lemon trees at Chula Vista, with tissue from diseased lesions*[1]

Experiment No.	Date of inoculation.	Date of observation.	Size of area.[2]	Gum formation.[3]
			Cm.	
		Mar. 24, 1912	—	3
		Aug. 2, 1912	25 by 15	3 (Pl. 1, A.)
		Sept. 19, 1912	30 by 30	3 (Pl. 1, B.)
1	Feb. 27, 1912	Nov. 15, 1912	33 by 30	1
		Mar. 11, 1913	48 by 80	—
		May 24, 1913	—	1 (Pl. 1, D.)
		Sept. 3, 1913	—	3 (Pl. 1, C.)
		Nov. 15, 1912	—	3 (Pl. 1, F.)
		Mar. 11, 1913	20 by 11	3
		May 24, 1913	38 by 11	3
2	Sept. 21, 1912	July 9, 1913	60 by 13	—
		Sept. 3, 1913	66 by 15	—
		Oct. 31, 1913	68 by 16.5	2
		Apr. 18, 1914	75 by 16.5	—
		Nov. 15, 1912	—	3 (Pl. 1, E.)
		May 24, 1913	49 by 22	2
		July 9, 1913	60 by 22	3
3	do	Sept. 3, 1913	66 by 22	—
		Oct. 31, 1913	70 by 22	—
		Apr. 19, 1914	84 by 22	—
		Feb. 6, 1913	—	3
		Mar. 11, 1913	13 by 8	—
		May 24, 1913	29 by 13	2
4	Nov. 16, 1912	July 9, 1913	46 by 33	3
		Feb. 6, 1913	—	0
		Mar. 11, 1913	7.5 by 5	1
		May 24, 1913	16.5 by 5	3
		July 9, 1913	16.5 by 5	—
5	do	Sept. 3, 1913	21.5 by 7.5	— (Pl. 7, A.)
		Oct. 31, 1913	23 by 10	3

[1] All experiments except No. 1 were made on trees growing in light sandy soil.
[2] The first number gives the greatest extension vertically and the second number the greatest extension horizontally. This applies also to similar data in the tables that follow.
[3] 0=none; 1=slight; 2=medium; 3=copious; a dash indicates that no data were obtained.

INOCULATIONS WITH DISEASED FRUITS

After it seemed probable that the fungus commonly associated with *Pythiacystis gummosis* was the same as that causing brownrot of lemon fruits, *Pythiacystis citrophthora*, it was of interest to determine whether the disease could be induced directly from the brownrotted fruits, either in wounds or on uninjured tissue.

Experiments were made (1) by inserting pieces of lemon fruits affected with the brownrot into incisions made in the bark of healthy lemon trees; (2) by placing similarly affected lemon fruits in contact with the roots or trunks of healthy lemon trees and covering them with moist soil; and (3) by placing affected lemons, as in experiment 2, at the root or trunk of orange trees budded on both the sour- and the sweet-orange stocks.

In a typical experiment of the first kind, a piece of diseased lemon rind was inserted into a cut in the bark of a 19-year-old lemon tree 20 cm. above the sweet-orange stock, on February 26, 1913. A control cut not inoculated healed rapidly without gumming, but in 45 days the inoculated cut showed definite evidence of infection. The killed bark area enlarged from 4 by 7.5 cm. in 70 days to 28 by 82 cm. in about 13 months after inoculation (Pl. 2, A, B, C). In about 18 months the killed area reached extreme dimensions of 35 by 125 cm., one-third of this length being upon the sweet-orange stock (Pl. 2, D); the leaves were now yellow and dropping on the affected side. Cultures were made from the bark and Pythiacystis was obtained, but only at the advancing discolored margins of the area where the bark was killed through to the wood. Specimens taken from other places in the diseased tissue, and from the live tissue just outside the diseased areas, either yielded other organisms or were sterile. Among the organisms found both inside and outside the area of recent invasion were species of Colletotrichum, Alternaria, and Cladosporium, and also species of bacteria.

The disease having been produced by inoculation from diseased fruits into cuts in the bark, it now became of interest to determine whether infection could originate from diseased fruit placed near uninjured bark.

On May 13, 1913, at Whittier, several lemon fruits affected with Pythiacystis-rot were buried in the soil near the trunk of a small lemon tree growing in a large earthen pot. The soil was heaped up against the trunk and kept moist by frequent watering. A similar tree in another pot received the same cultural treatment without the diseased fruit. During the summer of 1913 no results of infection were noted. When next examined, in March, 1914, the first tree was seen to be badly affected with typical Pythiacystis gummosis, while the control tree remained healthy.

In other tests on lemon and sweet-orange trees with decayed fruits, infection leading to Pythiacystis gummosis frequently but not invariably resulted. Sour-orange trees under the same conditions whether injured or uninjured failed to contract the disease.

These tests, taken in connection with numerous observations in orchards, show that the sour-orange tree is highly resistant to Pythiacystis gummosis and that sweet-orange and common-lemon trees, though easily infected through injuries, are not otherwise readily infected except under the most favorable conditions for invasion by the parasite.

INOCULATION WITH PURE CULTURES OF PYTHIACYSTIS CITROPHTHORA, AND REISOLATION

As a result of a large number of inoculations from pure cultures of *Pythiacystis citrophthora*, it was shown that this fungus was capable of invading the bark and bringing about all the characteristic effects that have been noted in naturally occurring cases or those produced by

inoculations with diseased bark or fruit. The following is a typical example.

On November 23, 1912, a lemon tree about 18 years old, having a trunk about 30 cm. in diameter, growing at Santa Paula, was inoculated by inserting a very small bit of mycelium from a culture of *Pythiacystis citrophthora* into a 2-cm. vertical cut made 15 cm. above the bud union. The surface of the bark had previously been washed with water and with alcohol and the inoculated cut was covered with oiled paper.

No effect from the inoculation was evident in 37 days, but in 42 days an invaded surface area of 5 by 9 cm. was observed, and this increased to 8 by 11 cm. in 76 days and to 8 by 35 cm. in 7 months, with copious exudation of gum (Pl. 3, A). A similar cut without inoculum, on the opposite side of the same tree, healed rapidly.

A strip of bark 10 by 68 cm., as seen in Plate 3, B, was cut out at this time, for the purpose of cultural examination and for enzym experiments, to be discussed in Part III of this paper. Cultures made from this strip of bark at various places, by cutting bits of tissue from the inner side, yielded *Pythiacystis citrophthora* at both the upper and the lower margin of the invaded zone (see white line shown on the plates) but not at points 25, 8, and 16 cm., respectively, above this killed area, within the outer gummous zone.

On removal of the bark, the cambium adjacent to the dark brown or blackened area appeared yellowish. This discoloration extended only slightly laterally but to a much greater distance from the margins of the cut upward and downward. In this outer gummous zone interior gum had formed in places 60 cm. or more upward from the killed and invaded margins. The causal organism, as indicated by isolation tests in numerous other cases, does not extend into this outer gummous zone. To remove the causal organism in treatment, therefore, it is necessary to cut away the bark only a short distance beyond the discolored region. Experiments have shown that when this is done the gum will cease forming, the further extension of the yellow, gummy zone will stop, and the bark over it will usually return to normal condition.

The cut-out area and surrounding bark shown in Plate 3, B, was painted with Bordeaux paste. Three weeks later, June 27, 1913, gum was seen to have continued to ooze out at the upper angle, but no further bark had died. The same tree is shown in Plate 3, C, in September, 1914, and in Plate 3, D, in June, 1920. No effect on the foliage or health of the tree, such as is usually seen in severe cases of Pythiacystis gummosis, was noticed in this tree. The organism was probably removed in time to obviate visible injurious effects.

The results of a number of other inoculations on lemon trees with cultures of *Pythiacystis citrophthora* are summarized in Table III; others on lemon, orange, and other Citrus varieties, will be found described under "Resistance" and "Mal di gomma." In all, about 90 inoculations with pure cultures resulted in Pythiacystis gummosis, from 20 of which the organism was reisolated.

TABLE III.—*Inoculations of Pythiacystis citrophthora on trunks of 19-year-old lemon trees at Santa Paula*

Experiment No.	Part of trunk inoculated.	Date of inoculation.	Date of observation.	Size of killed bark lesions.	Gum formation.[1]
1	30 cm. from bud union, 60 cm. from soil.	Nov. 23, 1912	Dec. 30, 1912	0	0
			Feb. 12, 1913	10 by 3.5	3
			Mar. 14, 1913	11.5 by 5	3
			Apr. 12, 1913	18 by 8	3
2	15 cm. above bud union.	.....do.....	Dec. 30, 1912	0	0
			Feb. 12, 1913	9 by 5	3
			Mar. 4, 1913	9 by 5	3
			Apr. 12, 1913	13 by 8	3
			May 7, 1913	13 by 8	3
			June 5, 1913	35 by 8	2 (Pl. 2, F, 3, A)
3	10 cm. above bud union.	.....do.....	Dec. 30, 1912	0	0
			Feb. 12, 1913	14 by 5	1
			Mar. 14, 1913	18 by 5	3
			Apr. 12, 1913	19 by 6.5	3
			May 7, 1913	19 by 6.5	–
			June 5, 1913	25 by 6.5	3
4	Trunk, 13 cm. above bud union.	Feb. 20, 1913	Apr. 1, 1913	.....	2
			May 7, 1913	7.5 by 4.0	3
			June 27, 1913	10 by 6	3
			July 28, 1913	15 by 8	3
			Oct. 23, 1913	33 by 15	3

[1] 0=none, 1=slight, 2=medium, 3=copious.

These typical examples show that the general development of the disease was the same with inoculations with pure cultures of *Pythiacystis citrophthora* as it was with the inoculation with bits of diseased bark or fruit previously described. A period of slow development for 2 to 4 months is followed by copious gumming and rapid development, resulting in large invaded areas in 6 to 10 months. Later the rate of increase is again comparatively slow and is frequently partially checked. It is believed that the accumulation of gum in the outer gummous zone is a material hindrance to the rapid advance of the fungus. This feature of the disease is discussed in Part III of this paper.

A partial representative list of inoculations from which *Pythiacystis citrophthora* was again isolated, with the date and the size of invaded areas at time of isolation, is shown in Table IV.

TABLE IV.—*Representative inoculations of Pythiacystis citrophthora*

Date of inoculation.	Date of reisolation.	Size of invaded area.
		Cm.
Nov. 16, 1912	Nov. 1, 1913	23 by 10
Do	July 10, 1913	46 by 15
Nov. 23, 1912	Apr. 12, 1913	47 by 8
Do	June 5, 1913	35 by 8
Do	.....do	25 by 6
Feb. 26, 1913	Feb. 7, 1915	95 by 46
Do	Oct. 22, 1913	33 by 13
July 13, 1913	May 9, 1914	48 by 15
Sept. 10, 1914	Oct. 9, 1914	15 by 5
Oct. 14, 1914	Apr. 7, 1915	25 by 8
Feb. 7, 1915	Sept. 3, 1915	8 by 5
Mar. 4, 1915	.....do	30 by 10
Do	.....do	46 by 13
Apr. 9, 1915	.....do	30 by

As is shown by Table IV, the fungus was recovered in different cases from 1 to nearly 12 months after inoculation and from margins of different invaded areas varying from 8 to 95 cm. in vertical extent. Since the fungus was isolated most frequently at the upper margin of the invaded area, the distances from the original point of inoculation at which the fungus was recovered was frequently more than one-half that of this vertical extension, because the areas usually enlarged upward much faster than downward or laterally, as previously pointed out.

The severe inoculation test to which this fungus was subjected in order to establish its causal relation to this type of gummosis is indicated by an example of the repeated isolations, inoculations and reisolations of one of the strains of this fungus, over a period of three years. Isolated in September, 1912, its record was as follows:

Date of inoculation.	Date of reisolation.	Size of lesions.
		Cm.
Nov. 23, 1912	Apr. 12, 1913	47 by 8
June 13, 1913	May 9, 1914	48 by 15
Mar. 4, 1915	Sept. 3, 1915	46 by 13

The fungus was thus in the bark of the three trees for periods of about 5, 11, and 6 months, respectively, and between these periods in cultures for about 2, 2, and 10 months, respectively. Transfers from the original culture which were kept alive for over 8 years, on cornmeal-agar medium, were still capable of producing brownrot of lemon fruits when tested in 1921.

INOCULATIONS WITH PYTHIACYSTIS CITROPHTHORA ON DIFFERENT PARTS OF SAME TREES

After the results of the first inoculations began to indicate that *Pythiacystis citrophthora* was the causal agent in this type of disease, it became important to find out the effect of the fungus when introduced into different parts of the same tree. Inoculations were made on roots, trunk, and branches of various ages, as shown in Table V.

TABLE V.—*Inoculations with Pythiacystis citrophthora July 13, 1913, at different locations on same tree*

Part of tree inoculated.	Length and width of killed bark areas.					Final extreme distance from place inoculated.	
	1913			1914			
	July 27.	Sept. 13.	Nov. 25.	Apr. 9.	May 9.	Upward.	Downward.
Large root (sweet-orange stock), 13 cm. below bud union (Pl. 4, B)	*Cm.* 2.5 by 1.8	.....	.....	*Cm.* 13 by 4	*Cm.* 14 by 5	*Cm.* 9	*Cm.* 5
Orange bark of stock 5 cm. below bud union (Pl. 3, D)	4 by 1.8	10 by 2.5	24 by 10	43 by 13	48 by 15	40	8
Lemon trunk, 13 cm. above bud union (Pl. 4, B)	5 by 2.5	25 by 10	50 by 25	53 by 25	68 by 25	46	22
Lemon trunk 88 cm. above bud union just below crotch	2.5 by 0.6	13 by 4	34 by 9	36 by 9	36 by 9	.....	.....
Limb 8 cm. in diameter	2.5 by 0.6	20 by 6	33 by 8	33 by 8	33 by 8	20	13
Limb 5 cm. in diameter	.....	.....	30 by 5	30 by 5	30 by 5	18	12
Limb 5 cm. in diameter	.....	.....	27 by 1.3	30 by 2.5	30 by 2.5	20	10
Limb 2.5 cm. in diameter	.....	10 by 5	10 by 5	15 by 5	15 by 5	10	5
Limb 3 mm. in diameter	.....	15 by 5	18 by 5	22 by 5	22 by 5	15	7
Controls, not inoculated	0	0	0	0	0	0	0

The control cuts not inoculated healed without gumming or dying of bark. All cuts inoculated with *Pythiacystis citrophthora* resulted in killing of the bark accompanied by exudation of gum. The areas on the branches had nearly reached their maximum size when examined on November 25, 1913, 104 days after inoculation. A slight increase in some of them took place previous to the next April, at which time they had ceased to enlarge and had become self-limited, as was shown by subsequent records in Table V. The fungus apparently died out of these limb lesions, as no evidence of it could be found later.

The inoculation on the orange root resulted in gumming and killing of some bark in two weeks and continued gumming for about four months. This lesion (Pl. 4, C, lower area) became self-limited after an area 14 by 5 cm. had been killed and later resembled self-limited typical footrot or mal di gomma lesions as they often occur naturally on orange trees in Florida.

The diseased area resulting from inoculation on the orange bark 5 cm. below the bud union enlarged at about the same rate as that on the root for the first two weeks. After it had spread to the lemon bark, however, at the bud union, the progress upward was rapid. By May 9, 1914, the killed area was 48 by 15 cm. (Pl. 4, A, B), only 8 cm. of this extension being downward on the orange bark. Of five cultures taken about 10 months after inoculation from the margin of the invaded area (see chalk line on Pl. 4, A), three developed *Pythiacystis citrophthora*. Further extension of the area was prevented by cutting out the bark (Pl. 4, B) and painting the trunk with Bordeaux paste.

The result of the inoculation made on the lemon bark on the opposite side of the same tree a few centimeters above the bud union is shown in Plate 4, C, the killed area being at this time (April 9, 1914) 53 by 25 cm. with large quantities of gum exuding on the surface.

COMBINED EFFECTS OF PYTHIACYSTIS CITROPHTHORA AND FUSARIUM SP.

During the examination of a large number of naturally occurring cases of Pythiacystis gummosis it was noticed that a species of Fusarium frequently accompanied and was closely associated with *Pythiacystis citrophthora* in the diseased tissue. The question arose as to whether the Fusarium played any part in the development or the severity of the disease.

Fusarium has been mentioned frequently in literature as having some possible relation to certain types of gum disease. Briosi (*11*) and McAlpine (*46*) concluded that *Fusarium limoni* Briosi played an important part in mal di gomma in Italy and Australia. Earle and Rogers (*21*), though not able to produce gummosis by inoculation with Fusarium, believed that under certain conditions it was probably a factor in a certain type of gum disease in Cuba. The writer had previously also found species of Fusarium repeatedly associated with mal di gomma or footrot in Florida, but inoculations with them had been negative.

Cuts, as before, into which bits of pure cultures of *Pythiacystis citrophthora* and *Fusarium* sp. were placed side by side were now made on lemon trees. At the same time inoculations into other similar trees were made with *P. citrophthora* alone and others with *Fusarium* sp. alone. The main results are given in Tables VI and VII.

TABLE VI.—*Inoculation tests with Pythiacystis citrophthora alone, compared with those with P. citrophthora plus Fusarium sp.*

INOCULATIONS MADE LOW ON TRUNKS OF 19-YEAR-OLD LEMON TREES, FEBRUARY 20, 1913

Date of observation.	*P. citrophthora* alone.		*P. citrophthora* and *Fusarium* sp.	
	Size of lesions.	Gum formation.[1]	Size of lesions.	Gum formation.[1]
1913.	*Cm.*		*Cm.*	
Apr. 1	0	1	0	1
May 7	7.5 by 3.8	3	6.3 by 2.5	3
June 27	10 by 6.3	3	13 by 4	3
July 28	15 by 7.5	3	30 by 9	3
Oct. 23	33 by 13 (treated).	3	No record	
Nov. 25			58 by 18	3

INOCULATIONS MADE HIGH ON TRUNKS OF 21-YEAR-OLD LEMON TREES, FEBRUARY 25, 1915

1915.	*Cm.*		*Cm.*	
Mar. 4	0	0	0	0
Apr. 21	2.5 by 2.5	1	5 by 2.5	3
May 18	6.5 by 2.5	2	9 by 7	3
Sept. 4	8 by 5	2	38 by 15	3

[1] 0=none; 1=slight; 2=medium; 3=copious.

TABLE VII.—*Inoculation with Fusarium sp. at Santa Paula.*

Experiment No.	Date of inoculation.	Maximum results as to—		
		Gum.[1]	Initially killed bark at inoculation point.[1]	Cracking of outer bark.[1]
1	Nov. 23, 1912	2	1	1
2	.....do	1	0	1
3	Feb. 20, 1913	0	1	0
4	.....do	0	1	2
5	Mar. 14, 1913	1	1	1
6	.....do	0	1	1
7	.....do	1	1	1
8	Feb. 25, 1915	0	1	0
Control cuts without inoculum, to correspond with each of the above		0	0	0

[1] 0=none; 1=slight; 2=medium.

The progress of the disease on the tree inoculated with Pythiacystis and Fusarium on February 20, 1913, is more completely shown by Plate 5. The invaded area which was 58 by 18 cm. on November 25, 1913 (Pl. 5, B), the last date given in Table VI, had increased to 90 by 41 cm. on February 6, 1915 (Pl. 5, C–F). Extension vertically remained at 90 cm., but the lateral extension had increased to 61 cm. by March 9, 1916; and subsequently the entire circumference was encircled and the tree killed by the disease.

In the comparative inoculations with Fusarium alone on February 20, 1913, and February 25, 1915, No. 3 and 8 (Table VII), only a narrow layer of tissue along the cuts was killed, without exudation of gum. A thin outer layer of bark subsequently died about one inoculation, but otherwise the effect was not different in either case from that produced by the uninoculated cuts on the same trees. In a number of other inoculations with species of Fusarium associated with gummosis shown in Table VII, only slight effects in killed tissue and only slight to medium effects in gum formation were obtained.

Although the experiments on this phase of the question have been too few as yet to justify definite conclusions, the results suggest that the severity of the disease may be slightly increased by adding *Fusarium* sp. to *Pythiacystis citrophthora* at the time of inoculation. The characteristics of the disease except in rapidity of development, however, were the same as when *P. citrophthora* was inserted alone.

RESISTANCE OF DIFFERENT SPECIES AND VARIETIES OF CITRUS TO PYTHIACYSTIS GUMMOSIS

Among the Citrus species and varieties that have been tested, the common lemon has the lowest resistance to Pythiacystis gummosis and the sour orange the highest. The sour orange usually is so resistant to Pythiacystis attack that even when the most favorable conditions are given by inoculation in wounds there is only a slight gumming with rapid healing of the wounded tissue and with total failure to produce a diseased lesion. The sour orange is also highly resistant to all other infectious gum diseases of importance. Mere gum formation, however, may be induced by suitable stimuli in sour orange as well as in other species and varieties. Of the forms which have been most used for stocks, the trifoliate orange probably stands next to the sour orange in resistance and the sweet orange next to the common lemon in susceptibility, with the pomelo and the rough lemon between these two. Because these stocks are grown from seed there is a large possibility of variation in resistance within each variety, due to differences between "strains," and observations have indicated that such variation actually exists. The following observations and experiments indicate in a rough way the relative resistance of some of the species and varieties.

A block of 5,000 sweet-orange seedlings about 2½ years old, growing in nursery rows on medium heavy clay loam soil, had been planted adjacent to a block of 15,000 sour-orange seedlings of the same age and having the same care. All the trees had been irrigated rather frequently and heavily. On October 21, 1914, four representative rows of sweet-orange trees showed the following percentage of *Pythiacystis gummosis:*

Row No.	Number of trees in row.	Number of affected trees.	Percentage affected.
1	222	52	23
2	213	73	34
3	212	63	29
4	180	53	29
Total	827	241	.....
Average	.....	.....	29

On some trees only a small lesion was evident with much gum exuding, on others the bark was killed to a distance of 15 to 30 cm. above the soil, with an abundance of gum, and still other trees were dead. Some trees showed a strong tendency to form ridges of callous tissue along the edges of the dead strips of bark. A thorough search in the block of sour-orange trees failed to reveal a single tree affected.

Differences in resistance are indicated further by an estimate made by W. M. Mertz in a nursery of Citrus seedlings about 2 years old grown at the Citrus Experiment Station. The following is the percentage of gum disease (probably *Pythiacystis gummosis*) which was recorded:

Species.	Number of trees.	Percentage with gummosis.
Citrus aurantium (sour orange)	1,000	0.3
Poncirus trifoliata (trifoliate orange)	1,000	1.0
Citrus grandis (pomelo)	1,000	2.5
Citrus sinensis (sweet orange)	2,000	10.0

The inoculations recorded in Table VIII were made on trees about 2 years old from seed, by cutting through the bark on the stem not far from the soil and inserting bits of the mycelium from cultures of *Pythiacystis citrophthora* grown on sterilized orange wood. Oiled paper was tied over the cuts.

TABLE VIII.—*Inoculations made May 15, 1916; observations July 2, 1915, when the plants were pulled up*

Host.	Number of plants.	Results.
Sour orange	3	No gum on exterior. One shows interior gum. All healing normally.
Rough lemon	5	No gum. Healing normally.
Sweet orange	4	Two trees gumming copiously; long strip of bark killed. Two trees no gum on surface; wounds healing.
Pomelo	3	Bark killed slightly on edges of cuts without gum on exterior; healing rapidly.

Of the four forms tested in this experiment, sweet orange showed the greatest effect, pomelo less, and sour orange and rough lemon[7] showed no appreciable effect from the inoculation. The weather was very hot and dry during the experiment and thus not very favorable to the fungus.

On February 6, 1915, inoculations into the trunks of 21-month-old seedling sour-orange and rough-lemon trees were made at Santa Paula. A branch of a young common-lemon tree close by was inoculated at the same time. The results on May 18, 1915, were as follows:

The inoculated sour-orange cuts healed rapidly, with slightly more gaping of the wound than in the controls, while the rough lemons showed a small amount of dead bark next to the inoculated cuts, which were also healing rapidly. A slightly lower resistance of the rough lemon than of the sour orange was indicated. On the common lemon, however, the leaves had withered on the inoculated branch, the bark was killed

[7] The rough lemon is thought to be a hybrid. It is used largely for stocks in Florida and should not be confused with the common lemon.

around the branch for a distance of 8 cm. below the wound, and a large quantity of gum had formed. The control cuts on all three varieties healed perfectly without gumming or evident injury to the trees.

On July 2, 1913, inoculations were made at Whittier, with *Pythiacystis citrophthora* in cuts on the bark of sour-orange, sweet-orange, and a number of young deciduous fruit trees. The results on September 26, 1913, are given in Table IX.

TABLE IX.—*Comparative inoculations with Pythiacystis citrophthora into Citrus and deciduous fruit trees. Inoculations made July 2, 1913; observations, September 26, 1913*

Inoculation No.	Host.	Results.
1	Sour-orange stock (Valencia orange scion above).	Slight gumming, but healing rapidly.
2	Sour-orange stock (Valencia above)...	Healed perfectly, no gumming.
3	Sour-orange stock (lemon above).....	Healed with swollen scar only.
4	Valencia orange scion, hardened tissue (sour-orange stock below).	Gumming copiously with killing of small amount of tissue.
5	Valencia orange, same tree as No. 4, younger tissue.	Gumming copiously; 15-cm. strip of bark on one side of twig killed.
6	Control cuts on sour-orange and on Valencia orange bark (not inoculated).	All healed perfectly without visible gumming on surface.
7	Almond (2 trees).....	Gum exuded. Tissue slightly killed beyond cut.
8	Almond (control, not inoculated).....	Healed perfectly without gumming.
9	Peach (12-mm. stem).....	Much exuded gum. Bark killed 5 by 1.3 cm.; killed wood 8 cm. long.
10	Peach (control, not inoculated).....	Healed perfectly with slight gumming
11	Burbank plum.....	Gumming, outer part of bark killed and sunken, but healing underneath.
12	Burbank plum (control not inoculated).	Healed perfectly, no gum.
13	Pear.....	Healed with enlarged scar.
14	Pear (control).....	Healed with slight scar.

As before, the sour-orange bark showed high resistance while the sweet-orange (Valencia) bark was considerably affected by the fungus. Of the deciduous fruits tested, peach was most affected, almond and plum slightly, and pear scarcely at all.

In Table X are shown the results of inoculation into branches of various species by placing bits of Pythiacystis-infected fruit into cuts.

TABLE X.—*Comparative inoculations on Citrus branches, October 9, 1914; observations October 22, 1914*

Experiment No.	Host.	Size of diseased lesions.	Gum.[1]
		Cm.	
1	Common lemon.....	5 by 1.3	3
2	.....do.....	5 by 1.3	3
3	Citron.....	2.5 by 0.5	3
4	.....do.....	2.5 by 0.3	3
5	Sweet orange (Valencia).....	4 by 1.0	3
6	Sweet orange (navel).....	3 by 1.0	3
7	Sour orange.....	1.2 by 0.5	2
8	Controls.....	0	0

[1] 0=none; 1=slight; 2=medium; 3=copious.

The lesions here, though small at the time of observation, give some indication of differences in resistance. The common lemon was most affected, followed by the sweet orange (Valencia and navel) and the citron. Sour orange, as before, was quite resistant, though showing some effect, but was already beginning to form callus at the cut.

Experiments to test the effect of *Pythiacystis citrophthora* on small roots of different species of seedlings were also made.[8]

On November 15, 1915, mycelium of Pythiacystis and lemon fruits affected with Pythiacystis in different tests were placed on the healthy roots (with and without punctures) of the common lemon, sweet orange, pomelo, and sour orange. When last examined, on May 11, 1916, only the roots of the common lemon, where inoculated with *Pythiacystis citrophthora* (with punctures) and where inoculated with diseased lemon fruit (without punctures), were killed. The fungus was reisolated from the lemon root which had been inoculated with *P. citrophthora*. This experiment gives additional weight to the correctness of previous observations that even small and medium sized roots of the common lemon are frequently attacked by *P. citrophthora*, but that the small roots of other varieties are quite resistant.

Inoculations reported in other sections of this paper also give further indication of differences in resistance, especially as between the common lemon and sweet orange. The reasons for these differences in susceptibility is an interesting question in itself, which has not as yet been investigated. Our inoculation experiments would indicate that the differences in resistance in sour orange, sweet orange, and lemon, at least, can not be confined to the superficial layers of cells. If it were so limited the insertion of the parasite into cuts should cause equal effects in each variety.

MAL DI GOMMA IN RELATION TO PYTHIACYSTIS GUMMOSIS

Mal di gomma, due to *Phytophthora terrestria* Sherbakoff, is a gum disease with close relationships to Pythiacystis gummosis. It has previously been pointed out that certain phases of the Pythiacystis gummosis occurring on or near the main roots of sweet-orange trees are similar to those of mal di gomma, or footrot.

For this reason, certain footrot-like forms due to *Pythiacystis citrophthora* in California have previously been known by the name of mal di gomma according to Smith and Butler (*56*), 1908, and Fawcett (*26*). Since the name mal di gomma was first used in Florida to designate a common Florida gum disease, which is now known to be due to *Phytophthora terrestria*, it is proposed to restrict its use (in this country at least) to the disease due to this fungus.

This type of gum disease affects, for the most part, the bark on the lowest portion of the trunk and the upper portion of the first main roots, mostly below the surface of the soil. Gum usually forms on the trunk of the tree above the soil. The inner bark and finally the wood under-

[8] Boxes were constructed with one side consisting of glass plates inserted into grooves and covered by a removablewooden door fastened by hooks to hold it against the glass. After the young trees had become established in these boxes, the glass plate was removed to make inoculations on roots that had grown out against it and was then replaced in its former position. The assistance of Mr. E. E. Thomas, for whom similar experiments with Fusarium were made, is here acknowledged.

neath frequently develop a disagreeable fetid odor.[9] The bark dies and breaks away in patches, leaving bare, dead areas, which spread in all directions, but mostly downward, on the main crown roots and laterally around the trunk. Trees thus affected bear heavy crops of fruit and the leaves become yellow.

Phytophthora terrestria Sherbakoff (*53*) was first isolated by the writer from gumming lesions of an orange tree at Lindsay, Calif., in 1912, and was considered at that time to be only a peculiar strain of *Pythiacystis citrophthora*. Later, in 1914, it was isolated from mal di gomma lesions, as follows: (1) from a grapefruit tree at Palmetto, Fla. (*26, fig. 5*); (2) from orange trees in Cuba; and (3) from a tangelo tree on the Isle of Pines. The Florida and Cuban cultures were still considered to be strains of *Pythiacystis citrophthora* and were referred to as such in publications (*25, 26*). Stevens (*61*) later made an extended survey and isolated this species in a large number of mal di gomma cases from widely separated localities representing the principal part of the Florida Citrus belt. In the meantime, Sherbakoff (*53*) had described *Phytophthora terrestria* as causing rot of tomatoes in Florida. The cultures previously isolated at Lindsay, Calif., and in Florida and Cuba were then examined by Sherbakoff, who concluded that they were all the same species. A culture isolated by George Fawcett from a lemon tree in Argentina and sent to the writer in October, 1916, was also determined to be similar to this species. Later, the writer (*27*) determined that *Phytophthora terrestria* had markedly different growth-temperature relations from those of *Pythiacystis citrophthora*. The vegetative growth on certain culture media, the method of forming sporangia, and the effect on the host when inoculated, are so similar, however, as to make it seem probable that the two are closely related species. (See inoculation experiments, Tables XI and XII.)

Phytophthora terrestria is believed by some authors to be identical with *P. parasitica* Dastur. Ashby (*4*) has recently described a leaf-stalk rot of coconuts in Jamaica as being due to this latter fungus and states that Dastur has compared *P. terrestria* with *P. parasitica* and found them to be identical. Ashby has also found this fungus on tobacco and pineapple plants. Dastur (*19*) originally described his species as causing a disease of castor oil plants in India and later (*20*) found it attacking *Vinca rosea*. If *P. parasitica* and *P. terrestria* are the same species, a wide distribution of this fungus is indicated.

Comparative Inoculations with Phytophthora Terrestria and Pythiacystis

Many different series of inoculation experiments with *Pythiacystis citrophthora* and *Phytophthora terrestria* were made under varying conditions, the results of some of which are given in Tables XI and XII.

[9] This rotting of the wood, as well as the bark, and the accompanying "fetid odor" are believed to be due mainly to secondary organisms setting up fermentation and decay below the surface of the soil after killing of the bark by the primary organism. While gum may be formed below as well as above the surface of the soil, it is dissolved readily by moisture and is usually less conspicuous below the soil surface.

TABLE XI.—*Comparative effects of various "strains" of Pythiacystis citrophthora and Phytophthora terrestria on lemon and orange bark—Eureka lemon trunks about 6.5 cm. in diameter, budded high on sweet-orange stocks, at Riverside, Calif. Inoculations April 17, 1913; examination May 20, 1913*

Experiment No.	Source of culture.	Host and place on tree inoculation.	Killed area.	Gum formation.[1]
			Cm.	
1	*Pythiacystis citrophthora; from* lemon bark, Whittier, Calif.	Eureka lemon above bud union.	3 by 1.3...	2
2	.....do.....	Orange stock below bud union, same tree.	2 by 1.....	2
3	*Pythiacystis citrophthora from* lemon bark, San Diego, Calif.	Eureka lemon above bud union.	2.5 by 1.5..	2
4	.....do.....	Orange below bud union..	0.....	0
5	*Phytophthora terrestria* from orange bark, Lindsay, Calif.	Eureka lemon above bud union.	4 by 2.5....	3
6	*Phytophthora terrestria*, same.	Same tree on orange stock below bud union.	5 by 2.....	2
7	*Pythiacystis citrophthora* from lemon fruit, California.	Lemon bark above bud union.	30 by 18 (Pl. 6, C).	3
8	*Pythiacystis citrophthora*, same	Orange bark below bud union.	46 by 18 (Pl. 6, C).	2
9	*Phytophthora terrestria*, from pomelo bark, Palmetto, Fla.	Lemon bark above bud union.	20 by 5 (Pl. 6, E).	3
10	*Phytophthora terrestria*, same.	Orange bark below bud union.	25 by 9 (Pl. 6, E).	3
11	*Phytophthora terrestria* from orange bark, Cuba.	Lemon bark above bud union.	6 by 5 (Pl. 6, D).	2
12	*Phytophthora terrestria*, same.	Orange bark below bud union.	23 by 6 (Pl. 6, D).	3
13	*Phytophthora terrestria* from tangelo, Isle of Pines.	Lemon bark above bud union.	19 by 5 (Pl. 6, B).	3
14	*Phytophthora terrestria*, same.	Orange bark below bud union.	23 by 8 (Pl. 6, B).	2
15	Control (cut without inoculation).	Lemon and orange bark....	(Pl. 6 A)..	0

[1] 0=none; 1=slight; 2=medium; 3=copious.

TABLE XII.—*Comparative effects of different "strains" of Pythiacystis citrophthora and Phytophthora terrestria in inoculations on trunks of 20-year-old Lisbon lemon trees at Santa Paula. Inoculations, April 9, 1914; examination, August 14, 1914*

Experiment No.	Original source of cultures.	Organism.	Size of diseased area.	Gum formation.[1]
			Cm.	
1	Lemon tree, Santa Paula, Calif...	*Pythiacystis citrophthora.*	23 by 10....	3
	Lemon tree, Whittier, Calif.....	.....do.....	8 by 5.....	2
2	Orange tree, Lindsay, Calif.....	*Phytophthora terrestria.*	2.5 by 1.3..	1
3	Pomelo tree, Palmetto, Fla.....	.....do.....	8 by 4.....	2
4	Orange tree, Cuba.....	.....do.....	6 by 2.5....	2
5	Tangelo tree, Isle of Pines.....	.....do.....	10 by 5.....	2

[1] 1=slight; 2=medium; 3=copious.

The lesions produced by the inoculations with the two fungi were identical in general appearance. The characteristic manner of killing the bark, the formation of the outer noninvaded gummous zone, and the exudation of gum were the same with both fungi. Cuts without inoculation healed without producing disease.

In addition to these inoculations, a number of other comparative inoculations with pure cultures of *Pythiacystis citrophthora* and *Phytophthora terrestria*, using fruits affected with the two fungi, were made on the large main roots and at the crown of old orange trees. In nearly all the successful inoculations of this kind, lesions resulting from the two species of fungi could not be distinguished clearly one from the other and resembled true mal di gomma or footrot lesions as they occur in Florida. Most of the lesions became self-limited in three to four months on old orange trees, after enlarging to a maximum of 8 by 10 cm., but some made with *Pythiacystis citrophthora* progressed longer and developed large patches, in one case spreading 25 cm. and killing a large main root.

CONTROL OF PYTHIACYSTIS GUMMOSIS

More rational control methods based directly upon a knowledge of the cause and development of the disease and upon the results of many experiments [10] growing out of this knowledge now became possible. Previous control methods, though based largely on a different explanation of the cause, were partially successful because they frequently removed entirely the conditions contributing to infection and development of the disease, which conditions were formerly thought to be the sole cause.

The most successful method of prevention for new plantings is obviously the use of the resistant sour-orange stocks budded high. This method was employed in Italy after the orchards were killed out by gummosis and has since been used largely in Florida and California. To prevent gummosis on susceptible stocks or on low-budded trees, the method now in common use in California on heavy soils is to pull back the soil from the base of the trunk, thus exposing the top of the first main roots and making a circular ridge to exclude irrigation water from standing in contact with the trunk of the tree. As an added preventive on soils especially subject to gummosis, the base of the trunk is painted with Bordeaux paste or other noninjurious fungicide. In one experiment with Bordeaux used in this way on about 100 acres of lemons the number of new cases decreased from 123 in 1912 to 16 in 1914. By a similar treatment in another locality with 23,837 lemon trees the number of new gummosis outbreaks decreased from 727 in 1912 to 113 in 1914 (from 3.5 to 0.5 per cent). In a third experiment with 560 lemon trees, one-half of which was treated, 9 per cent of those receiving Bordeaux spray two previous years developed disease, against 21 per cent of those not so sprayed.

The treatment of trees after they are diseased consists in removal of the bark tissue from the invaded zone and the application of a fungicide to kill out the fungus in small bits of tissue possibly left behind and to prevent reinfection (Pl. 3, B–D). No attempt need be made to cut beyond the large outer gummous zone, since it has been shown that this does not contain the invading parasite and will recover rapidly after the inciting cause for the gumming has been destroyed.

[10] The detailed results of experiments in prevention and control will be presented in a bulletin from the University of California Agricultural Experiment Station.

PART II.—GUMMOSIS DUE TO BOTRYTIS CINEREA AND OTHER FUNGI

BOTRYTIS GUMMOSIS

INTRODUCTION

Botrytis gummosis differs from Pythiacystis gummosis, discussed in Part I of this paper, in that it causes softening of the invaded bark in the early stages and produces conidiophores and spores in damp, cool weather. In the later stages the outer layer of bark is killed and becomes dry and hard much in advance of the inner layer, while there is a greater tendency than in Pythiacystis gummosis for the tree to renew the bark underneath the dead hard layer and there is usually also a less copious flow of gum. Botrytis gummosis is confined in California almost exclusively to lemon trees growing in the coastal regions and is usually seen on trees more than 10 years of age. This disease should not be confused with a desquamated-bark (shellbark) condition in which the outer bark on old lemon trees dies, cracks, and breaks away in longitudinal strips, a condition which is somewhat similar to that frequently brought about in the later stages of Botrytis gummosis. Neither disease should be confused with psorosis (scaly-bark) of sweet-orange trees.

The causal fungus, *Botrytis cinerea* Lk., depends to a much greater extent than does *Pythiacystis citrophthora* Sm. and Sm. on wounds or other conditions predisposing the bark to attack.

The writer's attention was first called to this type of gummosis early in February, 1912. After a period of moist, cool weather, patches of bark 15 to 30 cm. long and half as wide presented the gray furry appearance characteristic of the fruiting bodies of *Botrytis cinerea*. In a later survey of the Citrus districts of California, *Botrytis cinerea* was usually found associated with this type of gummosis and was isolated from a large number of diseased lesions. *Penicillium roseum* Link,[11] *Fusarium* sp., and several other fungi were also frequently conspicuous.

The important question at once arose, whether any of these fungi might break down sound tissue and initiate conditions leading to disease and gum formation or whether gumming and associated death of the bark might result primarily from injuries or other contributing conditions, the fungi playing only a minor or secondary part, as had previously been assumed. It seemed advisable, therefore, to attempt to answer this question experimentally.

INOCULATION EXPERIMENTS

Preliminary Tests

On March 7, 1912, at Santa Paula, a preliminary set of rough inoculations was made with diseased tissue and with fungi taken directly from the surface of the bark.

Sixteen Lisbon lemon trees 18 years old, on sweet-orange stocks, were used for the inoculation. The portion of the bark to be inoculated was washed first with water and then with alcohol, and the flame of an alcohol lamp was quickly passed over the surface. A cut about 3 cm. long was made through the bark with a heavy sterilized knife. Into these cuts the materials for inoculation were inserted. The cuts were made at varying

[11] Determination of this species was kindly made by Dr. Charles Thom.

distances, ranging from 5 to 60 cm. above the "bud union." With every inoculation a similar cut, to serve as a control, was made in the opposite side of the same tree. A sheet of paraffined paper was then tied against the surface of the bark over each cut and fastened with wax at the upper edge to exclude rain and dust.

The different kinds of inoculum and the number of trees were as follows: (1) Small pieces of sound bark and wood, four trees; (2) bit of diseased bark, two trees; (3) small bits of wood permeated with gum from outer gummous zone, two trees; (4) small portion of exuded gum, one tree; (5) bits of dead bark containing *Penicillium roseum* Lk., two trees; (6) hyphae and spores of *Botrytis cinerea*, taken from rotting diseased bark, three trees.

None of the inoculations with sound wood or bark, gum-filled wood from the outer gummous zone, or exuded gum produced any gumming or development of disease. All the inoculations from diseased tissue, however, showed gum exudation within 2 to 4 months, and in one case a lesion 8 by 15 cm. developed in 9 months. One of the inoculations with *Penicillium roseum* showed slight gum exudation in 4 months without development of a lesion, but the other cut healed without gumming. All three of the Botrytis inoculations resulted in gummosis of the same type as that from which the fungus was obtained. In one of them slight softening of the bark was noted in 10 days. A softened area of bark which measured 8 by 2.5 cm. in 50 days (with fruiting of Botrytis on the surface) had increased to 10 by 4 cm. in 4 months, with copious exudation of gum some of which was exuding through cracks 50 cm. directly above the cut in the outer noninfected gummous zone. About 1 year after this inoculation the space over which the bark was dead to the wood measured 10 by 2.5 cm., and this was surrounded by an irregular area over which only the outer bark was killed, making the entire area 30 by 27 cm. Botrytis was fruiting at various places over this area.

A second set of experiments was then carried out. These were planned to test the influence of various kinds of wounds, the influence of obstructions in the sap current, and finally, the influence of continued pressure upon the bark. These experiments were made in July, 1912, on sound trunks of 19-year-old Lisbon lemon trees at Santa Paula. The following methods were used both with and without contamination with pure cultures of *Botrytis cinerea:* Cuts vertically or horizontally through the bark, auger holes with and without glass or wooden plugs, bruises made by light and heavy blows from hammers, wounds made by slicing off both thin and thick layers of bark, wounds made by entirely cutting off large areas of the bark and so forth. Constant pressure was also exerted against the bark with wooden blocks tightened by means of screws in an iron collar.

It was difficult to make these inoculations or injuries in tree trunks in the open and keep them absolutely free from organisms. In attempting to overcome this difficulty the following method was adopted for the most important of these experiments. A cloth hood to be tied to the tree trunk was made by fastening a piece of fumigation-tent cloth to a wooden barrel hoop severed on one side. Strings were fastened to the cut ends of the hoop and to the corners of the cloth opposite the hoop. The hoop was allowed to hang down. The upper strings were tied to the trunk of the tree just below the branches and the hoop was fastened

below so as to cause the cloth to flare outward and thus protect the wound from falling dust and excessive currents of air. In order to prevent dust in dry weather, the under surface of the hood, the trunk, and the soil around the base of the tree were sprayed with water. The area of bark to be experimented on was washed, first with water and then with alcohol, and quickly flamed with an alcohol lamp. The augers, hammers, knives, or other instruments used were sterilized either by heat or by alcohol which was allowed to evaporate from their surfaces. Either grafting wax or glass slides sealed with putty were used as a covering in most of the experiments.

There was no gum exudation or development of disease in connection with any of the experiments with which attempts were made to keep the bark free from contaminations. With the experiments in which spores of *Botrytis cinerea* were used as a contamination, however (except where the bark was not injured) gum exuded, and in most cases typical Botrytis gummosis lesions developed around the place of injury.

The results of these experiments appeared to show that injuries were not sufficient in themselves to induce gum diseases. When the injuries were contaminated with pure cultures of *Botrytis cinerea*, however, under the same conditions, gum formation and death of the tissue were readily produced.

Inoculations with Pure Cultures of *Botrytis cinerea*

Many inoculations have been made with pure cultures of *Botrytis cinerea* and the following is typical of the results.

On July 12, 1912, spores and sporophores of *Botrytis cinerea* were inserted in a cut 2.5 cm. long, on the trunk of an 18-year-old lemon tree at Santa Paula. The wound was then covered with oiled paper. Gum was exuding rapidly by July 20 and continued to do so until a diseased area 4 by 5 cm. was noted on August 22. On November 21, there was a softened, dead area of bark, 15 by 5 cm., which on February 14, 1913, was 23 by 8 cm. in size and on June 27, 1913, 28 by 10 cm., with new gum, and Botrytis fruiting on a part of the surface. On July 28, 1913, the main area was 32 by 10 cm., and a number of smaller areas were scattered over the same side of the trunk. On scraping the trunk for treatment at this time, it was found that only a small area of bark was killed through to the wood, only the outer cortical layer of the remaining part being dead. Bordeaux paste was applied to one lateral half of the scraped portion. On November 25, 1913, the disease was seen to be arrested on the portion treated with Bordeaux, but further dying of outer bark had taken place on the other portion. A similar cut without inoculum made on the opposite side of same tree healed without gumming or any other apparent effect.

In all, about 40 inoculations with pure cultures of *Botrytis cinerea* were made on lemon trees, most of which resulted in gummosis of the type represented by the previous example. The general results of a number of these are given in Table XIII.

TABLE XIII.—*Inoculations with pure cultures of Botrytis cinerea, on 18-year-old lemon trunks except where noted*

Experiment. No.	Date of inoculation.	Host.	Gumming.[1]	Botrytis fruiting.[1]	Cracked outer bark area.[1]	Date of examination.	Area killed, outer bark.
							Cm.
1	July 12, 1912	Lemon	x	x	x	July 28, 1913	32 by 8
2	.....do.....	...do...	x	o	x	Mar. 9, 1916	30 by 8
3	Aug. 23, 1912	...do....	x	x	x	.....do.....	30 by 8
4	Dec. 23, 1912	...do....	x	x	x	.....do.....	10 by 8
5	Feb. 20, 1913	...do....	x	x	x	.....	
6	Mar. 14, 1913	...do....	x	x	x	Sept. 3, 1914	20 by 5
7	July 13, 1913	...do....	x	o	x	Apr. 7, 1914	Very small.
8	.....do.....	...do....	x	o	x	.....	
9	.....do.....	Orange .	o	o	o	Feb. 6, 1915	No effect.
10	.....do.....	Lemon..	x	.....	x	Apr. 2, 1914	Very small.
11	.....do.....	...do....	x	.....	x	.....do.....	Do.
12	.....do.....	...do....	x	.....	x	.....do.....	Do.
13	.....do.....	...do....	x	.....	x	.....do.....	Do.
Control cuts for each experiment.....			o	o	o		

[1] x=positive; o=negative.

With most of these inoculations made on old lemon tree trunks such effects as the softening and death of the bark through to the wood in the earlier stages, the appearance of fruiting bodies, formation of gum, and the subsequent development of an area of dead outer bark surrounding the initially invaded area and finally of an outer noninvaded gummous zone were found to be identical with those of cases of the disease occurring naturally.

Further inoculations into cuts on young bark of several species of Citrus and of deciduous trees are reported in Table XIV.

TABLE XIV.—*Inoculations with spores and mycelium from pure cultures of Botrytis cinerea on young trees of Citrus, and other hosts*

I. INOCULATED JULY 2, 1913; EXAMINED AUGUST 11, 1913

Experiment No.	Host.	Kind of tissue.	Results.
1	Valencia orange...	Young branch.....	Gumming slightly.
2	Sour orange.....	Small branch.....	Healing without gumming.
3	Lemon.....	Hardened bark.....	Cut filled with gum and healing back of it.
4	.....do.....	Young twig.....	Gum more copious than last; not healing.
5	.....do.....	Same as above without inoculum.	Healing without gumming.
6	Almond.....	Stem of young tree.....	Gumming with small portion of bark killed at cut, then healed.
7	Peach.....	.....do.....	Much gumming. Killed slightly on each side of cut. Bark area 6 cm. long in wood.
8	Plum (Burbank)..	.....do.....	Gumming at first and then healing.
9	Pear.....	.....do.....	Healed with enlarged scar.
10	.....do.....	Same as above without inoculum.	Healing perfectly.

TABLE XIV.—*Inoculations with spores and mycelium from pure cultures of Botrytis cinerea on young trees of Citrus, and other hosts*—Continued

II. INOCULATED APRIL 17, 1913; EXAMINED MAY 20, 1913

Experiment No.	Host.	Kind of tissue.	Results.
11	Common lemon...	Trunk 3 years old.....	Bark soft and decaying over areas of 0.5 by 1.5 inches. Much gum exuding.
12	Sweet orange.....	.....do.....	Bark slightly killed. Slight internal gumming.
13	Same as above without inoculum.	.....	No effect on bark.

III. INOCULATED FEBRUARY 6, 1915; EXAMINED MAY 18, 1915

14	Common lemon...	Branch about 2 years...	Gumming and fruiting of Botrytis on killed bark.
15	Rough lemon....	Trunk 2 years old.....	No effect.
16	Sour orange.....	.....do.....	Do.
17	Same as above without inoculum.	.....	Do.

With comparatively young common lemon, peach, and almond trees these inoculations with spores placed in cuts resulted in the formation of considerable gum. The gum was slight in sweet orange and plum. No gum was induced in sour orange, rough lemon, or in any of the control cuts except on peach. The death of the bark was slight and unimportant in extent except in the common lemon and peach.

In general the results of all these experiments showed that the effects of *Botrytis cinerea* were quite different from those of Pythiacystis gummosis. This fungus, unlike *Pythiacystis citrophthora*, was not able to make an entrance except through some wound or defect in the bark, and it was not able to progress so rapidly in killing the bark through to the wood. After a limited portion was killed to the wood, however, a larger surrounding area was involved, in which only certain outer layers of bark tissue were killed, leaving the cambium alive and capable of renewal. There was also in the Botrytis gummosis an outer gummous zone beyond the invaded zone, but this was usually less extensive and less rapidly formed than in Pythiacystis gummosis. Other conditions being equal, there was usually somewhat less gum formation in Botrytis gummosis than in Pythiacystis gummosis.

The fungus was reisolated from the softened invaded area of a large number of these lesions. Attempts to isolate the fungus from the outer gummous zone failed, just as they did in Pythiacystis gummosis. Only rarely was Botrytis isolated from the area where the outer bark was dead and hard. Cultures showed that this outer dead cortical layer following Botrytis inoculations is, under such conditions as prevail at Santa Paula, rapidly occupied by species of Alternaria, Cladosporium, Penicillium, Colletotrichum, Fusarium, and other fungi as well as bacteria.

FACTORS FAVORING BOTRYTIS GUMMOSIS

Many contributing conditions favor the occurrence and the severity of this disease. These are similar to those which favor Pythiacystis gummosis, mentioned in Part I of this paper, to be discussed in more

detail in a subsequent bulletin of the California Agricultural Experiment Station.

Injuries of various kinds to the bark, not only near the soil but anywhere on the trunk or large branches, may lead the way to infection and development of Botrytis gummosis when the conditions of moisture and temperature are also favorable. This disease is frequently severe on living tissue of trees that have been injured by frost. The fungus may become established in such trees first in a small portion of dead or dying tissue and then advance rapidly into tissue which appears to be sound.

A desquamated condition of bark, fairly common on old lemon trees in the California coastal regions, is also frequently accompanied by Botrytis gummosis. It furnishes dead outer bark tissue from which the fungus may advance. This desquamated condition is similar in appearance to that which usually follows inoculations with *Botrytis cinerea* on sound tree trunks and with which it is often confused, but which is thought to be due to other causes.

The previous use of "neat's-foot oil" in the treatment of gummosis also encouraged the growth of this fungus. Lemon trees previously treated at Santa Paula by scoring the bark and painting with neat's-foot oil were observed in February and March of 1912 to have their trunks fairly well covered with a gray coating consisting of the sporophores and spores of Botrytis. The bark on these trees was found to be in various stages of soft decay with the exudation of large masses of gum. Experiments also showed that this fungus grew better on bark treated with neat's-foot oil either before or after infection by the organism than on bark free from this oil. More recently the application of neat's-foot oil to Citrus trees has been largely given up, and the more severe stages of this disease, such as were previously seen, have not been observed.

CONTROL

The principles governing the control of this type of gum disease are similar to those of Pythiacystis gummosis: (1) The prevention of infection by avoiding injuries, and the use of fungicides, and (2) if that is not done, the elimination of the invaded tissue. As the result of many different experiments in which growers took a prominent part, a method consisting largely of scraping off the outermost layers of bark proved to be best adapted for treatment of this disease. The portion where the bark is totally killed is cut away, but beyond this, where only the outer layers of bark are dead, these outer layers only are scraped off, leaving the live inner layer next to the cambium intact. To prevent further invasion of bark it is usually found necessary to scrape the sound bark several inches beyond the margin of the affected region. A sharp curved tool made on the principle of a box scraper is in general use for this purpose. Where both types of gummosis are present on the same trees, as is frequently the case, this method is applicable to the combined lesions produced. The cut or scraped portions are then painted with a fungicide. Bordeaux paste, and some of the coal-tar products which contain only the heavier oils, have given good results in experimental work and in practice. A discussion of the experiments on which these control methods are based will be reported in a bulletin of the California Agricultural Experiment Station.

SCLEROTINIA GUMMING, DUE TO SCLEROTINIA LIBERTIANA

This disease, usually of minor importance, occasionally has been found associated with rapid dying of bark on the roots and trunks of Citrus trees growing in damp, cool situations, especially after periods of severe frost. The bark is at first soft, as if from an attack by *Botrytis cinerea.* Though the fungus usually advances more rapidly than Botrytis, it is soon checked, and callus begins to form as soon as gum accumulates. Later, as the bark dries, it is left in shreds and large black sclerotia are found within and under this bark. Its effect on Citrus twigs has been described by C. O. Smith (*54*), who refers to the gumming usually accompanying its attack. It appears to infect the young growth, usually at the blossoming period and frequently extends back into larger branches.

When found on the trunk or roots, observations have indicated that previous injury of the bark was usually necessary for the entrance of the fungus. It has frequently been found on young trees following frost injuries, apparently advancing from frost-injured tissue into tissue not killed by frost. It has been observed on a 20-year-old lemon tree where all the roots had been infected, probably from injuries made in digging about them and placing vetch straw near the crown in damp, cool weather. An old seedling orange tree was also observed with the bark on one side of the trunk killed by the fungus, which had apparently gained entrance through a slight sunburned area and had advanced into the live bark for some distance.

In order to obtain some idea of the ability of *Sclerotinia libertiana* to break down sound bark and induce gumming, several inoculations with pure cultures were made on healthy lemon trees, of which the following examples may be given:

On February 20, 1913, a bit of mycelium from a pure culture was inserted into a vertical cut in the bark of an 18-year-old Lisbon lemon tree. A similar cut to serve as a control was made on the opposite side of the same tree. These were then covered with oiled paper. The cut not inoculated healed normally, but the bark adjacent to the inoculation was soft on one side within 4 weeks. In 8 weeks there was a dead, soft area 15 by 8 cm., which had increased to 22 by 9 cm. in 11 weeks with a white mycelium conspicuous over a smaller area of 13 by 5 cm. About 4 months after inoculation (June 27, 1913) the area of dead bark measured 30 by 14 cm. and increased to 45 by 15 cm. by July 28, 1913, after which it soon ceased enlarging and became self-limited. The gum began to appear in 6 weeks; its rate of formation increased rapidly and reached a maximum about 3 months after inoculation. It ceased to increase about 5 months after inoculation. The sclerotia were seen to have formed in flat plates under and within the bark in 4 months and appeared to be alive for at least 2 years, but no subsequent activity was apparent in this lesion for a period of at least 3 years. The killed bark showed the characteristic shredded appearance. Without treatment, there was a complete stoppage of further invasion by the fungus.

A 2-year-old lemon tree was similarly inoculated 1 inch above the bud union on February 25, 1913. By May 7, 1913, the tree was girdled and showed gumming, the bark being killed 6 inches upward and 1 inch downward to the bud union. The invasion did not advance into the sour-orange tissue of the stock. The foliage at this time showed no effect, but by June 27, 1913, the leaves were wilted. In both these cases similar

cuts not inoculated, made as controls, healed without visible injury to the tree.

As indicated by these inoculations, the attack of *Sclerotinia libertiana* may be very severe and its progress very rapid for a comparatively short time, and then it may be quickly halted. If the tree trunk attacked is small, girdling and death may result, while on a large trunk with not more than one point of infection, self-recovery may take place. Observation shows that the halting of the invasion is usually coincident with the formation and exudation of considerable quantities of gum.

EXPERIMENTS TO TEST THE POSSIBLE RELATION OF OTHER ORGANISMS TO GUMMOSIS

A number of other organisms, most of which were found commonly on dead or decaying bark of Citrus trees, were used in inoculation experiments to ascertain their relation, if any, to gummosis.

The results of inoculations with various cultures of *Fusarium* sp., commonly found associated with Pythiacystis gummosis, have already been presented on page 205. The effect of Fusarium alone was insignificant. The death of the bark at the inoculated cuts was inconspicuous, and only slight death of the outermost layer of bark resulted over small areas. Only part of the inoculations produced gum, usually in small quantity.

Penicillium roseum was frequently found on dead bark affected with gummosis. The results of some of the inoculations with pure cultures of this fungus are presented in Table XV.

TABLE XV.—*Inoculations with Penicillium roseum at Santa Paula*

Experiment No.	Date of inoculation.	Kind of inoculation.	Gum.	Initially killed bark at inoculation point.[1]	Cracking of outer bark.[1]
1	July 12, 1912	Vertical slit 2 cm. long, covered with oiled paper	2	1	2
2	Aug. 23, 1912	Vertical slit	2	1	0
3	Feb. 21, 1913	Vertical slit not protected	2	1	2
4	.....do.....	Controls on same trees as above	0	0	0
5	Feb. 20, 1913	Vertical slit not protected	1	1	0
6	.....do.....	Control on same tree	0	1	2

[1] 0=none, 1=slight, 2=medium.

Gumming was induced in all the cuts inoculated with *Penicillium roseum* in Table XV. The dead bark adjacent to the inoculated cuts was slight in amount. The outer layer of bark surrounding two of the inoculated cuts and only one of the controls died and cracked.

Inoculations were made with a number of other organisms, as shown in Table XVI. All of these were found on Citrus trees except *Coryneum berynkii* and *Pseudomonas cerasius*.

TABLE XVI.—*Inoculation with miscellaneous organisms*

Experiment No.	Date of inoculation.	Variety and age of host.	Organism.	Gumming.[1]	Death of bark.[1]
1	Nov. 23, 1912	Lemon, 19 years..	*Alternaria* sp. from injured fruit.	1	1
2	Feb. 25, 1913	Lemon, 2 years....	*Alternaria* sp.....	0	1
3	May 24, 1912	Lemon 16 years...	*Coprinus atramentarius*, mycelium.	0	0
4	.....do.....	.....do.....	Spores of same.....	2	1
5	July 13, 1913	Lemon, 19 years...	*Coprinus atramentarius* mycelium.	2	1
6	May 24, 1912	Lemon, 16 years...	*Hypholoma* sp. spores...	1	0
7	Aug. 3, 1912	Lemon, 16 years (2 trees).	*Hypholoma* sp. mycelium.	1	0
8	Sept. 25, 1912	Lemon, 19 years...	*Cladosporium* sp.....	0	0
9	.....do.....	.....do.....	*Rhizopus* sp.....	0	0
10	Sept. 4, 1913	Lemon, 18 years...	*Penicillium digitatum*...	0	0
11	Aug. 3, 1912	Lemon, 16 years...	*Diplodia* sp.....	2	1
12	Nov. 23, 1912	Lemon, 19 years...	*Spegazzinia ornata*.....	0	0
13	July 13, 1913	.....do.....	*Coryneum berynkii* from peach.	2	1
14	.....do.....	Orange stock, same tree.	.....	1	0
15	July 12, 1912	Lemon, 19 years...	*Pseudomonas cerasius* Griffin.	0	0
16–25	.....do.....	.....do.....	10 different cultures of bacteria inoculated from bark killed by gummosis.	0	0
Controls, without inoculum to correspond with each of the foregoing experiments.				0	0

[1] 0=none, 1=slight, 2=medium.

The results show that several different organisms are usually capable of inducing the formation of a small quantity of gum and a limited amount of injury to cells adjoining a wound when inserted into cuts on sound tissue but are without any noticeable effect in producing definite diseases. This gum formation or death of tissue was, in these cases, as well as with *Fusarium* sp., and *Penicillium roseum*, insignificant in amount as compared with that produced in cases of either Pythiacystis or Botrytis gummosis.

PART III.—GUM FORMATION AND ITS RELATION TO THE DEVELOPMENT OF DISEASES

INTRODUCTION

In Parts I and II of this paper the relation of certain fungi to definite diseases in which gum formation was a conspicuous feature has been discussed. It was shown experimentally that the most destructive types of gum diseases on Citrus in California in which there is progressive dying of tissue over large areas are due to fungus invasion. Fungi, however, are not necessary to mere gum formation, since it has been shown that other agencies, such as chemical injections, may induce gum formation in the absence of microorganisms.

It is the purpose of Part III to discuss more especially the process of gum formation itself, the conditions facilitating its formation, and its relation to the development of diseases.

NATURE AND ORIGIN OF THE GUM

Although gum appears to be formed in many other plants continuously as a "normal" process, it is usually not formed in Citrus except under the influence of stimuli more or less injurious to the tree. Citrus gum is similar to cherry gum and gum arabic, the latter of which is known to retain the nature of an acid, the molecule being composed of a number of sugar residues grouped about an acid nucleus in such a way as to leave the acid group exposed.

These gums differ from resins in being for the most part soluble in water and insoluble in alcohol, while the opposite is true of the resins. This solubility in water also distinguishes them in a rough, imperfect way, from mucilages, which have a more slimy consistency and merely swell up in water. There are, however, all gradations between gums so defined and the mucilages. As to origin and chemical composition, the distinction between the vegetable gums and some of the mucilages can probably not be clearly maintained. These exuded gums, however, should probably be distinguished from a hard, vitreous gumlike substance known as wound gum, which Higgins (*40*) refers to as occurring in the wood elements in the vicinity of wounds as a general phenomenon in woody plants. It differs from the ordinary gums in not swelling in water and in giving the lignin test.

Although there has been considerable difference of opinion as to the direct origin of gums and mucilages in plants, most investigators, including Grafe-Wien (*35*), Czapek (*18*), and Lloyd (*45*), have concluded that the gums like those represented by gum arabic, cherry gum and Citrus gum, are derived mainly from the cellulose walls. Greig-Smith (*36*), however, has reported the formation of gums of this nature by the direct action of bacteria on certain culture media containing no cellulose. Gum related to the dextrins also has been obtained by the action of *Bacillus radicicola* on culture media containing sacchrose by Buchanan (*12*), who concludes that this gum arises from the diffluent wall of the bacterial cell. MacDougal, Richards, and Spoehr (*47*) have pointed out that in cacti the formation of mucilagelike gum results from the dehydration of sugars and condensation of their products as a normal process in this plant.

In Citrus, however, the gum appears to arise mainly from the hydrolysis of the cellulose walls. The initial gum originates, according to Butler (*13*) and Floyd (*31*), between the medullary rays usually in thin-walled xylem cells newly laid down. The protoplasm becomes more granular, the cells round out, separate from each other, and dissolve from the outside inward, and the space is finally occupied by a mass of gum. It may also be derived apparently from the breaking down of other and older tissues in connection with some of the severe types of Citrus gummosis.

PHYSICAL EFFECTS AND GUM FORMATION

MECHANICAL INJURIES

The writer has not been able to induce gum exudation on healthy Citrus trees by mechanical injuries alone, provided these injuries are

kept clean and reasonably free from contamination with microorganisms or unusual chemical substances.

A number of experiments were carried out to test (a) the influence of various kinds of wounds, (b) the influence of obstructions in the sap current by insertion of substances such as glass or wooden plugs, and finally (c) the influence of continued pressure upon the bark. Most of these experiments were performed with and without contamination with spores from pure cultures of *Botrytis cinerea* and are shown in Table XVII.

TABLE XVII.—*Experiments with injuries with and without contamination, started in July, 1912, on sound trunks of 18-year-old lemon trees at Santa Paula*

Treatment of tree.	Number of experiments.	
	Not inoculated.	Inoculated.
Tangential slice of outer bark cut off and microscopic slide fastened over with putty	2	2
Surface of bark lightly scraped and covered as above	.....	1
Bark not injured but covered as above	1	1
Long slits made through bark and covered with grafting wax	1	1
Areas of bark about 8 by 4 cm. cut away and covered with cold liquid grafting wax	5	5
Bark on trunk injured with heel of heavy boot, as if climbing tree	.....	1
Bark injured by throwing wire coal basket against trunk	.....	1
Bark injured by blow from blacksmith's hammer and covered with wax	2	2
Auger holes 1.3 to 2 cm. deep and 1 to 1.5 cm. in diameter filled with glass tubes and sealed in with wax	5	2
Pressure exerted against wooden blocks, on opposite sides of tree, by means of screws in an iron collar	3	.....

On none of the injuries shown in Table XVII, kept uninoculated, was there any gum exudation or development of the disease. All such injuries healed in the usual way. With all the experiments in which spores of *Botrytis cinerea* were used as a contamination, however, except where the bark was not injured and one of those with glass tube used as a plug, gum exuded and in most cases typical *Botrytis gummosis* lesions developed around the place of injury.

The results of these experiments appeared to show that injuries in themselves were not sufficient to induce gum formation. When the injuries were contaminated with pure cultures of *Botrytis cinerea*, however, under the same conditions, gum formation and death of the tissue readily took place. Other injuries of various kinds also failed to induce gumming in most cases even when no means were provided to keep the wounds free from chance contaminations from the air or from water during rains. These same wounds, however, when purposely infected with *Botrytis cinerea* or other injurious organisms resulted in gum formation. Mechanical pressure and obstructions placed in the conducting system likewise failed to induce gum in these lemon trees. In previous experiments conducted by Fawcett (*22*), clean injuries made by cutting through the bark of *Prunus persica*, *Prunus umbellata*, *Prunus serotina*. *Laurocerasus caroliniana*, *Xanthoxylum americanum*, and *Rhus glabra* in Florida failed to induce gum, but when similiar injuries in all these species were inoculated with *Diplodia natalensis*, gum formation resulted.

In Prunus and other plants, however, mechanical injuries in themselves have been considered as sufficient to induce gum formation. Butler (*13*) was successful in inducing gum formation on peach, cherry, and plum by bruising the bark with a mallet. No mention is made of similar experiments on Citrus.

The injuries on Citrus made by a number of insects are frequently followed by gumming, usually slight in amount. Small drops of gum may form on fruit at points of injury produced by the orange tortrix (*Tortrix citrana*), and on small tree trunks and limbs by grasshoppers, katydids, and other insects. To what extent this gumming may be due to secretions of the insects and to what extent to the entrance of microorganisms at the time of injury is uncertain. Our negative results from mechanical injuries on Citrus kept sterile and free from chemical stimuli would indicate that this gumming was probably not due to the injury or wound in itself.

BURNING

In the author's experience, burning was not in itself any more effective than mechanical injuries in producing gum formation in Citrus. The flame of an alcohol lamp was held against the trunk of young Citrus trees until the bark was severely injured, but no gumming resulted. Examination of many Citrus trees that have been accidentally injured by fire has shown that bark may be killed on one side of twigs and branches without resulting in gum formation. Severe injuries from sunburning on Citrus have also been observed to be free from gum formation, provided they are not followed by invasion of parasitic or wood-rotting organisms. Butler (*13*), however, reports the production of gum on young shoots of Prunus by burning with a hot iron.

FREEZING

Freezing has also been considered a frequent cause of gum formation. Observations of hundreds of Citrus trees in California in all stages of injury from frost have not indicated that freezing in itself is an important factor in initiating gum formation. Frost injury, however, may frequently be followed by invasion of organisms such as *Botrytis cinerea*, *Sclerotinia libertiana*, or other fungi, which after becoming established in the injured tissue may advance rapidly and induce gumming in tissue apparently sound.

DESICCATION

Drying or partial desiccation of cells in plants has been suggested as an important factor in gum formation. As the result of a number of controlled experiments with species of Prunus, drying of the tissue was considered by Higgins (*40*) as an important factor in the acceleration of gum formation. Sorauer (*59*, *p. 708*) cites Martin as mentioning the action of dry desert winds of autumn and winter as having a relation to gum formation in *Acacia senegal*. It has been pointed out by MacDougal, Richards, and Spoehr (*47*) that when Opuntia plants are subjected to long periods of drought, resulting in partial desiccation, the sugars, which have a low water-holding capacity, are converted into the pentosans or mucilages, which have a high imbibition capacity.

That this factor of partial desiccation operating alone can not account for excessive gum formation in Citrus seems to be indicated by the fact that such gum formation does not necessarily follow injuries from frost

or sunburn, nor does it necessarily result from drying of tissue in wounds if these are kept uncontaminated by microorganism or chemical stimulants.

Some of the results of the investigations reported in Parts I and II of this paper have a bearing on this subject. It was shown that gum formation could be induced in cuts inoculated by *Pythiacystis citrophthora* or *Botrytis cinerea* where these were covered with grafting wax or waxed paper, which presumably prevented undue loss of water from the surface. It is, of course, possible that dehydration of the cells invaded by the parasite might have taken place even in this case, without loss of water through the surface, by changes in internal conditions by which water was lost to the surrounding tissues. With Pythiacystis gummosis excessive water in the soil about the trunks, rather than drying or partial desiccation, is the condition favorable for the invasion of the bark by the causal organism that is instrumental in bringing about gum formation.

While partial desiccation in itself does not appear to be an important factor in initiating gum formation in Citrus, it is probably one of the factors in producing an acceleration of the process when the other necessary factors are already in operation. It has been noticed also that when a lesion is developing and gumming moderately, the coming of dry weather is frequently followed by increased exudation of gum for a brief period. If the weather is sufficiently dry, however, the enlargement of the invaded zone often ceases, probably due to the dying out of the parasite, and the rate of gum formation soon decreases to zero. It appears that in Pythiacystis gummosis, at least, the conditions of desiccation that tend to increase the gum flow are frequently those that hinder or kill out the causal agent, while furnishing a temporary stimulus to increased gum formation. If, as seems highly probable, partial desiccation of the cells is one of a group of factors which favor excessive gum formation in Citrus, it seems clear that other more important influences or stimuli must precede or accompany it.

CHEMICAL STIMULI AND GUM FORMATION

Many substances have been reported as inducing gum formation in Citrus. A study of gum formation was made by Floyd (*31*) by the introduction of 28 organic and inorganic substances into young Citrus trees. Thirteen of these induced gum formation. Most of them were acids, alkalies, or salts of heavy metals. In general, the salts of the heavy metals brought about the greatest amount of gumming. The gum formation was coincident with the injury from the chemicals; the gum was small in amount and was formed in proximity to the region of insertion of the chemicals except in case of some of the salts of the heavy metals. The gum originated in all cases in the live embryonic xylem tissue in regions or zones beyond the dead area produced by the chemical. None of the cuts or injuries used as controls, and not inoculated with chemicals, produced any gum formation. The dead area produced by the chemical is thus seen to be directly comparable to the invaded zone in Pythiacystis gummosis, and the region of chemically induced gum formation beyond this is directly comparable to the noninvaded "outer gummous zone" of the disease.

Experiments on Citrus had been made previously with a limited number of chemicals. Butler (*13*) induced gum formation with sul-

phuric, phosphoric, nitric, and lactic acids, and with potassium hydrate but failed to induce it with acetic acid or kerosene; and Fawcett (*23*) induced gum with nitric, sulphuric, acetic, citric, and phosphoric acids, copper sulphate, mercuric chlorid, and ammonium lactate but failed to induce it with carbolic acid.

Among the chemical stimuli that have been seen to result in gum formation occasionally in Citrus orchards may be mentioned: (1) liquid hydrocyanic acid spilled on the soil too near the roots of trees; (2) hydrocyanic acid gas used in fumigation; (3) spray mixtures containing copper sulphate not properly neutralized with lime, or containing other toxic substances; (4) ant poison containing arsenic.

It has not been possible, however, to duplicate entirely by chemical stimuli any of the typical maladies produced by certain organisms. While the gum formation induced by chemicals may be slight or extensive, the stimulus is soon at an end and the wound usually begins to heal promptly without the long-continued, progressive killing and gumming characteristic of Pythiacystis and Botrytis gummosis. Much confusion and apparent differences in results regarding gummosis have arisen from a failure to distinguish between the severe types of gummosis induced through the agency of microorganisms and those more temporary and usually milder types brought about by chemical or other stimuli. While the nature of the gum exuded may be the same, the manner in which the accompanying injury develops is usually very different.

ENZYMS AND GUM FORMATION

Only a small amount of experimental work on the rôle of enzyms in Citrus gummosis appears to have been done. The results of the writer's experiments will be presented here merely as suggestive of certain possibilities in connection with this subject.

Savastano (*52*) concluded that in the form of Citrus gummosis due to *Bacterium gummis*, a toxin secreted by the organism, spreads out considerable distances beyond the invaded tissue, stimulating the gum formation. In line with this view, Higgins (*39*) in the study of plum wilt, concluded that the fungus *Lasiodiplodia triflorae* Higgins secreted a toxic substance which reacted directly or indirectly on the zymogen of the host cells and brought about the production of a gum-forming enzym. Butler (*13*), on the other hand, had concluded that enzyms had no part in gum formation in Citrus or Prunus. This view, it seems, was based mainly on the origin and histology of the gum pockets and not on microchemical tests or experiments to detect the presence of enzyms. (See criticism of this view by Wolf) (*65*). Floyd (*31*) made certain microchemical tests with material taken from gum pockets on Citrus branches affected with exanthema and concluded that the enzyms present were probably hemicellulase and pectinase.

To get definite information as to the possible influence of enzyms or other filterable substances on gum formation in Pythiacystis gummosis, the following experiments were carried out.[12]

EXPERIMENT 1.—On October 1, 1912, lemon bark containing Pythiacystis gummosis lesions was cut from two large trees at Whittier, Calif., and 350 gm. of green material were ground, first in a meat grinder, then

[12] The assistance of H. D. Young, formerly of the Southern California Pathological Laboratory, in carrying out these experiments is acknowledged.

in a mortar with sand and water. One liter was decanted off and filtered through a sterilized clay filter. No organisms were found in the filtrate. Part of this filtrate was boiled and the other part left unheated. Two small holes slanting downward were bored, one in each side of a large lemon tree, and burettes holding about 50 cc. of the liquid were inserted into these holes and sealed. One burette contained a portion of the boiled filtrate just referred to, and the other unboiled filtrate. In each case the solution was taken up by the tree in a few hours. On November 5, 1912, a considerable quantity of gum was observed pushing up into the burette which had contained the unboiled filtrate. No gum was observed in the other burette. No further development had taken place in either case on November 30, 1912.

EXPERIMENT 2.—On April 16, 1913, filtrate from a large piece of lemon bark containing a lesion 35 by 8 cm. produced by inoculation with *Pythiacystis citrophthora* (Pl. 3, B) was obtained as described in the previous experiment. At the same time, a filtrate from a large piece of sound bark from a healthy tree was obtained in the same manner, as a control. The filtrate from the diseased bark was placed in a separatory funnel and was allowed to be absorbed by a young lemon tree (Pl. 8) through a small hole in the bark. About 100 cc. were taken up in five days. The filtrate from the sound bark was placed so as to be taken up by another lemon tree of the same size.

On May 6, 1913, thin gum was observed pushing up into the lower end of the funnel tube through which diseased filtrate had been introduced. Gum undoubtedly had begun to form internally long before this time. In about two weeks the gum (about 10 cc.) had accumulated in the lower enlarged portion to a height shown in Plate 8, after which the pressure resulted in this breaking through the rubber connections at the bottom. Very little gum was exuded after June 3, 1913, when the funnel was removed. The bark remained alive around the opening. During this entire time no gum appeared in the funnel through which the filtrate obtained from the sound bark had been introduced.

The results of these experiments indicate that there is a substance in the diseased bark which is capable of passing through a fine clay filter and of inducing gum formation. In the experiments, however, gum formation soon ceased without serious injury to the bark, such as is produced by the invasion of *Pythiacystis citrophthora*. It would seem, therefore, that the gumming is induced by some substance that is formed either by the fungus or by the interaction of the host and parasite. The fungus during its invasion of the host probably secretes a substance which stimulates either directly or indirectly the production of a gum-inducing enzym by the host cells themselves. This substance doubtless also passes out into adjacent cells, and into the conducting tissue, and thus brings about gum formation at long distances from the invaded zone. This substance passing out from the invaded regions probably accounts for the large outer noninvaded gummous zones which so readily recover when the inciting cause, the parasitic fungus, is removed. This substance, capable of inducing gum formation, but not capable of bringing about the formation of lesions characteristic of Pythiacystis gummosis, appears to be destroyed by boiling and may, therefore, be an enzym. Further investigation as to the real nature of this gum-inducing substance is needed.

PARASITIC ORGANISMS AND GUM FORMATION

Although there are various agencies or diverse stimuli that contribute directly or indirectly to gum formation in Citrus, the more serious and progressive types of gummosis (in California at least) appear to be initiated by the invasion of certain parasitic fungi. With Pythiacystis gummosis, for sample, the bark and cambium region are first invaded and killed by the fungus which appears to advance most rapidly in the inner bark adjacent to the cambium. Soon after a small portion is killed by the initial invasion of the fungus, an influence is exerted bringing about gum formation in the xylem region surrounding the dead portion. After some time gum begins to form rapidly within the xylem for long distances vertically, but usually for only short distances laterally, from the invaded zone. The parasite, therefore, must stimulate such gum formation in an indirect manner, through setting into action some substance which passes out into the conducting system, and which moves probably in the sieve tubes as well as in the wood vessels. This gummy degeneration within the outer gummous zone usually does not kill the bark nor prevent its recovery, provided the parasite does not invade it later. A thin outer layer of wood, not penetrated nor killed deeply by the parasite, becomes infiltrated with gum, and occasionally gum pockets are formed within it but usually near the cambium.

The seriousness of excessive gum formation in itself has been overemphasized by some investigators because it was erroneously held that the death of the bark was the usual cause of gum formation instead of being the result of fungus invasion. Gum formation appears to diminish, rather than to increase the seriousness of the diseases. As has been previously mentioned, the excessive gum formation in connection with Pythiacystis gummosis is frequently accompanied, or is soon followed, by a stoppage in the further advance of the invading parasite. It is highly probable that the infiltration of tissue by gum in the outer gummous zone tends to hinder the further advance of the mycelium into this tissue. In Diplodia gumming, Earle and Rogers (*21*) state that their observations have convinced them that gum flow in Citrus serves as a "protective device," and in plum wilt, Higgins (*39*) concluded that the gum served to hinder the advance of the organism by being formed in its path of advance. In the disease due to *Sclerotinia libertiana* on Citrus twigs, C. O. Smith (*54*) states that with the appearance of the gumming of the twig the further enlargement of the lesion is checked.

The gum in connection with Pythiacystis gummosis lesions, in addition to being a probable hindrance to the organism, serves in dry weather as a seal or covering to the wood beneath the killed bark, thus preventing the entrance of wood-destroying organisms and preventing excessive drying-out of the exposed wood. The gum being soluble in water, however, it is of little value for this purpose after the rains appear.

BRIEF GENERALIZATION ON GUM FORMATION

The explanation of gum formation itself, which seems to fit in best with the known facts and results obtained with Citrus, is that a substance of an enzymatic nature may be brought into action through various instrumentalities, such as substances acting under the influence of invading organisms, introduced or induced chemical stimuli, etc. It appears to be evident that the enzym immediately responsible for gum formation is

produced by the host tissue itself; otherwise it would be difficult to account for the gum formation induced by such a variety of chemical substances and agencies. In Pythiacystis and Botrytis gummosis, for example, there would seem to be two possible explanations of the manner in which the gum formation is brought about: (1) A substance secreted directly by the fungus sets in motion the gum-forming enzym or (2) a substance resulting from the interaction of parasite and host furnishes the means by which the gum-forming enzym is brought into action.

In case of the action of chemicals, such as corrosive sublimate and sulphuric acid, the substance might act also in either of two ways analogous to that given for the parasite, either directly in the same way as a secreted substance, or indirectly through a substance produced by the interaction of the plant tissues and the introduced chemical. The experiments with sterile filtrate from diseased and healthy bark previously described, taken in conjunction with the work of Floyd, Higgins, and others, appear to show that whatever may be the indirect factors in gum formation, the immediate cause or stimulus to its formation is a filterable substance of the nature of a heat-sensitive enzym. It is evident that many important phases of the subject of gum formation in Citrus remain for further investigation.

SUMMARY

(1) A destructive form of Citrus gummosis attracted attention first in the Azores in 1834. A similar gum disease appeared in Italy as early as 1863; in Portugal, 1865; in Australia, 1867; in Spain, 1871; in United States of America, 1875; and in most other Citrus regions before the year 1890.

(2) Pythiacystis gummosis, the most widespread and destructive type of Citrus gummosis in California, is characterized by copious exudations of gum and dead patches of bark on the trunk and main roots. The gum may arise not only from the margin of the invaded area but also from a large contiguous, outer, noninvaded zone.

(3) It has been shown that the disease with all its usual symptoms may be readily transmitted to healthy trees by inoculation with bits of bark tissue cut from the advancing margins of killed regions of bark. It is not transmitted, however, by tissue from surrounding outer gummous zones or by killed tissue not recently invaded.

(4) Cultural tests have shown that live mycelium of *Pythiacystis citrophthora* Sm. and Sm., formerly known as the cause for lemon brownrot, is present in this narrow band or fringe at the advancing edges of the killed region of bark (invaded zone) but is absent or dead elsewhere.

(5) Numerous inoculations with pure cultures of *Pythiacystis citrophthora* into healthy trees under various conditions have shown that this fungus is capable of inducing gummosis with all the characteristic symptoms of naturally occurring cases. The fungus has been reisolated from many lesions after it has remained in the bark from 1 to 11 months.

(6) Lemon fruits affected by brownrot due to *Pythiacystis citrophthora* have been shown to be capable of inducing the same type of gummosis as that produced by the fungus from gummosis lesions.

(7) The organism appeared to die out more readily and the lesions became self-limited more quickly after inoculation in branches or large roots than after inoculation in the trunk.

(8) A limited number of inoculation tests gave some indication that *Fusarium* sp., found commonly in connection with Pythiacystis gum-

mosis lesions, increased the severity of the disease when associated with *Pythiacystis citrophthora* but that Fusarium alone was not capable of initiating this gummosis.

(9) Observations and inoculation experiments both indicated that the order of resistance of species and varieties to *Pythiacystis citrophthora* from highest to lowest, is, sour orange (*Citrus aurantium* Linn.), trifoliate orange (*Poncirus trifoliata* Raf.), rough lemon (a resistant variety of *Citrus limonia* Osbeck), pomelo (*Citrus grandis* Osbeck), sweet orange (*Citrus sinensis* Osbeck), and common lemon (*Citrus limonia* Osbeck). The first is almost immune, the last very susceptible.

(10) Inoculation of small roots of young trees indicated that common-lemon roots are somewhat susceptible but that sweet-orange, pomelo, and sour-orange roots are resistant.

(11) Mal di gomma due to *Phytophthora terrestria* Sherb. has been shown to be similar to certain phases of Pythiacystis gummosis, especially to the form the latter takes at the junction of the main roots and trunk of old orange trees in California.

(12) Inoculations with *Phytophthora terrestria* and with *Pythiacystis citrophthora*, under the same conditions, produced lesions which showed no characteristic differences.

(13) Experiments have shown that in cases where excess of moisture and other contributing conditions cannot be entirely avoided the disease may be prevented largely by applying Bordeaux mixture or other fungicides to the trunks.

(14) It has also been shown that the progress of the disease may be readily prevented by dissecting out the bark invaded by the causal organism and applying a fungicide. It was not found necessary to remove the bark in the outer gummous zone, since this bark would finally recover after the advancing fungus had been removed.

(15) Botrytis gummosis of lemon trees is characterized in the early stages by a soft area of invaded bark killed to the wood, with exudation of gum on the trunk. Later this soft area becomes surrounded by a larger, firmer area in which only the outer layer of bark is killed, leaving a layer next to the cambium region alive. There is also, as in Pythiacystis gummosis, a noninfected outer gummous zone from which copious gum exudation may take place.

(16) A strain of *Botrytis cinerea* Lk. has been found commonly associated with this type of gummosis and has been isolated from numerous lesions.

(17) Pure cultures of the fungus, as well as bits of the diseased bark, were found capable of inducing the disease when inoculated into cuts and other kinds of injuries on healthy lemon trees.

(18) Attempts made to induce gum formation by various kinds of wounds on lemon tree trunks invariably failed when these wounds were kept clean and free from contamination with injurious organisms or chemical substances.

(19) The experimental results show that the disease may be prevented by avoiding injuries to the bark and by using a fungicidal coating on its surface. The treatment found effective consists in cutting or scraping away the dead bark, leaving as much of the live bark as possible, and painting the treated area with a fungicide.

(20) *Sclerotinia libertiana* is occasionally found associated with rapid dying of bark and copious gum exudation on trunks and roots. The

bark is at first soft, later dries out into long shreds, and usually contains flat sclerotia within it.

(21) Inoculations with pure cultures have shown that this fungus may also kill the bark rapidly and bring about the results just mentioned on healthy lemon tree trunks.

(22) Inoculation experiments with a large number of other organisms showed that some of them were capable of inducing gum formation, but this effect was usually slight as compared to that of Pythiacystis, Botrytis, and Sclerotinia. Those which produced some gum exudation in cuts were *Penicillium roseum*, *Fusarium* sp., *Diplodia* sp., *Coryneum berynkii*, *Coprinus atramintarius*, *Alternaria* sp., and *Hypholoma* sp. The effect in killing of bark was insignificant, and no definite diseases resulted. Several other organisms produced no effect whatsoever either in inducing gum exudation or killing tissue.

(23) In Parts I and II of this paper the relation of certain fungi to definite types of diseases in which gum is a conspicuous feature has been discussed.

(24) Gum in Citrus is similar to gum arabic and cherry gum and appears to originate mainly in the xylem tissue by hydrolysis of the cellulose walls.

25) Attempts to induce gum formation in lemon trees by various mechanical injuries, by obstructions placed in the conducting tissue, and by pressure on the bark, failed when the tissues experimented upon were free from parasitic organisms or unusual chemical stimuli.

(26) Injuries from certain insects have been observed to result in slight gum formation, which is probably due to secretions by the insects or to contaminating organisms.

(27) Experiments and observations indicate that burning and freezing are not important factors in inducing gum formation in Citrus but merely serve to open up the way for parasitic and wood-rotting fungi, which afterwards induce gumming.

(28) Partial desiccation appears to be merely a factor in the acceleration of the process of gum formation in Citrus and not a necessary condition to its initation.

(29) Certain chemical substances are capable of inducing gum formation when injected into Citrus bark. It has not been possible, however, to reproduce all the typical symptoms of any of the gum diseases by chemical injections. The invasion of certain parasitic organisms appears to be the chief factor in initating gum formation under natural conditions. These organisms may bring about, as do certain chemical substances, gum formation over considerable areas of bark surrounding the portions killed by either the organism or the chemical substance.

(30) Comparative experiments with boiled and unboiled filtrates from diseased and healthy tissue show that the diseased bark contains a substance capable of passing through a fine clay filter and of inducing gum formation when unboiled. When boiled, however, this capacity to stimulate gum is destroyed. This indicates the presence of a heat-sensitive enzym.

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PLATE 1

Pythiacystis gummosis on lemon trees, from inoculation with diseased bark tissue.

A.—Tree inoculated on February 27, 1912, the gum formation showing the development of disease on August 2, 1912.[a]

B.—Same tree, September 19, 1912, over 6 months after inoculation.

C.—Same tree about 1 year later, view at right angles to B. Only 5 cm. of live bark then prevented complete girdling.

D.—Same view as A and B, on May 24, 1913, 15 months after inoculation. The gum first formed has been dissolved away by winter rains and the dead bark has dried and shrunk. Gum was exuding farther around, as in C, at this time.

E.—Lemon tree inoculated with diseased bark tissue, September 21, 1912, showing excessive exudation of gum 2 months later.

F.—Lemon tree inoculated with diseased bark under a glass slide held with putty, September 21, 1912, showing gum formation 2 months later.

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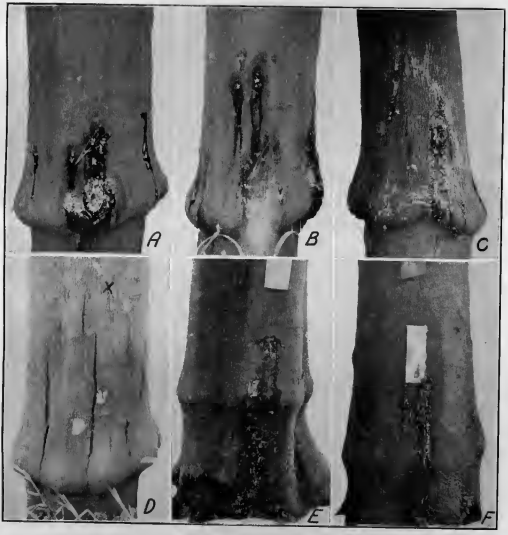
[a] A majority of the trees in this same orchard had the same "overgrowths" at the union of stock and scion as observed in this illustration.



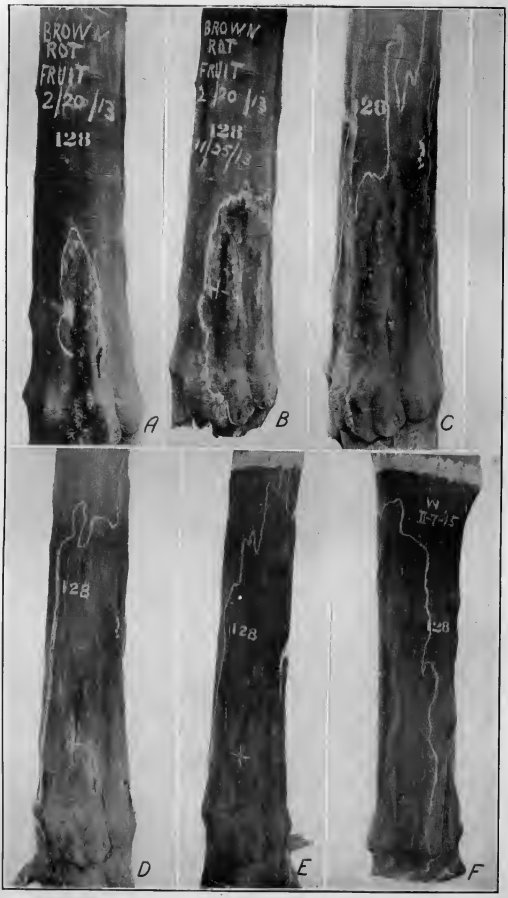

BROWN
ROT
FRUIT
2/20/13
128
A
BROWN
ROT
FRUIT
2/20/13
128
11/25/13
B
20
C
128
D
128
E
W
11-7-15
128
F

PLATE 2

Pythiacystis gummosis on lemon tree from inoculation made on February 20, 1913, with diseased fruit tissue affected with Pythiacystis-rot.

A.—Extent of invaded bark and gum formation about 5 months after inoculation.

B.—Same tree about 7 months after inoculation, showing increase in the invaded area.

C.—Same tree about 13 months after inoculation, showing cracking of dead bark over invaded area and its large increase in size.

D.—Same tree 18 months after inoculation, showing a still larger area affected.

E, F.—Two views of the same tree, about 2 years after inoculation. Figure F taken on opposite side of tree from figure E.

PLATE 3

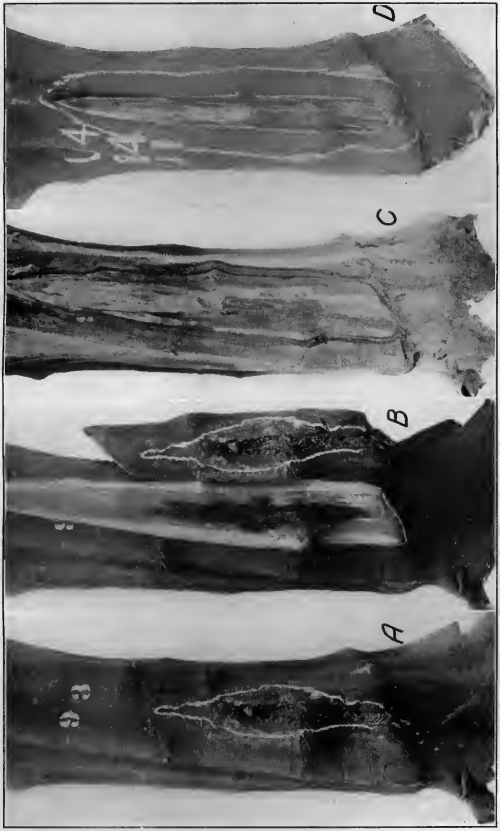
Pythiacystis gummosis on lemon tree from inoculation on November 23, 1912, with pure culture of *Pythiacystis citrophthora*.

A.—Extent of invaded portion (inside of chalk line) and gum formation on June 6, 1913. The fungus was isolated from several places near chalk line at this date.

B.—Same tree after bark was cut, showing invaded zone (black) and a part of the outer gummous zone (shaded), which extended upward and downward under live bark.

C.—Same tree in September, 1914, showing new bark pushing in over wound.

D.—Same tree in June, 1920, showing increase in the new bark covering the edges of the original wound.


A
B
C
D

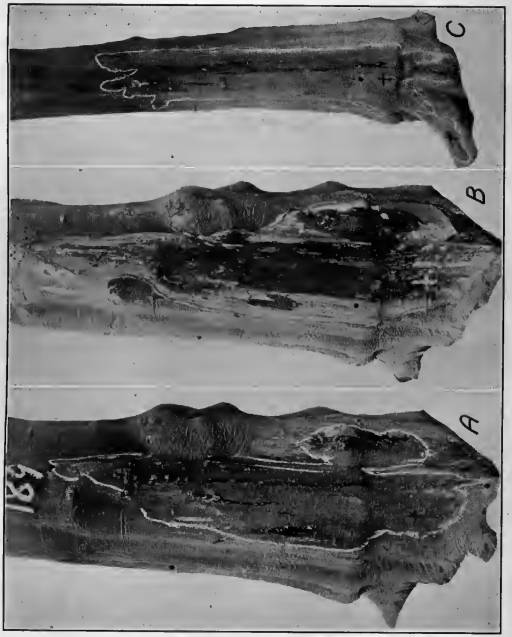

C
B
A
183

PLATE 4

Pythiacystis gummosis from inoculations made July 13, 1913, with pure cultures of *Pythiacystis citrophthora* on different parts of the same lemon tree.

A.—Extent of invaded zone and gumming from an inoculation on the sweet-orange stock. Photographed May 9, 1914. The fungus was isolated from upper portions near chalk line at this date, about 10 months after inoculation.

B.—Same, with bark removed, showing darkening at cambium region over invaded zone. Original inoculation was at the place marked +, from which the invasion of the fungus proceeded mostly upward, over the "bud union" (swollen ring) into the lemon bark.

C.—Opposite side of same tree on same date, showing portions invaded (chalk line) as result of two inoculations, one on lemon bark above and one on a large crown root below the bud union.

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PLATE 5

Pythiacystis gummosis which was apparently increased in severity by inoculation with both *Fusarium* sp. and *Pythiacystis citrophthora* February 22, 1913, showing progress of disease over a period of about 3 years. This tree was finally girdled and killed by disease starting from one inoculated cut.

A.—Exuded gum and extent of invaded zone (chalk line) July 28, 1913, 5 months after inoculation. Arrow indicates point of inoculation.

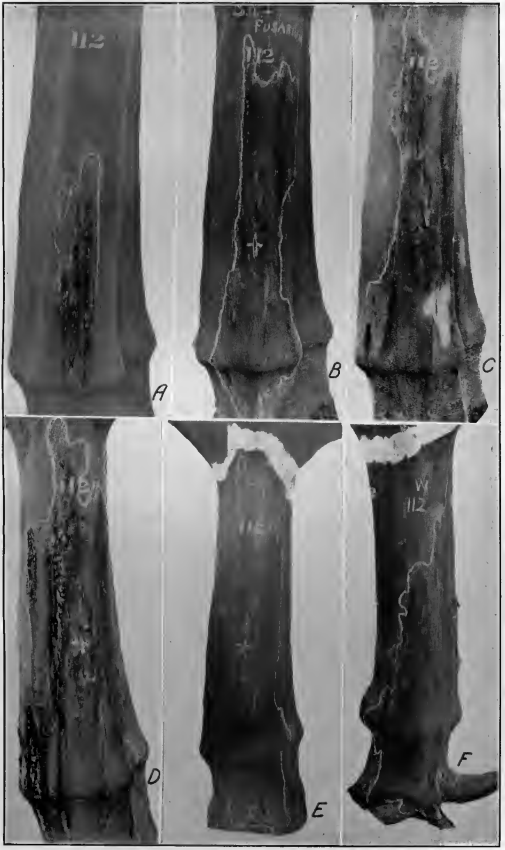
B.—Extent of the lesion on same tree November 25, 1913, 9 months after inoculation. The infection has extended downward nearly as far as upward.

C.—Extent of lesion and cracking of bark on April 9, 1914, more than 13 months after inoculation. The gum of figures A and B has been dissolved by rains.

D.—Large quantity of exuded gum and a great enlargement of invaded zone and cracking of bark, on September 4, 1914, about 17 months after inoculation.

E.—Further cracking of dead bark on February 6, 1915, nearly 2 years after inoculation. The gum of figure D has been dissolved by further rains.

F.—Trunk on opposite side from figure E, on same date. Only a small strip of live bark here remains, which was later invaded, this resulting in the death of the tree.


112
FUSARIUM
112
A
B
C
D
E
F

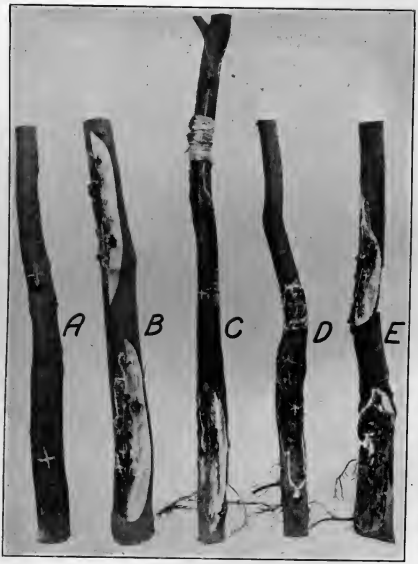

A
B
C
D
E

PLATE 6

Results of inoculation with pure cultures of different strains of *Phytophthora terrestria* and with *Pythiacystis citrophthora* on young lemon trees (orange stock at lower inoculated cut). Inoculated April 20, 1914, photographed August 26, 1914. (See Table XI.)

A.—Control cuts without inoculum.

B.—Inoculated with *Phytophthora terrestria* from Isle of Pines. Bark cut away to show extent of lesions.

C.—Inoculated with *Pythiacystis citrophthora* from California.

D.—Inoculated with *Phytophthora terrestria* from Cuba.

E.—Inoculated with *Phytophthora terrestria* from Florida.

PLATE 7

Method of cutting away diseased bark in treatment of a severe case of Pythiacystis gummosis.

A.—Result of inoculation with diseased bark containing *Pythiacystis citrophthora* on November 16, 1912. Photographed September 3, 1913. Invaded area 21.5 by 7.5 cm.

B.—Bark cut away September 3, 1913, on a similar tree in treatment.

C.—Same tree after painting with Bordeaux paste.

D.—Same tree on March 17, 1916, showing growth at edges of wound and asphalt paint on exposed wood.

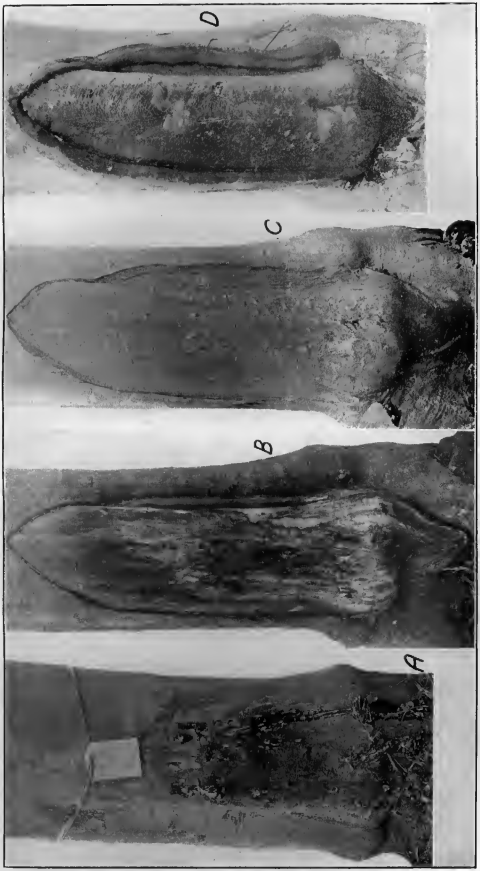

D
C
B
A

PLATE 8

Gum pushed up into bottom of funnel from which sterile, unheated, and filtered extract from bark affected with Pythiacystis gummosis had gone into the tree about 4 weeks before.

OCCURRENCE AND SIGNIFICANCE OF PHLOEM NECROSIS IN THE IRISH POTATO[1]

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INTRODUCTION

In a recent publication Esmarch (*5*)[2] makes the following generalization in regard to the condition of the phloem:

> Necrosis of the phloem is found in both normal and diseased plants and is always present in mature organs; if necrosis is observed during the early life of the plant, it is an indication of premature ripening.

In support of this theory, which is based on his own observations and supplemented by the data of other writers (*7*), Esmarch compares the changes in the phloem of the potato to those taking place in the secondary phloem of woody plants. Here, with the disintegration of the nucleus and the formation of callus deposits over the plates, the sieve tubes become inactive. The empty elements, deprived of their turgor, collapse and are crushed by the surrounding tissues. As obliteration progresses the old phloem often becomes so changed structurally and chemically as no longer to resemble its former state. However, while the obliteration of the phloem in woody plants, as has been shown by numerous investigators (*3*), is comprehensible on structural and physiological grounds, similar changes in the phloem tissue of herbaceous plants may not be expected, nor is their frequent occurrence reported in literature. Indeed, our present knowledge is restricted to two short notes by Boodle and Schuman. Boodle (*4*) observed in *Helianthus annuus* the occurrence of sieve tubes and companion cells whose walls were lignified and whose content gave reactions resembling those of lignin. Schuman (*8*) mentions that sclerosis of the phloem takes place in some few woody Composites and gives *Scorsonera hispanica* and *Aster thyrsiflorus* as examples. A systematic study of the mature phloem, carried on recently in the botanical laboratory of Cornell University, indicates clearly that most herbaceous plants do not exhibit any changes in the phloem upon maturation; where changes do take place, the phloem elements become lignified without obliteration.

A consideration of anatomical changes in diseased plants has often been fruitful in providing diagnostic symptoms for the identification of plant diseases, provided, however, that the ontogeny of the normal parts was fully understood. It is imperative, indeed, to distinguish clearly between normally expected tissue changes, such as take place

[1] Accepted for publication July 11, 1922.
[2] Reference is made by number (italic) to "Literature cited," p. 245.

in the phloem of woody plants, and induced abnormal states, the latter alone being truly pathological. The apparent discrepancies noted by different investigators may, and usually do, become intelligible if we consider all pathological changes and trace the causes of the various types of necrotic conditions, if possible, to their sources.

A classical example of such discrepancies is afforded by the researches on the pathological anatomy of the potato in connection with leafroll. To be sure, the views of the different writers are substantiated by observations, but since statistical data are incomplete at best, only a purposeful and systematic investigation of the problem can provide adequate data, even though factors which govern plant growth be not altogether neglected in the analysis of results.

To arrive at some definite basis as to what constitutes a healthy potato plant from the viewpoint of the anatomist, and under what conditions the phloem will remain normal, an investigation of a large number of plants of both cultivated varieties and indigenous South American forms was carried on. Such studies have been in progress since the summer of 1916, and the data obtained have been embodied in part in earlier publications (*1*, *2*) and have been extended and partly modified in the present paper. Further work on the pathological anatomy of potato diseases will doubtless bring forth new interesting facts and greatly aid in analyzing these complicated potato disorders of which the cause is only vaguely understood.

THE NORMAL PHLOEM

The vascular tissue of the potato plant shows a bicollateral arrangement of its elements, a condition most clearly seen in the larger stem bundles (fig. 1). The primary phloem, external to the cambium, is made up of small groups of cells more or less continuous; the groups constituting the inner phloem are very variable in size and scattered. Groups of primary phloem appear also in the interfascicular region, where they may be seen on both sides of a well-developed cambium. Through branching and anastomoses the individual groups in each region communicate with one another, while through branch and leaf gaps a similar connection is effected between the inner and outer phloem.

During the early vegetative development only groups of primary phloem are seen in a cross-sectional area of the stem. However, when tuber formation is under way, the cambium gives rise to a broad band of phloem (fig. 2). The amount of this secondary phloem varies with the location. In the nodal region the amount exceeds that found in the internode, and in a given cross-sectional area, the larger amount is always found on the face of the larger stem bundles.

While secondary phloem elements become differentiated and take part in the translocation processes, the primary phloem groups remain active until the plant is mature. The walls of the cells thicken slightly, and occasionally callus deposits cover the plates of the sieve tubes. Otherwise, there are no noticeable changes, either structural or chemical, characteristic of the phloem of the mature plant.

Local necrotic changes in the parenchymatous tissue, however, may be observed in any potato plant. The diseased areas are usually restricted and do not extend vertically for any appreciable distance. In the distal stem region, especially the node, such small pathological areas are of frequent occurrence, but they usually disappear in the maturing

organs. The first appearance of such a diseased area is exhibited by a swelling in the cell wall, often accompanied by discoloration. At a later stage, browning of the walls is noted, while at the same time the

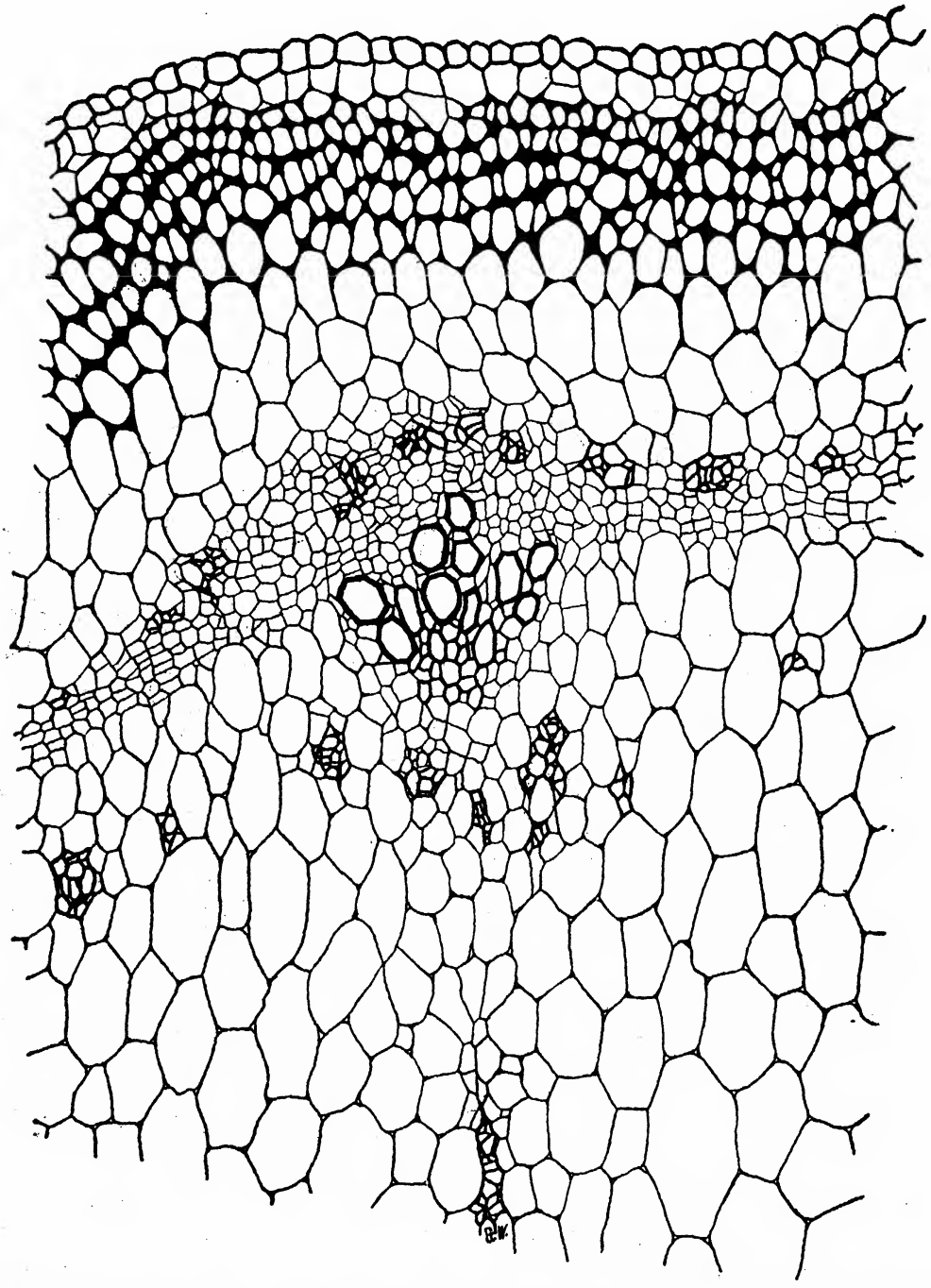

FIG. 1.—Stem section of young normal potato plant, showing general anatomical arrangement of vascular and fundamental tissues.

lumen is partly or entirely filled with a gummy deposit which is homogeneous or granular in nature. The parenchyma cells in the region where phloem fibers are differentiating show commonly an abnormal swelling, local in nature or extending laterally to include the walls of the perihperal phloem cells.

Any theory which could be postulated to explain the pathological disturbances in seemingly healthy individuals would naturally contain much of the hypothetical and be antecedent to our present knowledge of physiological phenomena. Temporary and local changes in the metabolism may cause the production of substances toxic to young and delicate cells. Microorganisms, which have occasionally been isolated from normal plants, could, under certain conditions, cause physiological

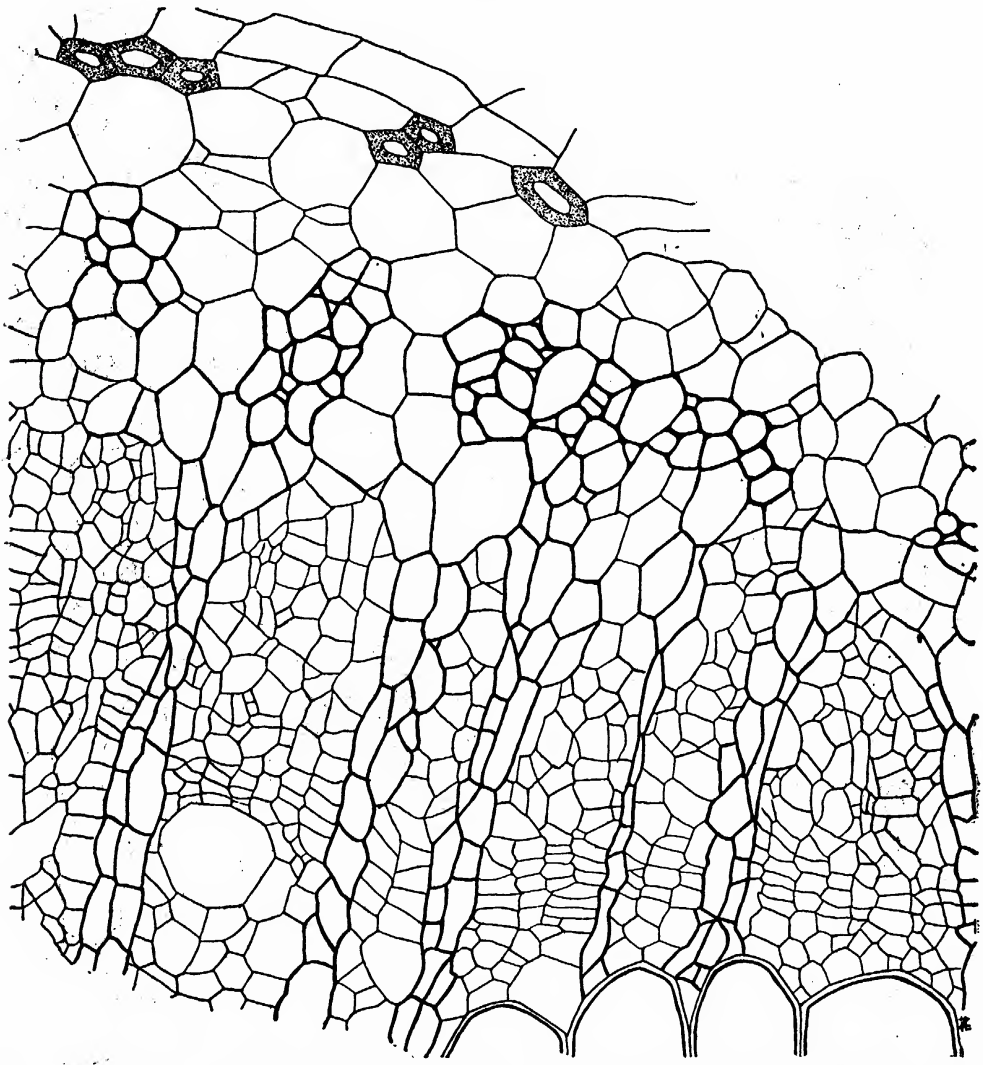

FIG. 2.—Mature normal stem section. Secondary phloem is well developed and by far exceeds in amount the primary tissue.

disturbances with accompanying cytological changes. Mechanical injuries may directly or indirectly produce similar effects. However, in the latter case it should be possible to trace the origin of the diseased area and to determine whether the cause was external or internal, and this has often been accomplished.

INFLUENCE OF ENVIRONMENT

An insight into the effect of environmental factors on the anatomical structure was obtained by studying a number of potato varieties grown at the high altitude station at Fort Lewis in Colorado. By taking ad-

vantage of the natural great diversity of ecological conditions, almost any desired combination of soil, moisture, and climatic factors could be had. Only where the natural agencies were inadequate or where a special combination of factors seemed advantageous was artificial control of these factors resorted to. Material for the study of the effect of progressively increasing water supply was taken from a field a part of which had become inundated by seepage from an adjoining mesa.

Plants suffering from an oversupply of water showed anatomical changes resembling those observed in foot diseases. The primary xylem and parts of the secondary elements of the wood were discolored and the lumen was filled with a brown, gummy deposit. The phloem part of the vascular tissue was commonly unaffected, and in cases where necrosis of the phloem was observed the material came from doubtful stock. The diseased phloem groups, which were occasionally observed in such material, did not take the lignin stain but showed the typical brownish discoloration which is characteristic of necrosis in general.

A greatly reduced water supply, or alternate wetting and drying, produced a denser and more strongly lignified wood. It neither inhibited nor accelerated the production of pathological changes.

The effect of shading was found to be closely bound up with the photosynthetic activity of the plant. Plants which grew to maturity in the shade of the river bottom vegetation were rank and slender. The xylem was greatly reduced and lignification of the cells less pronounced. The phloem remained normal throughout the life of the plants.

Thus, qualitative changes in the phloem, as a result of extremes of environmental conditions, are uncommon. When present, the diseased groups are localized and are connected with or are under the influence of necrotic areas in adjacent tissues. The pathological changes in the phloem tissue consists in a browning, rarely a typical lignification of the cell walls.

These observations, together with the fact that obliteration of the phloem, as a natural phenomenon in normal herbaceous plants, is discredited on theoretical grounds and disproved by extensive systematic studies, emphasized once more the fact that generalizations based on inadequate observations may lead us astray in the interpretation of true pathological changes.

NECROSIS FOLLOWING EXTERNAL INJURIES

By far the most common source of internal stem lesions is external injury produced by insects. The ensuing cellular changes may either constitute a direct response to the mechanical injury or be the result of irritations causing abnormal cell division and necrotic changes in the affected areas.

The lesions become externally visible as abnormal swellings of the size of small pustules barely discernible to the naked eye (Pl. 1, A) or they are of the nature of large intumescences which may be seen in various stages of development. The oldest swellings are conspicuous by their brownish color and the fissured surface of the epidermis. The location of the lesions is the apical stem region and the young petiole. However, swellings may be found in any region regardless of the age and relative development of the organ. Concomitant with the formation of these intumescences is a change in the morphological development of the parts above the points of injury. The leaves are deformed and discolored. The leaflets fold along the midrib or roll in tubular fashion. The inter-

nodes are shortened and often appear almost telescoped. Withal, the morphological changes are of a type akin to the symptoms of leafroll and foot diseases.

An examination of a section through a swollen area discloses to the naked eye a dark discoloration of the tissues. A microscopic study shows further that both vascular and cortical tissues are affected and that aside from necrotic changes there are regions of abnormal cellular activity. Sections through the advancing margin of a young pustule show that the first evidence of pathological changes is found in the cambium region. The xylem has matured irregularly, giving the peripheral region of the tissue a jagged appearance. In places one may observe mature groups of xylem embedded in parenchymatous tissue and completely separated from the vascular ring. In the more central part of the swelling the tissues appear completely disorganized. In cortex and pith are small groups of brown cells surrounded by concentric rings of thin-walled periderm cells. This latter type of necrosis is, however, frequently met with in normal plants, or is found in connection with other injuries.

The phloem tissue, in the affected areas, shows the same pathological condition observed in general. The cell wall and content show a brownish discoloration, and in extreme cases entire cells or cell complexes may be obliterated.

NECROSIS IN CONNECTION WITH STEM STREAK

Potato tubers of the variety Irish Cobbler which were grown in the greenhouse developed extensive stem lesions on both young and maturing shoots. The lesions may best be referred to as "streak," the affected areas being elongated and brownish in color. Their advance along the stem is acropetal. In the nodal region the browning is especially pronounced and extends into the lower part of the petiole.

The lesions are the result of necrotic changes in the cells of the collenchyma and adjacent tissues. The hypodermis is often still green while the collenchyma cells underneath are brown and in a state of obliteration. Soon, however, being cut off from the water supply, both epidermis and hypodermis die and the walls take on the same brown color as the cells underneath. In advanced lesions the necrotic areas extend through cortex and vascular tissue into the pith. In the nodal region the entire pith is reduced to a cavity lined with obliterated and discolored parenchyma cells. If the epidermis from an advanced lesion is removed and cleared in chloral hydrate, the microscopic examination shows scattered through the homogeneous mass of brown cells small areas of dark-colored cells. These areas usually cluster around a stomate and extend centripetally into the pith.

The behavior of the phloem cells is of special interest in this connection. Severe necrosis may be observed in both inner and outer phloem (Pl. 2). The cells are brownish in color, the lumen distinct or partly closed by the pressure of the surrounding tissue. Characteristic intercellular spaces are also formed, and progressive lignification of the type diagnostic of leafroll is not uncommon. The necrotic phloem groups, however, seem to be restricted and limited by the extent of the external symptoms, there being no regularity either in their vertical or lateral distribution. It still remains to be shown whether this disease is definitely connected with leafroll, and, if in part distinct, whether certain aspects of the disease are not the result of secondary infection by the leafroll virus.

TRUE PHLOEM NECROSIS AND THE LEAFROLL PROBLEM

In the microscopic examination of potato vines one observes, occasionally, pathological changes associated with certain types of diseased plants which by virtue of their peculiar external appearance are grouped under the collective name of leafroll. Quanjer (*6*), while investigating the leafroll disease, noticed this correlation, and as the result of subsequent studies arrived at the following conclusion:

> The lignification of the phloem is a dependable diagnostic symptom for the identification of leafroll, and the physiological disturbance, occasioned by the destruction of the conducting system for plastic materials, accounts for the change in the morphological structure and for the reduction in yield.

Stem sections of a typical leafroll plant exhibit, as a diagnostic internal symptom, a necrosis and lignification of the phloem groups. In case of severe external symptoms the diseased groups pervade the entire plant with the occasional exception of the underground organs. The distal stem region is commonly affected, and in nearly every instance the necrotic changes are of an extreme type. The basal stem region always shows necrotic changes when external symptoms become evident while the plant is still young. As a rule necrosis of the phloem in the lower stem means general necrosis of the plant throughout its extent, but the symptoms may decrease toward the distal end or disappear altogether. At any given height of the stem the node is typically more severely affected than is the internode. This condition is especially observed in the initial stages of the disease, but during subsequent development either region may be equally affected.

In petiole and midrib necrotic phloem groups may appear at a much later period, but the extent of necrosis is as a rule correlated with the severity of the rolling and the discoloration of the foliage. In the young leaf most advanced necrosis is observed in the middle part of the petiole probably because this part of the leaf is ontogenetically the oldest. In mature leaves, however, the midrib is often the organ which is most severely affected.

In the underground organs of diseased plants the phloem strands are usually normal, but in severe cases both stolons and tubers will show evidence of necrosis.

The lateral distribution of phloem necrosis is also subject to a great deal of variation. This is true for the phloem groups of a certain region, but the difference becomes even more apparent when outer and inner phloem are compared. In a given cross sectional area one may observe phloem groups in various stages of degeneration. Entire groups may be affected in whole or in part. Often one notices perfectly healthy groups side by side with diseased ones, which seems the more remarkable if one recalls how closely the groups are connected with each other through branching and anastomoses. In the apical stem region the first stages of necrosis are found in the external phloem and only later in both regions. In the basal stem either region is found to be diseased, but often the inner phloem alone is completely destroyed while the outer phloem is altogether normal or shows, at the most, only initial stages of disorganization.

In petiole and midrib, necrosis is primarily restricted to the outer phloem. In advanced stages the bundles flanking the corners of the large lateral bundles are necrotic, and occasionally all the inner phloem is also diseased.

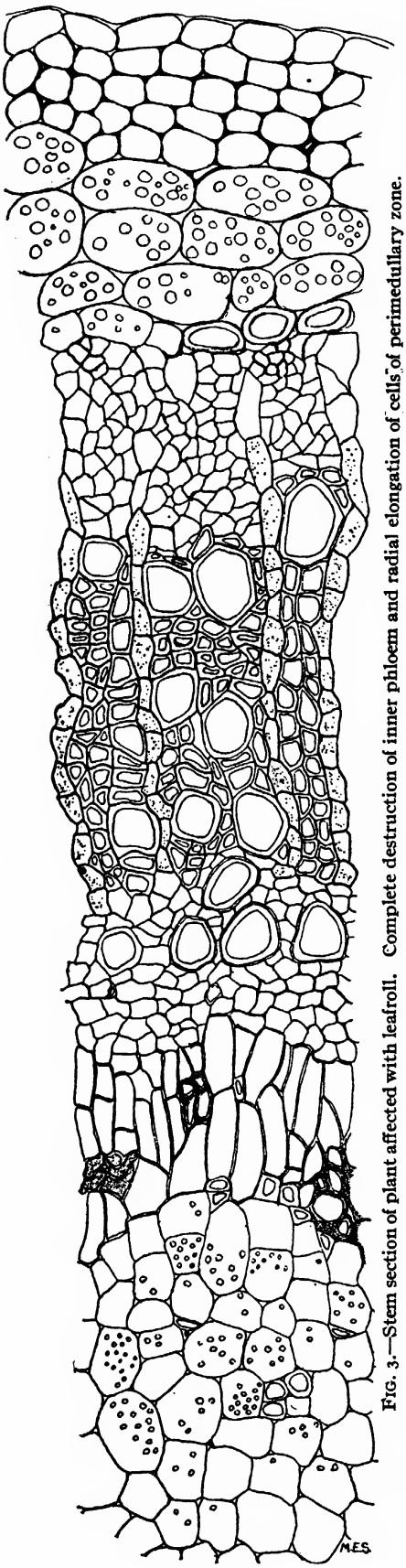

FIG. 3.—Stem section of plant affected with leafroll. Complete destruction of inner phloem and radial elongation of cells of perimedullary zone.

The progressive lignification of the phloem tissue can best be studied in sections taken through the distal stem region. Here one usually finds a complete series of changes from the first cell response to complete disorganization of the elements. Before there is any detectable evidence of lignification the development of the vascular tissue shows a deviation from its normal course, which is indicated by a more irregular maturing of the xylem than is found in healthy plants (Pl. 1, B). If such a section is stained with phloroglucin and hydrochloric acid, one notices upon close observation that the phloem cells centrifugal to the depression in the cambium show a slight amount of lignification in parts of the wall.

The cells of the pericycle in this region differ also from the normal type in having a greater radial diameter. As lignification progresses the entire cell wall or entire groups of cells become affected, while at the same time the radial elongation of the pericyclic cells of the outer phloem and the parenchyma of the perimedullary zone of the inner region becomes more pronounced (Pl. 3). The cells which first show lignification are commonly found adjacent to the fibers, but now and again lignification in a phloem group may start at the center and extend in a centrifugal direction.

Prior to lignification of the phloem, to be tested microchemically, a swelling of the walls of the diseased cells takes place. Ferrous sulphate and potassium ferrocyanid at this stage impart a deep blue color to the walls, indicating the presence of large quantities of pectic substances. The swelling of the walls extends from the region of the fibers centrifugally (Pl. 4, B). Gradually the cells lignify and progressively cells and groups of cells are withdrawn from active participation in conduction. In severe cases most or all of the primary phloem becomes destroyed (fig. 3; Pl. 5). In the initial stages of lignification of certain phloem groups the primary walls of adjacent cells swell and separate. The intercellular space

thus formed becomes filled with a brown deposit, which at a certain stage takes the lignin stain. Following the swelling of the wall there is often a disintegration of the wall substance. The swollen wall appears lamellate (Pl. 4, A) and between the lamellae spaces are formed which are filled with a gummy substance. The cell content also degenerates and disappears in part or becomes transformed into a substance giving reactions similar to the interstitial substance described above. Following the death of the cell and the subsequent loss of turgor, the phloem elements collapse, unless rapid lignification lends sufficient rigidity to the walls and prevents their being crushed by the surrounding tissues.

CONCLUSIONS

The phloem of the potato, like that of the majority of herbaceous plants, remains normal throughout the vegetative period and up to late maturity of the plant. Although it is not affected by extremes in environmental conditions, it nevertheless undergoes pathological changes under the effect of certain metabolic disturbances such as probably exist in the leafroll disease. These changes consist in lignification or obliteration of cell wall and content. However, while obliteration of the phloem is always observed in connection with leafroll, it is also an accompanying phenomenon in other diseases. It is not so much its mere presence as its universality in distribution, coupled with the absence of necrosis in other tissues, which gives it a real diagnostic value.

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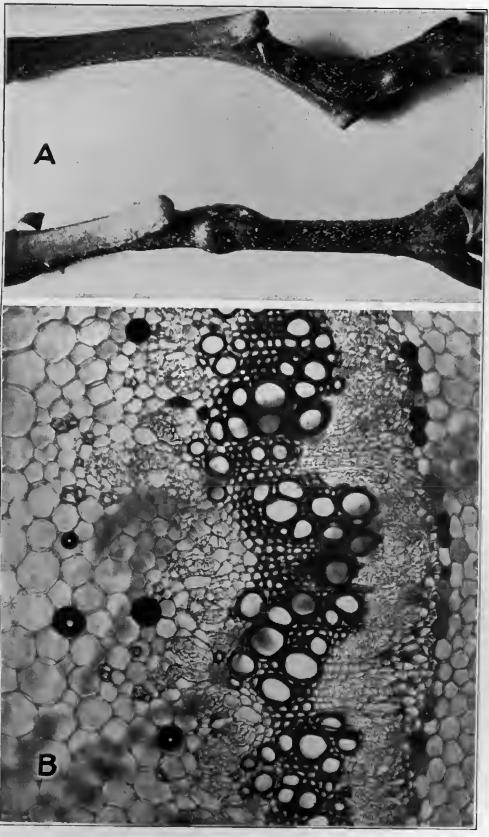
PLATE 1

A.—Distal stem region. The swellings are due to insect injuries; they show as internal symptoms necrosis of vascular and cortical tissue.

B.—Hand section of potato stem affected with leafroll. The xylem is maturing very irregularly, which gives the cambium zone a jagged appearance.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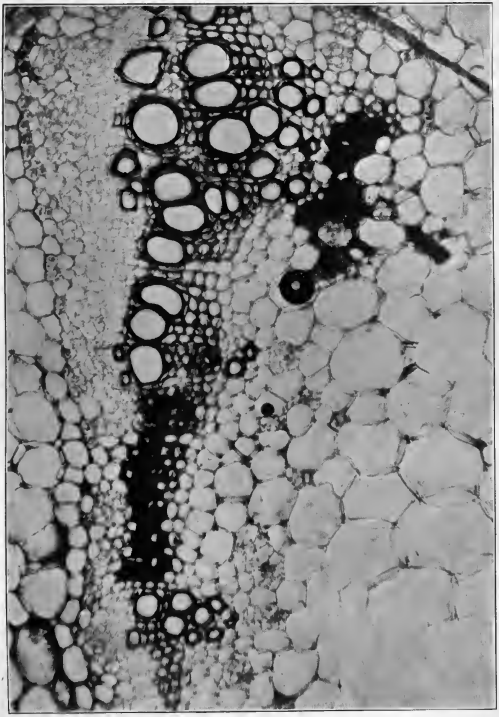

PLATE 2

Hand section of potato stem affected with "stemstreak." Both xylem and phloem show necrosis. The necrosis of the phloem, however, is not a typical lignification.

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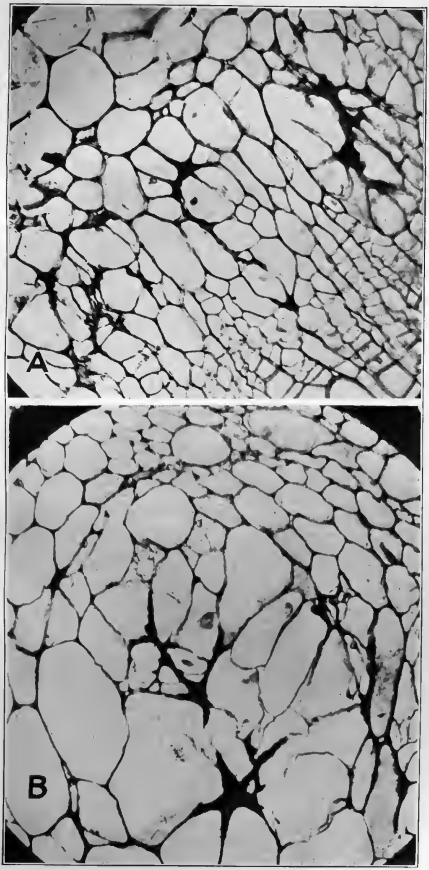
PLATE 3

A.—Advanced necrosis of outer phloem in leafroll stem.
B.—Advanced necrosis of inner phloem.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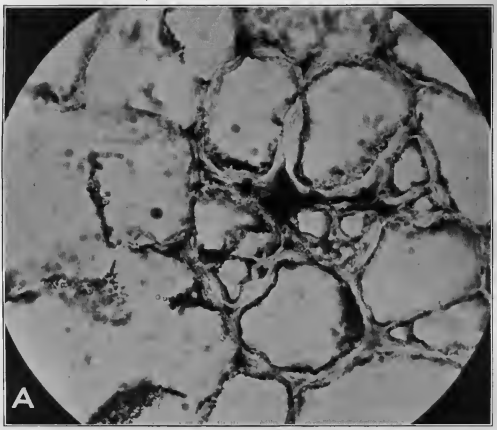
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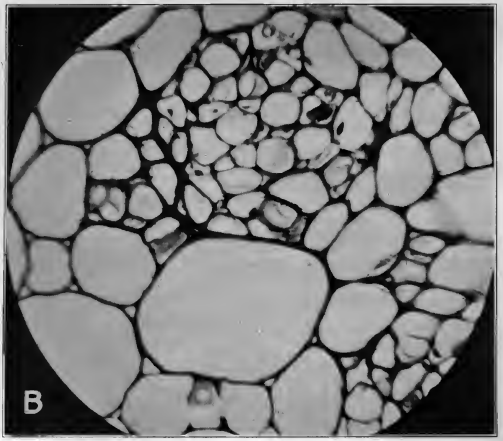
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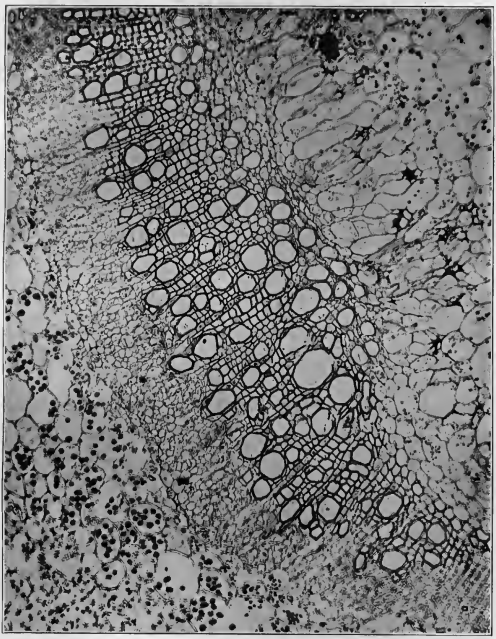
PLATE 4

A.—Enlarged view of inner phloem group, showing the minute pathological changes in the structure of the cell wall. (Gold chlorid stain.)

B.—Inner phloem group of potato stem, showing beginning stages of necrosis. Note the swelling of the walls of certain of the cells in the region of the fibers.

PLATE 5

Section through a large stem bundle of a mature stem affected with leafroll. The inner phloem is completely destroyed while the outer phloem, on the other hand, is normal.



CULTIVATED AND WILD HOSTS OF SUGAR-CANE OR GRASS MOSAIC[1]

By E. W. BRANDES, *Pathologist*, and PETER J. KLAPHAAK, *Assistant Pathologist*, *Office of Sugar-Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture*

PURPOSE OF THE INVESTIGATION

Since 1919, when it was demonstrated by the senior writer that grasses other than sugar cane are susceptible to the so-called sugar-cane mosaic (*3, 4, 5*),[2] a large number of experiments have been performed to determine whether grasses in general are susceptible. It was especially important to learn whether the forage and field crops of the South are affected and, if so, to what extent damage is caused. On account of the comparatively recent introduction of the disease on sugar cane into this country, it was expected that crops in the vicinity of diseased sugar cane would be more likely to be affected. Accordingly these crops were closely scrutinized for symptoms similar to those on sugar cane, and when suspected cases were observed the plants were tested experimentally at Washington. Other closely related cultivated plants were tested, whether or not they showed signs of natural infection in the fields.

Another class of grasses, namely, the weeds or wild grasses found in sugar-cane fields, was held under observation, and any species which showed signs of disease were similarly tested. The importance of having exact knowledge of the susceptibility of wild grasses to grass mosaic lies in the fact that the disease may be carried over winter in perennial grasses and may reinfect the susceptible annual grass crops in the spring.

The rôle of wild perennial hosts in preserving the virus of mosaic through the winter has not been fully established, but they must be regarded as a potential source of danger, and our studies are justified on these grounds. In the South, even the annual grasses may be found in the growing state during the whole year. Sugar cane itself is a perennial, and the virus of mosaic is known to survive the winter in the stubble, as the ratoon plants invariably come up diseased in the spring if the plants of the previous year were infected. The attempt has been made in some regions to eradicate the disease by destroying all infected cane stools or by plowing out whole fields and planting to other crops for a year or more. The labor of course would be wasted if the disease were perpetuated in some unobserved wild perennial host. This method of eradication has been successful, however, in at least one sugar-cane region, where the disease was early observed and prompt and vigorous measures were taken to stamp it out. This region is the peninsular section of Florida where the disease no longer exists to our knowledge.

More than 40 grasses have been tested experimentally, either by artificial inoculation or by means of insect inoculation or both.

[1] Accepted for publication Dec. 1, 1921.

[2] Reference is made by number (italic) to "Literature cited," p. 262.

EXPERIMENTAL INOCULATIONS

As a result of these experiments it has been conclusively proved that at least 13 species of grasses are subject to attack by the identical virus causing sugar-cane mosaic. Undoubtedly there are many more, but with the limited space at the disposal of the writers it was impossible to carry on the work on a larger scale. Species which have thus far given negative results may in the future prove to be susceptible. Many factors have entered which no doubt have diminished the chances for infection following these inoculations. Optimum growing conditions for many of the grasses do not prevail at Washington. Sometimes whole series of inoculations including known susceptible control plants failed, owing to unknown causes. Insect carriers became parasitized by other insects or died as a result of unfavorable conditions in the greenhouse, and they were not available or were not in a normal state when the plants were in the best condition for inoculation. In other words, the positive results attained must naturally carry more weight than the negative results, and in listing grasses as not susceptible it is merely meant that they did not become infected in these experiments.

Though it is possible that there is more than one mosaic disease affecting grasses, and there is some reason for believing this to be so, this paper is concerned with one only, the well-known type which has recently proved so destructive to sugar cane in Porto Rico and the southern United States.

The plants tested and found susceptible are shown in the following list:

Cultivated crops:
- Perennial—
  - Sugar cane.....*Saccharum officinarum.*
- Annuals—
  - Corn.....*Zea mays.*
  - Sorghum.....*Holcus sorghum.*
  - Pearl millet.....*Pennisetum glaucum.*

Ornamentals:
- Perennial—
  - Eulalia.....*Miscanthus sinensis.*

Wild grasses:
- Perennial—
  - Wild sugar cane.....*Saccharum narenga* (S. P. I. No. 38332).[3]
- Annuals—
  - Bull-grass.....*Paspalum boscianum.*
  - Crab-grass.....*Syntherisma sanguinalis.*
  - Yellow foxtail.....*Chaetochloa lutescens.*
  - Giant foxtail.....*Chaetochloa magna.*
  - Barnyard grass.....*Echinochloa crusgalli.*
  - Panicum.....*Panicum dichotomiflorum.*
  - Brachiaria.....*Brachiaria platyphylla.*

[3] A variety of *Saccharum narenga* is cultivated in China, according to Mr. Frank N. Meyer

The grasses tested with negative results are given in the following list:

Cultivated crops:
- Perennials—
  - Madake bamboo.....*Phyllostachys quilioi.*
  - Edible bamboo.....*Phyllostachys pubescens.*
  - Para grass.....*Panicum barbinode.*
  - Napier grass.....*Pennisetum purpureum var.*
  - Merker grass..... Do.
  - Australian giant-grass..... Do.
  - Johnson grass.....*Holcus halepensis.*
- Annuals—
  - Wheat, Power Fife (C. I. 3697).....*Triticum aestivum.*
  - Oats, Swedish Select (C. I. 134).....*Avena sativa.*
  - Rye, Von Runker No. 2 (C. I. 174)....*Secale cereale.*
  - Barley, Marionet.....*Hordeum vulgare.*
  - Rice, Blue Rose (C. I. 1962).....*Oryza sativa.*
  - Teosinte.....*Euchlaena mexicana.*
  - Redtop.....*Agrostis palustris.*
  - Timothy.....*Phleum pratense.*
  - Bluegrass.....*Poa pratensis.*
  - Ragi millet.....*Eleusine coracana.*

Ornamentals:
- Perennials—
  - Variegated Eulalia.....*Miscanthus sinensis variegatus.*
  - Do.....*Miscanthus sinensis zebrinus.*
- Annual—
  - Job's-tears.....*Coix lachryma-jobi.*

Wild grasses:
- Perennials—
  - Broom sedge.....*Andropogon virginicus.*
  - Broom sedge.....*Andropogon elliottii.*
  - Little bluestem.....*Andropogon scoparius.*
  - Indian reed.....*Sorghastrum nutans.*
  - Gama grass.....*Tripsacum dactyloides.*
- Annuals—
  - Green foxtail.....*Chaetochloa viridis.*
  - Red sprangletop.....*Leptochloa filiformis.*

It is noticeable that although plants have been selected for experiment from the entire grass family, the ones which proved susceptible are without exception confined to the tribes Paniceae, Andropogoneae, and Tripsaceae. In his phylogenetic arrangement of the genera of grasses, Hitchcock (9) divides the grass family into 13 tribes and places the 3 tribes here mentioned together as representing the highest type of development. It is significant that this arrangement, based on morphological characters, should prove so regular with respect to susceptibility to this disease. Since it is to be expected that closely related species are more likely to be affected by the same diseases than species located remotely from one another, the relationships are corroborated in an interesting and novel manner.

The possibility of the existence of more than one type of mosaic among the grasses has been mentioned. A type of mottling in the edible bamboo (*Phyllostachys pubescens*) from China resembles our mosaic very strikingly. It was first observed in one plant out of a lot of four being held in the detention house at Washington. Subsequently two other plants became affected. The writers have no experimental evidence that this is an infectious disease. It was found impossible to infect this species of bamboo with the sugar-cane mosaic. Furthermore, it is not nearly related to any other grass found susceptible to our mosaic but stands at the extreme opposite end of the list of tribes of the grass family.

A specific mosaic of *Nicotiana viscosum* distinct from the mosaic of *N. tabacum* has been reported by Allard (1), who states that the mosaic of

N. viscosum is more difficult to transmit artificially than the common tobacco mosaic. *Datura stramonium* was the only solanaceous plant found susceptible to both mosaic diseases. Allard suggests that results of inoculations with tobacco mosaic by various European investigators which are inconsistent with his own may be due to the existence of different types of mosaic in the Solanaceae.

COMPOSITE RECORD AND RESULTS OF EXPERIMENTS

The technic of the work here reported has been exactly the same as that described in a previous paper (*3*). All inoculations were made with one-half to 2 cc. of cell sap, obtained by squeezing young stalks in a powerful press under mineral oil. The virus or healthy sap was injected near the growing point by means of Leur all-glass hypodermic syringes provided with Yale 24-gauge needles. The results of the experiments are given in support of the facts presented in the preceding pages. In order to save space, composite records of the experiments are shown in tabular form.

Table I includes tests made for artificial transmission.

TABLE I.—*Composite record of tests of the artificial transmission of grass mosaic*

Species.	Number of plants.	Date inoculated.	Inoculum.	Results.	
				Date. examined.	Condition.
Saccharum officinarum var. Louisiana Purple.	4	Oct. 4, 1920	Virus from sugar cane variety B6450.	Oct. 18, 1920	3 mosaic.
Do	4	.....do.....	Sap from healthy sugar variety B6450.	Nov. 15, 1920	All healthy.
Do	4	.....do.....	Virus from B6450 passed through Berkefeld filter.	Oct. 18, 1920	3 mosaic.
Do	4	.....do.....	Virus from B6450 mixed with equal part of 0.2 per cent phenol.	.....do.....	1 mosaic.
Do	4	.....do.....	Virus from B6450 mixed with equal part of 0.05 per cent $CuSO_4$.	Nov. 15, 1920	All healthy.
Do	4	.....do.....	Virus from B6450 mixed with equal part of 5 per cent formalin.	.....do.....	Do.
Do	4	.....do.....	Virus from B6450 mixed with 1 per cent Carrol Dakin solution	.....do.....	Do.
Do	4	.....do.....	Virus from B6450 mixed with 0.1 per cent $HgCl_2$.	.....do.....	Do.
Do	4	.....do.....	Virus from B6450 diluted 1/100 with distilled water	.....do.....	Do.
Do	4	.....do.....	Virus from B6450 diluted 1/1,000 with distilled water.	.....do.....	Do.
Do	4	.....do.....	Virus from B6450 diluted 1/10,000 with distilled water.	.....do.....	Do.
Do	8	Aug. 4, 1920	Virus from Louisiana Purple.	Aug 28, 1920	8 mosaic.
Zea mays var. U. S. Selection No. 188.	3	.....do.....	.....do.....	Sept. 29, 1920	All healthy.
Holcus sorghum.....	2	.....do.....	.....do.....	Aug. 28, 1920	2 mosaic.
Syntherisma sanguinalis.	6	.....do.....	.....do.....	.....do.....	5 mosaic.
Coix lachryma-jobi.....	2	.....do.....	.....do.....	Sept. 29, 1920	Both healthy.
Pennisetum glaucum..	6	.....do.....	.....do.....	Aug. 28, 1920	6 mosaic.
Saccharum officinarum var. Louisiana Purple.	8	.....do.....	Sap from healthy sugar cane, variety Louisiana Purple.	Sept. 29, 1920	All healthy.
Zea mays.....	3	.....do.....	.....do.....	.....do.....	Do.
Holcus sorghum.....	2	.....do.....	.....do.....	.....do.....	Do.
Syntherisma sanguinalis.	6	.....do.....	.....do.....	.....do.....	Do.
Coix lachryma-jobi.....	2	.....do.....	.....do.....	.....do.....	Do.
Pennisetum glaucum..	6	.....do.....	.....do.....	.....do.....	Do.

It will be noticed that in the experiments the virus was treated in various ways. In at least one experiment virus which had been passed through a Berkefeld filter [4] caused infection in 75 per cent of the plants inoculated. Other similar experiments have failed to give such convincing results, but owing to the fact that control plants inoculated with untreated virus also show a low percentage of infection, indicating a less potent virus, the writers are inclined to attach considerable importance to the successful experiment and believe that the virus will pass through certain diatomaceous earth filters.

In some experiments the virus was shaken in bottles with various bacteriacidal chemicals one hour before being injected into the plants. With one exception none of the virus treated in this way caused infection. One plant in a series of four inoculated with virus treated with weak phenol solution became mosaic in 14 days. The same lot of virus diluted with distilled water in various proportions gave negative results. Virus capable of causing infection when used immediately after being expressed from diseased stalks was found in one experiment to be without effect when injected 24 hours later. The virus of grass mosaic is less stable or more sensitive to the influence of its environment than that of many other similar diseases, notably the tobacco mosaic. In these experiments it has been found very refractory and difficult of physical manipulation or chemical treatment without loss of virulence.

The results of the experiments on insect transmission of the disease are given in Table II. "Virulent" insects were obtained from mosaic sugar cane or from mosaic sorghum artificially inoculated with virus from sugar cane. "Nonvirulent" insects were from healthy cane or sorghum. These experiments were performed in insect-proof compartments in a mosaic-infested greenhouse.

TABLE II.—*Composite record of the tests of the insect transmission of the grass mosaic*

Species.	Number of plants.	Date exposed.	Character.	Insect.	Results.	
					Date examined.	Condition.
Saccharum officinarum var. Louisiana Purple.	3	Feb. 4, 1920	Virulent....	*Aphis maidis*..	Feb. 28, 1920	2 mosaic.
Do.....	3	.....do.....	Nonvirulent	.....do.....	Mar. 14, 1920	All healthy.
Do.....	6	.....do.....	Virulent....	*Draeculacephala molipes.*	.....do.....	Do.
Do.....	6	.....do.....	Nonvirulent	.....do.....	.....do.....	Do.
Zea mays var. U. S. Selection No. 182.	6	Mar. 12, 1920	Virulent....	*Aphis maidis*..	Apr. 6, 1920	4 mosaic.
Do.....	6	.....do.....	Nonvirulent	.....do.....	May 1, 1920	All healthy.
Do.....	20	Mar. 30, 1920	Virulent....	.....do.....	May 28, 1920	15 mosaic.
Do.....	20	.....do.....	Nonvirulent	.....do.....	.....do.....	All healthy.
Triticum sativum var. Power Fife.	50	May 20, 1920	Virulent....	.....do.....	Aug. 1, 1920	Do.
Do.....	50	.....do.....	Nonvirulent	.....do.....	.....do.....	Do.
Hordeum vulgare var. Marionet.	50	.....do.....	Virulent....	.....do.....	.....do.....	Do.
Do.....	50	.....do.....	Nonvirulent	.....do.....	.....do.....	Do.
Secale cereale var. Von Runker No. 2.	50	.....do.....	Virulent....	.....do.....	.....do.....	Do.
Do.....	50	.....do.....	Nonvirulent	.....do.....	.....do.....	Do.
Avena sativa var. Swedish Select.	50	.....do.....	Virulent....	.....do.....	.....do.....	Do.
Do.....	50	.....do.....	Nonvirulent	.....do.....	.....do.....	Do.

[4] This was a rather coarse filter. When immersed in water it withstood a pressure of only 3 pounds before passing bubbles.

TABLE II.—*Composite record of the tests of the insect transmission of the grass mosaic*—Continued

Species.	Number of plants.	Date exposed.	Character.	Insect.	Results.	
					Date examined.	Condition.
Oryza sativa var. C. I. No. 1962.	50	May 20, 1920	Virulent	*Aphis maidis.*	Aug. 1, 1920	All healthy.
Do	50	do	Nonvirulent	do	do	Do.
Eleusine coracana var. S. P. I. No. 43190.	50	do	Virulent	do	do	Do.
Do	50	do	Nonvirulent	do	do	Do.
Agrostis palustris	40	May 15, 1920	Virulent	do	do	Do.
Do	40	do	Nonvirulent	do	do	Do.
Poa pratensis	40	do	Virulent	do	do	Do.
Do	40	do	Nonvirulent	do	do	Do.
Holcus halepenses	40	do	Virulent	do	do	Do.
Do	40	do	Nonvirulent	do	do	Do.
Phleum pratense	40	do	Virulent	do	do	Do.
Do	40	do	Nonvirulent	do	do	Do.
Coix lachryma-jobi	6	do	Virulent	do	June 15, 1920	All mottled; not clear case of mosaic.
Do	6	do	Nonvirulent	do	do	All healthy.
Miscanthus sinensis	16	May 20, 1920	Virulent	do	July 15, 1920	5 mosaic.
Do	6	do	Nonvirulent	do	do	All healthy.
Pennisetum purpureum.	4	May 30, 1920	Virulent	do	June 30, 1920	Do.
Do	4	do	Nonvirulent	do	do	Do.
Merker grass	4	do	Virulent	do	do	Do.
Do	4	do	Nonvirulent	do	do	Do.
Australian giant grass.	4	do	Virulent	do	July 6, 1920	Do.
Do	4	do	Nonvirulent	do	do	Do.
Syntherisma sanguinalis.	10	June 1, 1920	Virulent	do	July 29, 1920	6 mosaic.
Do	10	do	Nonvirulent	do	do	All healthy.
Zea mays var. U. S. Selection No. 182.	50	do	Virulent	do	July 28, 1920	2 mosaic.
Do	50	do	Nonvirulent	do	do	All healthy.
Paspalum boscianum.	3	July 31, 1920	Virulent	do	Aug. 31, 1920	3 mosaic.
Do	3	do	Nonvirulent	do	do	All healthy.
Saccharum officinarum var. Louisiana Purple.	10	Aug. 3, 1920	Virulent	do	Aug. 28, 1920	3 mosaic.
Do	10	do	Nonvirulent	do	Sept. 15, 1920	All healthy.
Zea mays	10	do	Virulent	do	Aug. 28, 1920	3 mosaic.
Do	10	do	Nonvirulent	do	Sept. 15, 1920	All healthy.
Holcus sorghum	5	do	Virulent	do	Aug. 28, 1920	5 mosaic.
Do	5	do	Nonvirulent	do	Sept. 15, 1920	All healthy.
Coix lachryma-jobi	5	do	Virulent	do	do	Do.
Do	5	do	Nonvirulent	do	do	Do.
Syntherisma sanguinalis.	5	do	Virulent	do	Aug. 28, 1920	5 mosaic.
Do	5	do	Nonvirulent	do	Sept. 15, 1920	All healthy.
Miscanthus sinensis.	2	do	Virulent	do	Oct. 3, 1920	2 mosaic.
Do	2	do	Nonvirulent	do	do	All healthy.
Saccharum officinarum var. Louisiana Purple.	10	do	Virulent	*Kolla similis*	Sept. 15, 1920	Do.
Do	10	do	Nonvirulent	do	Sept. 9, 1920	Do.
Zea mays	10	do	Virulent	do	do	Do.
Do	10	do	Nonvirulent	do	do	Do.
Holcus sorghum	5	do	Virulent	do	do	Do.
Do	5	do	Nonvirulent	do	do	Do.
Coix lachryma-jobi	5	do	Virulent	do	do	Do.
Do	5	do	Nonvirulent	do	do	Do.
Syntherisma sanguinalis.	5	do	Virulent	do	do	Do.
Do	5	do	Nonvirulent	do	do	Do.
Miscanthus sinensis.	2	do	Virulent	do	do	Do.
Do	2	do	Nonvirulent	do	do	Do.
Saccharum officinarum var. Louisiana Purple.	10	do	Virulent	*Draeculacephala molipes.*	do	Do.
Do	10	do	Nonvirulent	do	do	Do.
Zea mays	10	do	Virulent	do	do	Do.
Do	10	do	Nonvirulent	do	do	Do.
Holcus sorghum	5	do	Virulent	do	Sept. 15, 1920	Do.
Do	5	do	Nonvirulent	do	do	Do.
Coix lachryma-jobi	5	do	Virulent	do	do	Do.
Do	5	do	Nonvirulent	do	do	Do.

TABLE II.—*Composite record of the tests of the insect transmission of the grass mosaic*—Continued

Species.	Number of plants.	Date exposed.	Character.	Insect.	Results.	
					Date examined.	Condition.
Syntherisma sanguinalis.	5	Aug. 3, 1920	Virulent	*Draeculacephala molipes.*	Sept. 15, 1920	All healthy.
Do	5	do	Nonvirulent	do	do	Do.
Miscanthus sinensis.	2	do	Virulent	do	do	Do.
Do	2	do	Nonvirulent	do	do	Do.
Brachiaria platyhylla.	7	July 13, 1920	Virulent	*Aphis maidis*	Aug. 13, 1920	5 mosaic.
Do	5	do	Nonvirulent	do	do	All healthy.
Syntherisma sanguinalis.	2	Aug. 16, 1920	Virulent	do	Sept. 20, 1920	Both mosaic.
Do	2	do	Nonvirulent	do	do	Both healthy.
Paspalum boscianum.	2	do	Virulent	do	do	Both mosaic.
Do	2	do	Nonvirulent	do	do	Both healthy
Leptochloa filiformis.	1	Aug. 17, 1920	Virulent	do	do	Healthy.
Do	1	do	Nonvirulent	do	do	Do.
Saccharum officinarum.	3	Sept. 2, 1920	Virulent	*Draeculacephala molipes.*	Nov. 1, 1920	All healthy.
Zea mays	3	do	do	do	do	Do.
Holcus sorghum	2	do	do	do	do	Do.
Coix lachryma-jobi	2	do	do	do	do	Do.
Syntherisma sanguinalis.	2	do	do	do	do	Do.
Miscanthus sinensis.	2	do	do	do	do	Do.
Leptochloa filiformis.	6	do	do	*Aphis maidis*	Nov. 18, 1920	Do.
Saccharum officinarum.	10	Oct. 1, 1920	Exposed on ledge. [a]		Feb. 25, 1921	9 mosaic.
Holcus sorghum	15	Feb. 3, 1921	do		Apr. 2, 1921	6 mosaic.
Chaetochloa lutescens.	3	do	do		do	3 mosaic.
Syntherisma sanguinalis.	4	do	do		do	All healthy.
Paspalum boscianum.	6	do	do		do	6 mosaic.
Saccharum officinarum.	3	do	Virulent	*Draeculacephala molipes.*	May 6, 1921	All healthy.
Do	3	do	Nonvirulent	do	do	Do.
Echinochloa crus-galli.	3	Apr. 7, 1921	Exposed on ledge. [a]		May 25, 1921	2 mosaic.
Miscanthus sinensis zebrina.	3	Apr. 13, 1921	do		June 15, 1921	Healthy.
Miscanthus sinensis variegata.	4	do	do		do	Do.
Sorghastrum nutans	3	do	do		do	All healthy.
Chaetochloa magna	4	do	do		do	2 mosaic.
Paspalum boscianum.	4	May 25, 1921	Virulent	*Aphis maidis*	June 20, 1921.	4 mosaic.
Chaetochloa magna	2	do	do	do	do	2 mosaic.
Chaetochloa lutescens.	8	do	do	do	do	All healthy.
Andropogon elliottii	6	do	do	do	do	Do.
Andropogon virginicus.	6	do	do	do	do	Do.
Andropogon scopiarus.	6	do	do	do	do	Do.
Syntherisma sanguinalis.	3	do	do	do	do	2 mosaic.
Brachiaria platyphylla.	4	do	do	do	do	All healthy.
Panicum dichotomiflorum.	2	do	do	do	do	2 mosaic.
Sorghastrum nutans.	2	do	do	do	do	Both healthy.

[a] These plants were merely exposed to natural infection by being placed on a ledge in a greenhouse containing infected plants with which they were not in direct contact.

The percentage of plants infected in both artificial-inoculation and insect-transmission experiments is not high in many cases, but the results are conclusive owing to the conditions under which all experiments were performed. All grasses for experimental purposes were grown in a greenhouse at Washington hundreds of miles from any known

cases of natural infection. When suitable for inoculation they were removed to another greenhouse where the inoculations were performed. In practically every case equal numbers of plants were inoculated with nonvirulent juice as controls. Insect-transmission experiments were conducted in cages in a third greenhouse with plant material raised in the greenhouse first mentioned. In these experiments also equal numbers of control plants were placed in adjoining cages. No case of mosaic has occurred among the control plants in any experiment performed.

In some experiments a method of handling aphids was developed by which the tedious operation of lifting individual insects with a camel's hair brush was eliminated. Small bits of infected sorghum leaves covered with the insects were clipped off with scissors and placed on or tied to the plants to be tested. Similar pieces of leaves from which the insects had been carefully removed were tied to a series of control plants, and healthy leaves with nonvirulent aphids were tied to a second series of control plants. Although it is necessary to run two sets of control plants in order to check all factors properly, this method was found to be of great convenience. The experiments noted in Table III were performed in the manner described.

TABLE III.—*Results of a test of the insect transmission of grass mosaic by the new method.*

Species.	Number of plants.	Date exposed.	Treatment.	Results.	
				Date examined.	Condition.
Saccharum officinarum var. Louisiana Purple	3	Aug. 3, 1920..	Aphids on mosaic leaf.....	Aug. 24, 1920.	2 mosaic.
Do	3	.....do.....	Mosaic leaf, no insects.....	Sept. 15, 1920.	All healthy.
Do	3	.....do.....	Aphids on healthy leaf....	.....do.....	Do.
Zea mays	3	.....do.....	Aphids on mosaic leaf.....	Aug. 30, 1920.	2 mosaic.
Do	3	.....do.....	Mosaic leaf, no insects.....	Sept. 15, 1920.	All healthy.
Do	3	.....do.....	Aphids on healthy leaf....	.....do.....	Do.
Holcus sorghum	2	.....do.....	Aphids on mosaic leaf.....	Aug. 26, 1920.	Both mosaic.
Do	2	.....do.....	Mosaic leaf, no insects.....	Sept. 15, 1920.	Both healthy.
Do	2	.....do.....	Aphids on healthy leaf....	.....do.....	Do.
Miscanthus sinensis...	1	.....do.....	Aphids on mosaic leaf.....	Aug. 30, 1920.	Mosaic.
Do	1	.....do.....	Mosaic leaf, no insects.....	Sept. 15, 1920.	Healthy.
Do	1	.....do.....	Aphids on healthy leaf....	.....do.....	Do.
Syntherisma sanguinalis.	2	.....do.....	Aphids on mosaic leaf.....	Aug. 30, 1920.	Both mosaic.
Do	2	.....do.....	Mosaic leaf, no insects.....	Sept. 15, 1920.	Both healthy.
Do	2	.....do.....	Aphids on healthy leaf....	.....do.....	Do.

Although a considerable number of species of insects have been tested for their ability to act as carriers of grass mosaic, it has been demonstrated only for *Aphis maidis* (*A. adusta*) by the writers. Our results on the transmission of the disease with this insect have been corroborated by Ledeboer (*13*),[5] who has also succeeded in transmitting it by the use of *A. sacchari*. Owing to its abundance and omnivorous habits, *A. maidis* has been found very convenient for use in ascertaining the susceptibility to mosaic of various grasses. Several grasses, notably corn and millet, have been found by the writers to be very difficult or even impossible to infect by artificial inoculation, but it is comparatively easy to bring about infection by the use of virulent corn aphids. The corn aphids, of course, do not feed on all the grasses equally well, and on some it was found quite impossible to establish them. They are not abundant on sugar cane.

[5] Since this paper was prepared, nearly two years ago, the work reported in Ledeboer's preliminary report has appeared: WILBRINK, G. "EEN ONDERZOEK NAAR DE VERBREIDING DER GELESTREPENZIEKTE DOOR BLADLUIZEN." *In* Archief Suikerindus. Nederl.-Indië, Meded. v. h. proefst. v. d. Javasuikerindus. 1922, no. 10, p. 413–456. Dr. Wilbrink confirms *A. maidis* as a vector of mosaic but not *A. sacchari*.

A. maidis has been reported on sugar cane, however, (*5*, *8*, *18*)[6], and on other crops from many sugar-cane regions.

While it is usually difficult to establish *Aphis maidis* on sugar cane in the greenhouse by transplanting them from other grasses, they sometimes migrate naturally to the cane and are found on it in large numbers. One of the worst infestations of any grass by *A. maidis* ever seen by the writers was on sugar-cane seedlings about 5 months old. It was brought to our attention by Dr. B. T. Galloway, who was growing the seedlings in one of the Washington greenhouses. In some of our experiments where *A. maidis* failed to become established and disappeared within 2 or 3 days, the plants nevertheless were infected by them and showed symptoms after the usual incubation period of 14 to 20 days. Corn, sorghum, and pearl millet are favorite food plants for *A. maidis*, which is frequently found on them in enormous numbers. The insect is an ideal carrier in the case of these three crops. Several species of leafhoppers have been held under suspicion as vectors of grass mosaic on account of strong indirect evidence, but no positive proof of such capacity on their part was developed.

SOME ECONOMIC ASPECTS OF GRASS MOSAIC

TESTS WITH CANE VARIETIES OF THE NORTH INDIA TYPE

Practically all of the well-known varieties of sugar cane are susceptible to grass mosaic. Prof. F. S. Earle noticed, however, that the so-called Kavangire, a variety of the slender North India type, was not affected under conditions favorable to the transmission of the disease (*15*). Later observations have indicated that the Uba, grown extensively in Natal, and Cayana No. 10, in the sirup sections of this country, are apparently immune. These varieties are of the same type, and Prof. Earle has declared that Kavangire is identical with the old well-known Uba (*6*). A collection of varieties of this type from various parts of the world was brought together in Washington to determine whether immunity to mosaic is characteristic of the whole group. They were placed in a greenhouse exposed to natural infection, with the results indicated in Table IV.

TABLE IV.—*Susceptibility to the mosaic disease of the varieties of the North India type of sugar cane*

Variety.	Date exposed.	Result.	
		Date examined.	Condition.
Uba	Jan. 6, 1921	July 1, 1921	All healthy.
Kavangire	.....do.....	.....do.....	Do.
Cayana No. 10	.....do.....	.....do.....	Do.
Saccharum narenga (S.P.I. No. 38332).	.....do.....	Jan. 25, 1921	All mosaic.
Khera (S.P.I. No. 33242)	.....do.....	.....do.....	Do.
Merthi (S.P.I. No. 33243)	.....do.....	July 1, 1921	All healthy.
Kinar (S.P.I. No. 33245)	.....do.....	.....do.....	Do.
Chikusho (S.P.I. No. 29106)	.....do.....	Jan. 25, 1921	All mosaic.
Var. from Kagawa Ken (S.P.I. No. 29107).	.....do.....	.....do.....	Do.
Kikaigashima (S.P.I. No. 29108)	.....do.....	.....do.....	Do.
Oshima (S.P.I. No. 29109)	.....do.....	July 1, 1921	All healthy.
Chikucha (S.P.I. No. 30464)	.....do.....	Jan. 25. 1921	All mosaic.

[6] Mr. A. C. Baker reports *Aphis maidis* on sugar cane in the quarantine greenhouse of the Federal Horticultural Board at Washington, March 11, 1920 (in letter to the writers, December 2, 1921), and Mr. Geo. W. Wolcott reports finding this species on sugar cane in Porto Rico (in letter to Dr. C. O. Townsend, December 30, 1921).

With the exception of the plants labeled *Saccharum narenga*, these varieties are all very similar, and some of them may be identical. They are of the slender North India type and probably have a common origin, but this experiment proves that not all varieties of this type are immune to mosaic. Varieties from India, China, and Japan proved to be susceptible. They are apparently scarcely injured by the disease. The leaf symptoms are much less conspicuous than in the thick-stalked varieties of sugar cane, and there is no evidence of stunting. In this respect they are like the well-known Java seedling varieties resulting from crosses between the Chunnee ♂ and Striped Preanger ♀ and between Chunnee ♂ and Black Cheribon ♀.

TESTS WITH CORN VARIETIES

That some varieties of corn are severely injured by grass mosaic was shown by the senior writer in 1920 (*4*). In 1921 a large number of corn varieties were tested in southern Georgia for immunity or resistance to the disease. The first experiment included 40 varieties of field, sweet, and pop corn from all of the corn sections of the United States.[7] About 25 plants of each variety were grown near the center of a field of first ratoon Louisiana Purple sugar-cane plants, more than half of which were mosaic. Seeds were planted in the field on April 15, 1921. On July 15, 1921, the plot was examined and notes were taken on the percentage of infected plants in each variety. The results are given in Table V.

TABLE V.—*Results of tests for resistance to mosaic of varieties of corn planted on April 15, 1921*

Variety.	Source of seed.	Percentage of mosaic on July 15, 1921.	Variety.	Source of seed.	Percentage of mosaic on July 15, 1921.
Native	Georgia	20	Clarage (U. S. Selection No. 125).	Maryland	0
Pope Prolific	Florida	20	Boone County (U. S. Selection No. 159).	Nebraska	0
U. S. Selection No. 165	Texas	35	St. Charles (U. S. Selection No. 202).	do	0
U. S. Selection No. 170	do	40	Lancaster Surecrop	Illinois	0
Laguna	do	10	U. S. Selection No. 160	California	15
Brazos	do	5	Orange County Prolific	do	15
Arlington Prolific	Mississippi	17	U. S. Selection No. 204	South Dakota	10
Red Cob	do	10	Northwestern	North Dakota	0
Millpond Prolific	Georgia	20	Gehu	do	0
Whatley Prolific	do	15	Pearl	do	0
Gerrick	South Carolina	12	U. S. Selection No. 133	Wisconsin	0
U. S. Selection No. 201	Arkansas	12	Hall Gold Nugget (U. S. Selection No. 193).	New York	0
Cuban Yellow	Florida	20	Arlington Peruvians	Virginia	15
Station Yellow	Alabama	0	Pueblo Black	New Mexico	0
Singleton	Texas	0	White Rice	Virginia	4
Huffman	Tennessee	10	Yellow Pearl	do	4
U. S. Selection No. 230	Virginia	5	Hull-less	Michigan	0
Boone County (U. S. Selection No. 119).	do	10	Golden Bantam	Virginia	0
U. S. Selection No. 120	do	0	Country Gentleman	do	0
U. S. Selection No. 182	do	10			
Woodburn (U. S. Selection No. 77).	Ohio	0			

Twenty-three of the 40 varieties became affected with mosaic in this experiment. All varieties from the Southern States excepting 4, which later proved susceptible, were more or less affected by the disease; but

[7] The seed of these varieties was furnished through the courtesy of the Office of Cereal Investigations, U. S. Department of Agriculture.

the varieties from Northern and Western States were conspicuously free from it. This result may be due in part to the fact that the northern and western varieties were subnormal in vigor. They were for the most part only half as tall as the southern varieites. It is now well known that a slight shortening of the accustomed length of day may check vegetative growth and hasten maturity in some varieties of plants (7), and this fact may account for the subnormal development of these varieties. It has been our experience that stunted plants are more difficult to infect experimentally than normal ones. The explanation for the low percentage of infection in the varieties which were out of their proper environment may therefore be due to this fact rather than to any innate character of resistance.

A second experiment on a much larger scale with southern varieties of field corn was started on May 15, one month after the first experiment. About 3 acres of corn, approximately equally divided among 17 varieties, was planted in a field immediately adjacent to badly diseased first ratoons of Louisiana Purple sugar cane. On July 15 the whole planting was carefully examined and a large proportion of all varieties was discovered to be already affected by the disease. The percentage of diseased plants on that date is given in Table VI.

TABLE VI.—*Results of tests for resistance to mosaic of varieties of southern field corn planted on May 15, 1921*

Variety.	Source of seed.	Percentage of mosaic on July 15, 1921.	Variety.	Source of seed.	Percentage of mosaic on July 15, 1921.
Native	Georgia	15	Whatley Prolific	Georgia	40
Pope Prolific	Florida	75	Gerrick	South Carolina	50
U. S. Selection No. 165	Texas	30	U. S. Selection No. 201	Arkansas	60
U. S. Selection No. 170	do	50	Cuban Yellow	Florida	45
Laguna	do	24	Station Yellow	Alabama	66
Brazos	do	40	Singleton	Texas	60
Arlington Prolific	Mississippi	50	U. S. Selection No. 230	Virginia	45
Red Cob	do	25	Boone County (U. S. Selection No. 119).	do	70
Millpond Prolific	Georgia	85			

A much higher percentage of infection was found in these plants than in the same varieties planted one month earlier. All of the corn was heavily infested with *Aphis maidis*, but no other insect was noticeably abundant.

In order to obtain data on the damage inflicted by this disease, 10 each of mosaic and apparently healthy plants in each variety were tagged for identification at harvest time. It is realized that many plants marked "healthy" on July 15 may have become infected before harvest, so that any decrease in yield indicated by this method does not represent the real extent of the loss sustained. The loss due to mosaic is without question greater than that indicated by our data. All tagged plants with legible inscriptions were harvested on September 22, and the ears were examined and weighed. The number of ears, total weight, and average weight of ears of healthy and mosaic plants for each variety are given in Table VII.

TABLE VII.—*Weight of healthy and mosaic ears of corn varieties planted May 15, 1921*

Variety.	Source of seed.	Condition.	Ears on 10 plants.	Weight.		Decrease due to mosaic.
				Total.	Average.	
			Number.	*Gm.*	*Gm.*	*Per cent.*
Native	Georgia	Healthy.	5	535	107	.....
Do	do	Mosaic...	7	470	67.1	37.3
Pope Prolific	Florida	Healthy.	8	822	102.7	.....
Do	do	Mosaic...	5	402	80.4	21.8
U. S. Selection No. 165	Texas	Healthy.	4	665	166.2	.....
Do	do	Mosaic...	9	780	82.2	50.6
U. S. Selection No. 170	do	Healthy.	6	995	165.8	.....
Do	do	Mosaic...	6	830	138.3	16.6
Laguna	do	Healthy.	5	765	153.0	.....
Do	do	Mosaic...	5	735	147.0	4.0
Brazos	do	Healthy.	6	1,115	186.0	.....
Do	do	Mosaic...	7	815	116.4	37.5
Arlington Prolific	Mississippi	Healthy.	10	1,100	110.0	.....
Do	do	Mosaic...	6	470	78.2	29.0
Red Cob	do	Healthy.	4	555	138.7	.....
Do	do	Mosaic...	5	640	128.0	7.8
Millpond Prolific	Georgia	Healthy.	7	750	107.1	.....
Do	do	Mosaic...	10	595	59.5	44.5
Whatley Prolific	do	Healthy.	7	790	112.8	.....
Do	do	Mosaic...	11	880	80.0	29.1
Gerrick	South Carolina	Healthy.	7	580	82.9	.....
Do	do	Mosaic...	12	890	74.1	20.7
U. S. Selection No. 201	Arkansas	Healthy.	8	910	113.5	.....
Do	do	Mosaic...	6	518	86.3	24.0
Cuban Yellow	Florida	Healthy.	5	815	163.0	.....
Do	do	Mosaic...	12	1,140	95.0	41.8
Station Yellow	Alabama	Healthy.	8	900	112.2	.....
Do	do	Mosaic...	8	755	94.3	16.0
Singleton	Texas	Healthy.	7	784	112.0	.....
Do	do	Mosaic...	6	670	111.6	.4
U. S. Selection No. 230	Virginia	Healthy.	2	318	159.0	.....
Do	do	Mosaic...	3	317	102.3	35.7
Boone County (U. S. Selection No. 119)	do	Healthy.	2	205	102.5	.....
Do	do	Mosaic...	6	435	72.5	29.3

This data indicates that with corn, as with sugar cane, some susceptible varieties tolerate the disease without greatly injurious results, while other varieties are severely injured by it. Plate 1, B, illustrates a variety not noticeably injured by mosaic. It was pointed out in a former publication (*4*) that in the case of "White Creole" corn in Louisiana the ears of affected plants are not only stunted but they are poorly filled, some being quite sterile. The rows of kernels in affected ears are likely to be very irregular as compared with the straight parallel rows of normal ears. In nearly all varieties in the present experiments the same condition was apparent and is well illustrated in Plate 1, A, and also in Plates 2 to 4. It will be noticed that some of the ears marked "healthy" are small and some show irregularity in the rows, but by no means to the same degree as in the mosaic ears. This may be due partly to the fact previously noted that plants labeled healthy in the field may have become infected later. The writers believe that grass mosaic is a serious disease of corn where conditions are favorable for infection. Since the disease is apparently not transmitted in the corn seed, as will be briefly considered

later, the infection must come each year from some diseased perennial grass. In all sugar-cane plantations, corn is invariably used in the crop rotation, and one of the required conditions for infection of corn is present if the disease exists in the nearby cane.

Corn mosaic has now been reported from Porto Rico, the United States, Guam, the Hawaiian Islands, and Trinidad.

In a publication by Kunkel (*12*), just received from Hawaii, a very complete cytological investigation of corn mosaic is recorded. Certain large bodies found in the cells of diseased tissues are described and suggested as the possible cause of the disease. Such cell inclusions have been noticed and recorded by early investigators of mosaic, but this was not mentioned and possibly was overlooked by Kunkel. Practically identical bodies were carefully described and accurately illustrated by Iwanowski (*10*) in his researches on tobacco mosaic published nearly 20 years ago. The latter, however, was cautious about ascribing to these bodies any etiological significance merely on the basis of their association with the disease. The paper by Kunkel referred to above contains some data of value on susceptibility of corn varieties to mosaic. His presentation of the history of our knowledge of this disease in corn is very misleading. One of the present writers contributed the original paper on corn mosaic (*4*). This paper, which was based on careful observations and experiments established the type of disease, its infectious nature, its identity with sugar-cane mosaic, and the natural agents of transmission. Within its pages ample credit was given to two previous investigators for observation of a possibly similar condition in corn. Their observations were of a very indefinite nature and were summed up in the following words by the only one of the two who published on it (*16*):

> The trouble was ascribed to various causes by different people, *but it appears to be the same baffling general condition observed in Hawaii rather than any specific disease.*[8]

On the basis of this candid admission and other less reliable evidence Kunkel, perhaps unintentionally, leads the reader to believe that the disease was well known and well understood prior to the present writers' contribution.

FIELD OBSERVATIONS ON SORGHUM AND PEARL-MILLET MOSAIC

Although mosaic on sorghum was early noted by one of the writers in experiments at Washington (*2*) and afterwards proved to be identical with sugar-cane mosiac (*3*), it was not observed in the field until the summer of 1920. At that time many fields of sweet sorghum were noticed to be naturally infected in the vicinity of Cairo, Ga. All such fields were within half a mile of affected sugar cane. No accurate data were obtained on injury to sorghum, but the leaf symptoms were strongly marked, the nondevelopment of chlorophyll being about the same as in severely injured sugar cane, and the plants were decidedly stunted. It was noticed that when plants were infected soon after germinating, the injury was far greater than in the case of late infection. A few notes were taken on varieties affected and on the percentage of infected plants in various fields.

The variety Honey appears to be especially susceptible. Several fields of this variety, known locally as Sugar Drip, were observed to contain 6

[8] The italics are ours.

to 30 per cent of diseased plants. Fields of the so-called Texas-Ribbon variety (probably Gooseneck) contained from 0.5 to 15 per cent of affected plants, or uniformly less than the first-named sort under the same conditions. No infection was observed in a field of Early Amber, but this variety has proved to be susceptible under greenhouse conditions at Washington. A number of grain-sorghum varieties recently introduced from Africa were planted in a greenhouse at Washington exposed to natural infection, but they have showed no signs of the disease. About 50 large stools of mosaic sugar cane were present in this house, and *Aphis maidis* was abundant.

During the summer of 1921 a patch of pearl millet (*Pennisetum glaucum*), sold under the name Georgia cat-tail millet, was planted for soiling purposes at the Sirup Experiment Station, Cairo, Ga. On July 15 this planting was noticed to be severely attacked by mosaic. More than 50 per cent of the plants were affected and were noticeably smaller than healthy plants. This species also had been previously proved to be susceptible to grass mosaic under controlled conditions at Washington. (See Table II.)

FIELD OBSERVATIONS ON MOSAIC OF WILD GRASSES

Collections of wild grasses affected with mosaic have been made in Louisiana, Georgia, and Florida. In all cases the plants were found in or near affected sugar-cane fields. That the mosaic appearing naturally in the field in these species is identical with sugar-cane mosaic has been verified experimentally under controlled conditions. (See Tables I and II.) A list of mosaic grasses collected with dates and localities is given in Table VIII.

TABLE VIII.—*Collections of wild grasses affected with mosaic*

Species and locality.	Date of collection.	Species and locality.	Date of collection.
Syntherisma sanguinalis:		*Paspalum boscianum:*	
Cairo, Ga.	Sept. 11, 1919	Cairo, Ga.	Sept. 11, 1919
New Orleans, La.	Oct. 15, 1919	Marianna, Fla.	Aug. 16, 1920
Do.	Sept. 6, 1920	Cairo, Ga.	July 27, 1920
Chattahoochee, Fla.	Sept. 1, 1920	Reno, Ga.	July 29, 1920
Plaquemine, La.	Aug. 21, 1920	*Chaetochloa magna:*	
Marianna, Fla.	Aug. 14, 1920	Dade County, Fla.	Dec. 11, 1920
Reno, Ga.	July 31, 1920	*Brachiaria platyphylla:*	
		New Orleans, La.	Aug. 11, 1920
		Do.	Sept. 6, 1920

No complete record of individual observations on mosaic of wild grasses has been kept. The foregoing list is sufficient to show that mosaic of these species is common near affected sugar cane under natural conditions.

EXPERIMENTS ON SEED TRANSMISSION OF GRASS MOSAIC

It seems appropriate to include in the present paper a brief account of experiments to determine whether the disease is transmitted by means of seeds, because of the relation of such transmission to the infection of one species with inoculum from a different species. In regions where the vegetative parts of annual grasses are completely killed during winter,

the virus would be more likely to persist in seeds, if it survives at all, than in any other state. The possibility of its being carried over in the bodies of insect vectors is rather remote, since there is no evidence that such insects function as intermediate hosts, but rather as mechanical carriers, and as such they would not furnish the special conditions neces sary for long-continued survival of the virus. This view is supported by our results with artificial inoculations, which proved that development of the virus within the body of an insect is not necessary. We have fairly conclusive proof that the virus does not survive in plant trash or soil, even in tropical countries. Our evidence that the virus is not carried over in seeds (*2*) has been strengthened by the results of subsequent experiments with corn, sorghum, and wild-grass seeds from mosaic parents, all of which gave rise to healthy plants. The results are shown in Table IX.

TABLE IX.—*Experiments to determine the transmission of disease by means of seeds from mosaic corn, sorghum, and wild grasses*

Species.	Date planted.	Number germinated.	Results.	
			Date examined.	Condition of seedlings.
Zea mays var. White Creole.	Nov. 30, 1920	44	Jan. 25, 1921	All healthy.
Zea mays var. U. S. Selection No. 182.	.....do.....	81	.....do.....	Do.
Zea mays var. White Creole.	Dec. 15, 1920	100	Feb. 15, 1921	Do.
Zea mays var. U. S. Selection No. 182.	.....do.....	90	.....do.....	Do.
Holcus sorghum var. Honey.	Sept. 15, 1919	181	Nov. 30, 1919	Do.
Do.....	Nov. 30, 1920	94	Jan. 26, 1921	Do.
Holcus sorghum var. Goose-neck.	.....do.....	75	.....do.....	Do.
Paspalum boscianum.....	Jan. 20, 1921	47	Mar. 12, 1921	Do.
Brachiaria platyphylla.....	.....do.....	52	.....do.....	Do.
Syntherisma sanguinalis.....	.....do.....	42	.....do.....	Do.

Observations by Vander Stok (*14*), Kobus (*11*), and Wilbrink and Ledeboer (*17*) in Java indicate that sugar-cane seedlings from mosaic parents are healthy at the start and remain so unless infected from outside sources in the usual way. All available evidence, therefore, points to the conclusion that in this disease, as in tobacco mosaic, the virus is not transmitted from generation to generation by means of seeds.

SUMMARY

(1) Thirteen species of grasses have been proved by inoculation experiments to be susceptible to the disease known as sugar-cane mosaic or, more properly, grass mosaic.

(2) Certain varieties of sugar cane belonging to the slender North India type, formerly regarded as immune, have proved susceptible to mosaic.

(3) Data on the yield of 17 varieties of southern field corn show a decrease in weight of 0.4 to 50.6 per cent, due to mosaic.

(4) Field observations indicate that natural infection of sorghum, pearl millet, crab-grass, bull-grass, *Chaetochloa magna*, and *Brachiaria platyphylla* is widespread near affected cane in the sugar-cane belt.

(5) All species tested for seed transmission of mosaic gave negative results.

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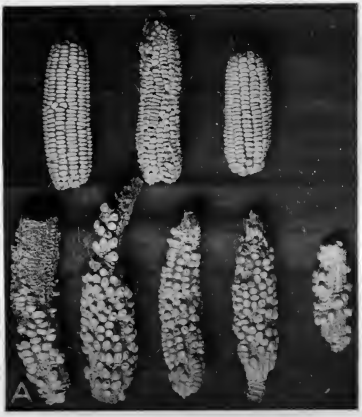
PLATE 1

A.—Ears of healthy (upper row) and mosaic (lower row) corn of Arlington Prolific variety.

B.—Ears of healthy (upper row) and mosaic (lower row) Laguna corn, a resistant variety.


A
B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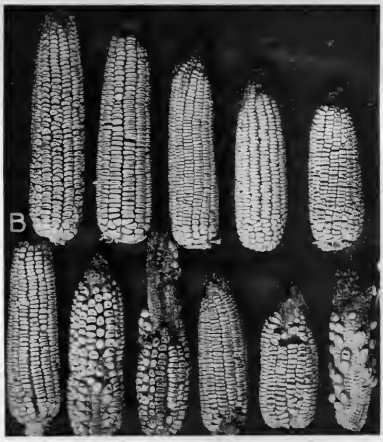

B

PLATE 2

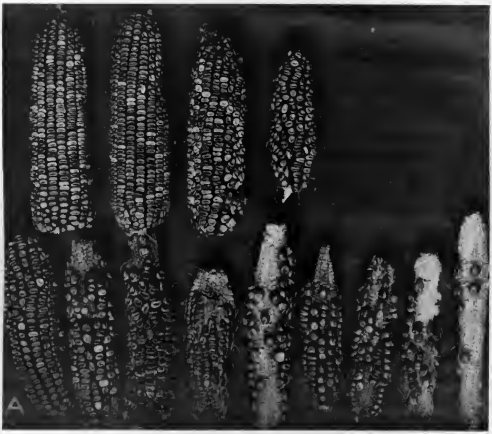
A.—Ears of healthy (upper row) and mosaic (lower row) corn of U. S. Selection No. 119.

B.—Ears of healthy (upper row) and mosaic (lower row) corn of U. S. Selection No. 170.

PLATE 3

A.—Ears of healthy (upper row) and mosaic (lower row) corn of Millpond Prolific variety.

B.—Ears of healthy (upper row) and mosaic (lower row) corn of U. S. Selection No. 165.


A


B

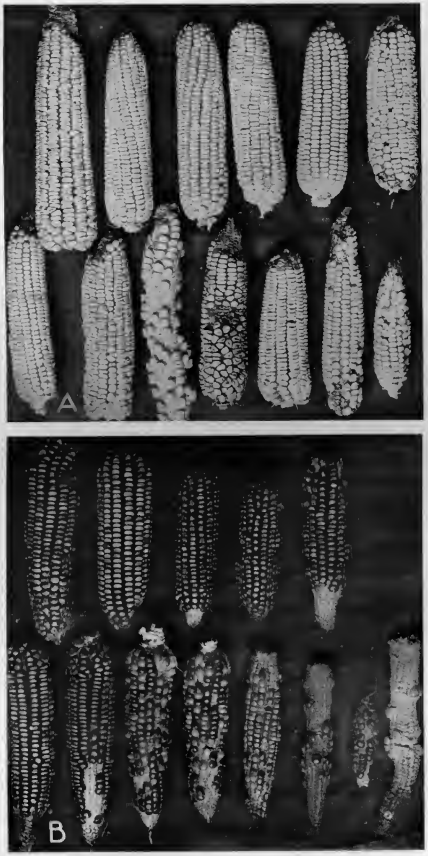

A
B

PLATE 4

A.—Ears of healthy (upper row) and mosaic (lower row) corn of Brazos variety.
B.—Ears of healthy (upper row) and mosaic (lower row) corn of Cuban Yellow variety.

PROTEIN SYNTHESIS BY AZOTOBACTER [1]

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The metabolic process of protein synthesis is common to all microorganisms. The rate, form, and total quantity of protein synthesized, however, as well as the type of food substances available for synthesis, varies widely.

An idea of the relative protein content of microorganisms can be obtained from the results recorded by various investigators; but the multiplicity of the methods employed in the cultivation, collection, and analysis of microbial cells makes a comparative study difficult. A high nitrogen content for most bacteria is reported. If all of this nitrogen be considered as protein nitrogen, the protein content of such cells is large. Wheeler (*8, p. 65*),[2] whose results seem to be comparative for such analyses, reports a nitrogen content varying from 5.964 per cent to 11.765 per cent for 12 bacteria examined. The nitrogen content of yeasts is probably similar to that of bacteria. Nicolle and Alilaire (*5*) report a nitrogen content of 10.0 per cent for a Froberg yeast.

The high protein content of yeasts, their rapidity of growth, and their practical method of cultivation justify their wide exploitation as a food product within recent years. The value of such yeast food as a protein concentrate appears to be firmly established. During the recent war the demand for this yeast concentrate in Germany far exceeded the supply.

Interest in the yeast industry has been accentuated with the development of the present vitamine theory. The high content of water-soluble B vitamine in yeast cells has apparently opened a new field for its use.

Several factors tend to influence the practicability of utilizing microorganisms as a source of protein. Mechanically, the problem is dependent upon the development of practical methods for securing a maximum degree of growth, as well as means for the collection and care of the product. Economically, the yield, rate of growth, and the food requirements of the organism are important considerations. The substances used as food for the organism should be in the nature of waste products, and to justify the process the synthesized product must have a greater value than the substances used in its production. Finally, if the industry is to be of value, the product must have a nutritional value.

The high protein content of the Azotobacter cell and its relatively simple food requirements suggested the possibility of utilizing it as a means for synthesizing a protein which could be used either as a stock food or as a fertilizer. The employment of this organism for such a purpose appeared to offer some important advantages on account of its nitrogen-assimilating ability. This would necessitate the use of a solution having a carbohydrate only as the important constituent.

On the other hand, the apparent slow development of Azotobacter seemed to present a serious difficulty. However, it has been demonstrated

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[2] Reference is made by number (italic) to "Literature cited," p. 274.

that a prompt and heavy growth of Azotobacter can be secured by a vigorous aeration of a liquid culture in a manner similar to that used in stimulating yeast growth.

The experimental data which follow are merely a report of progress in an investigation having for its object the utilization of waste products by Azotobacter for protein synthesis.

METHOD OF CULTIVATION

A rapid and vigorous growth of Azotobacter was produced by passing a current of air continually through a culture solution. The method used was identical with that described in a previous publication (*14*).

Aeration was accomplished by attaching the culture flask equipped with Folin's aerating tubes to a vacuum system. Contamination and evaporation of the culture were reduced to a minimum by filtering the air through a sterile cotton filter and two or three flasks of sterile water. The air was thus filtered through cotton and rinsed in water before entering the culture flasks.

Several flasks of culture media could be easily aerated at the same time. Usually a cotton filter and a wash bottle were placed between the culture flasks. Little trouble occurred from contamination. A good grade of rubber tubing and tight fitting connections are a necessity, however. Flasks containing from 200 cc. to 2,500 cc. of medium were used. The quantity of air passed through the cultures was not measured, but a vigorous bubbling of the liquid was maintained continuously.

AZOTOBACTER CULTURES

Cultures of Azotobacter were isolated from samples of soil received from various parts of Kansas, Colorado, California, Iowa, and Mississippi. Flasks containing dextrose-Ashby or mannite-Ashby medium were inoculated with the soil samples. Upon the formation of the characteristic surface growth, Ashby agar plates were streaked. Dextrose-Ashby agar slants were inoculated from well-isolated colonies and repeated streakings of the cultures upon Ashby agar plates were made. A large number of the cultures were streaked consecutively from 1 to 12 generations. The utmost care was exercised in attempting to obtain and maintain pure cultures.

The inoculum used for seeding the medium was prepared by adding a portion of the emulsion from a young dextrose-Ashby culture to a flask containing dextrose-Ashby broth. This was aerated for two to four days. The quantity of this starter usually used for inoculating the experimental medium was 0.5 per cent to 1.0 per cent of the medium seeded. In all cases a morphological examination of the starter was made as a test for purity before use. The temperature for incubation was 30° C.

MEDIUM

The medium used in the following experiments, unless otherwise noted, was made from the following formula:

	Gm.
Tap water	1,000.0
K_2HPO_4	.5
$MgSO_4$	.2
NaCl	.2
Dextrose	10.0

The reaction of this solution was readjusted to a P_H of 7.0 to 7.4, filtered if necessary. The required quantities were placed in flasks and sterilized in the autoclav at 20 pounds pressure for 30 minutes.

METHODS OF ANALYSIS

Total nitrogen determinations and sugar analysis of the Azotobacter cultures were made at frequent intervals.

In all cases the total nitrogen was determined by the usual standard methods. Unless otherwise noted, 50 cc. portions of the medium in duplicate were used. The figures referred to in the tables denote the average of the duplicate analyses. The net gain in nitrogen is recorded in all cases, unless otherwise stated, as milligrams of nitrogen in each 100 cc. of medium. In other words, the figures refer to the quantity of nitrogen fixed per gram of dextrose, since each 100 cc. of medium contains this quantity of sugar.

Sugar determinations were made according to the method proposed by Shaffer (*9*). The copper resulting from the reduced Fehlings was determined by colorimeter readings. Duplicate readings were made each time, and the average of these was recorded. As a general rule, 50 cc. of the medium were used. This was diluted to 100 cc., and 20 cc. of this filtrate were used for reduction. The figures given, unless otherwise stated, refer to grams of dextrose per 100 cc. of medium.

COMPOSITION OF THE AZOTOBACTER CELL

The composition of the Azotobacter cell has been determined by several investigators. Gerlach and Vogel (*1*) report a protein content of 80 per cent.

Lipman (*2*) reports the nitrogenous composition of the Azotobacter membrane as 10.45 per cent total nitrogen, 6.39 per cent nonbasic nitrogen, 2.76 per cent basic nitrogen, and 0.98 per cent ammonia nitrogen. He observed that lead acetate precipitated practically all of this nitrogen in young or old cultures, while phototungstic or tannic acid would not precipitate nearly as much. He believed that in young cultures this nitrogen substance is in a soluble form and not precipitated by phosphotungistic acid, but as the culture grows older this soluble nitrogenous material is converted into an insoluble and more complex protein.

Stoklasa (*4*) cultivated Azotobacter in liquid cultures. The growth was collected on a filter and washed. The nitrogen content of the washed cells was 11.3 per cent.

Hoffmann and Hammer (*6*) studied the composition of Azotobacter and report analyses much lower in protein. The organism was grown on Ashby agar plates. The growth was scraped off, washed, and dried. The maximum protein content recorded was 17.75 per cent. They suggest that the wide difference in their results, as compared with those of other investigators, was due to the jellylike material which in liquid cultures is filtered out of solution. In their method this material is included in the analysis. This increases the total residue, and since this substance is thought to be carbohydrate, the total percentage of nitrogen is decreased. The phosphorus content (P_2O_5) varied from 2.51 per cent to 2.97 per cent. Increasing with the age of the culture. Stoklasa (*4*) reports the P_2O_5 content to be 4.93 per cent.

Omeliansky (*7*) grew Azotobacter on dextrose mineral salt agar for six days at a temperature of 30° C. An analysis of this growth is

reported as 6.63 per cent moisture, 4.16 per cent ash, and 12.92 per cent protein. This protein he considers similar to other plant proteins.

While the exact method of cultivation is not reported by all investigators, it appears in general that the nitrogen content of Azotobacter will vary according to the method of cultivation. The nitrogen of the growth scraped off Ashby agar media is reported much lower than in the residue filtered from the liquid cultures.

The composition of seven cultures of Azotobacter cultivated on dextrose-Ashby agar was determined in this laboratory. The growth at the end of two to four days incubation at 30° C. was scraped off the surface of the agar, air dried, and analyzed for the total nitrogen and moisture content. The results are given in Table I.

TABLE I.—*Nitrogen content of Azotobacter cells grown on Ashby agar*

Culture No.	Total nitrogen.	Moisture.
	Per cent.	*Per cent.*
12 B	3.48	9.12
232	3.78	8.55
6 A	3.78	10.00
5 B	3.09	6.89
1 B	3.74	8.10
216	4.55	4.69
10 B	3.74	9.48
Average	3.73	8.12

The percentage of total nitrogen noted in the seven cultures was uniform, averaging 3.73 per cent for all cultures and ranging from 3.48 per cent to 4.55 per cent. If this nitrogen were calculated as protein, the average protein content of the cultures would be 23.31 per cent.

An analysis of a composite sample of these cultures gave the following results: Moisture 8.58 per cent; total nitrogen 3.55 per cent; albuminoid nitrogen 1.89 per cent; ash 12.99 per cent; phosphorus 0.57 per cent; and potassium 1.43 per cent. These figures indicate that only 53.1 per cent of the total nitrogen is protein. This gives a protein content of 11.81 per cent instead of 23.31 per cent, as calculated from the total nitrogen.

The chemical composition of the growth obtained from liquid cultures was determined for comparison. Culture No. 232 was aerated from two to four days at 30° C. in the dextrose-Ashby medium. The growth of cells was obtained by centrifuging in a Sharples laboratory supercentrifuge. A composite sample taken from the growth of several culture flasks was used for analysis. The composition was: Moisture 2.67 per cent; total nitrogen 5.15 per cent; albuminoid nitrogen 4.89 per cent; ash 4.62 per cent; phosphorus 0.24 per cent; and potassium 1.2 per cent.

A comparison of this analysis with that obtained from the growth on Ashby agar reveals considerable difference. In the first place, a higher total nitrogen content is observed in the growth from the liquid culture. The most marked effect, however, is the high albuminoid content of the liquid culture, it being 94.9 per cent of the total nitrogen. This gives a protein content of 30.56 per cent, as compared with 11.81 per cent for the growth obtained from Ashby agar. The liquid culture produced a growth with a lower ash content than did the culture on agar.

SOURCES OF ENERGY

There is a wide variety of substances available as energy for Azotobacter which differ greatly in their value as sources of energy. Not only is there a difference in the value of the sources of energy for nitrogen fixation, but the efficiency of the same material for azofication varies, according to different investigators. This is illustrated in the work of Löhnis and Pillai (*3*), Hoffmann and Hammer (*6*), and Mockenridge (*10*). There appears to be, however, a rather uniform opinion that a mannite solution will furnish energy for the fixation of the largest quantity of nitrogen. As a result, mannite is the carbohydrate employed almost universally in azofication experiments.

As a preliminary study, the comparative value of various substances as sources of energy for Azotobacter was determined. One per cent of the test material was substituted for mannite in Ashby's medium containing calcium carbonate. Flasks of each medium were inoculated with a pure culture of Azotobacter and aerated at 30° C. for six days. The average quantity of nitrogen fixed per gram of test substance for the two cultures studied were as follows:

	Mgm.
Potassium acetate	8.0
Dextrose	7.8
Saccharose	7.7
Mannite	7.2
Molasses	3.0
Lactose	2.4

Dextrose has been preferred in this laboratory as a source of energy for studying the nitrogen-fixing ability of different cultures and their rate of fermentation. Quantitative determination can be easily made and, as shown by these experiments with aerated cultures, it is an efficient source of energy for azofication.

While no special endeavor has been made to study the nitrogen-fixing ability of different Azotobacter cultures with the object of classifying them on such a basis, over 20 strains have been studied. The nitrogen fixation for these cultures grown in dextrose medium has varied from 7.20 mgm. to 18.72 mgm. per gram of dextrose. Many of the same cultures have been repeatedly used for the past two years, and the variations in the quantity of nitrogen fixed at different times by the same cultures is as much as the difference between various cultures. This variation, or at least a large portion, may be attributed to the different intensities of aeration to which the cultures were submitted. The aim was to aerate all cultures alike, but such is impossible unless the aeration is mechanically controlled. This suggests that the optimum quantity of air necessary for the maximum growth of Azotobacter should be determined.

YIELD OF AZOTOBACTER GROWTH

Cultures of Azotobacter cultivated by vigorous aeration in a liquid medium will exhibit a vigorous growth within two to four days. The sugar is rapidly consumed, and a corresponding increase in the nitrogen content of the liquid is noted. If the culture produces pigment, coloration of the medium is observed. In some cultures this coloration is black; in others brown. In those cultures which fail to produce pigment the solution becomes thick and milky in appearance. Pure cultures maintain an alkaline reaction in the absence of calcium carbonate in the medium throughout growth and emit a rather pleasant odor.

A rather interesting phenomenon was often noted in these liquid cultures when treated with a lead-acetate solution. If the heavy white membraneous precipitate which forms is collected on a filter, it gradually darkens and within several minutes is black. The reaction resembles an enzymatic process. No attempt was made to ascertain whether or not this process is connected with the characteristic pigment production in the living cells.

The yield, or amount of growth, was determined by centrifuging 50 cc. of the culture until the supernatant fluid was clear. This was decanted off, care being taken not to disturb the precipitate cells. The centrifuge tube containing this precipitated growth was placed in a drying oven and desiccated for eight hours at 75° to 80° C. to a constant weight. Duplicate determinations of each culture were made. The yield is recorded as a percentage of sugar originally present in the medium.

A daily comparison of the nitrogen assimilation, dextrose fermentation, and the yield of three cultures of Azotobacter is summarized in Table II. Four flasks containing 250 cc. of dextrose medium were used for each culture. All were aerated vigorously at a temperature of 30° C. One flask of each culture was removed daily for analysis. The medium contained 1 gm. of dextrose per 100 cc.

TABLE II.—*Yield rate of dextrose fermentation and nitrogen fixation by Azotobacter*

	1 day.				2 days.			
	Culture No.			Average.	Culture No.			Average.
	10 B	19	19–399		10 B	19	19–399	
Nitrogen per 100 cc. (mgm.)	1.82	4.31	5.60	3.91	8.77	8.21	10.20	9.06
Dextrose per 100 cc. (gm.)	.....	.49	.67	.....	.478	0	.06	.....
Yield per 50 cc. (gm.)	.0920	.0743	.0319	.....	.0901	.1939	.1281	.....
	.0750	.0717	.0154	.....	.0784	.1741	.1198	.....
Yield (per cent)	16.70	14.50	5.73	12.31	16.85	34.80	24.79	28.81

	3 days.				4 days.			
	Culture No.			Average.	Culture No.			Average.
	10 B	19	19–399		10 B	19	19–399	
Nitrogen per 100 cc. (mgm.)	9.36	13.72	12.50	11.86	12.0	14.43	12.90	13.11
Dextrose per 100 cc. (gm.)	.05	0	0	.....	0	0	0	.....
Yield per 50 cc. (gm.)	.0841	.1487	.1234	.....	.1043	.1317	.1017	.....
	.....	.1595	.....	.....	.0889	.1528	.1149	.....
Yield (per cent)	16.82	30.82	24.68	24.10	19.32	28.45	21.66	23.14

There occurs a gradual increase in the quantity of nitrogen fixed. The average quantity fixed for the three cultures was for the first day 3.91 mgm., for the second day 9.06 mgm., for the third day 11.86 mgm., and for the fourth day 13.11 mgm., per 100 cc. of medium. The characteristic rapid disappearance of dextrose occurred. It was practically all consumed by the three cultures within two days.

The average yield of the three cultures for four consecutive days was as follows: 12.31 per cent, 28.81 per cent, 24.10 per cent, and 23.14 per cent of the dextrose originally present in the medium.

The effect of different quantities of dextrose upon nitrogen fixation and the yield was determined for media containing 0.5 per cent and 1.0 per cent dextrose. Flasks containing 250 cc. of medium were seeded with Azotobacter and aerated for four days at 30° C. Total nitrogen and yield determinations were made at the end of the incubation period. The results for the two cultures studied are recorded in Table III.

TABLE III.—*Effect of varying quantities of dextrose upon yield and nitrogen fixation by Azotobacter*

Culture No.	0.5 per cent dextrose.			1 per cent dextrose.		
	Weight.	Yield.	Nitrogen per 100 cc.	Weight.	Yield.	Nitrogen per 100 cc.
	Gm.	*Per cent.*	*Mgm.*	*Gm.*	*Per cent.*	*Mgm.*
19	0.0413 .0437	17.0	9.88	0.1014 .1153	21.66	16.38
232	.0322 .0357	13.58	7.57	.1143 .1282	24.24	16.65
Average	.....	15.29	8.72	.....	22.95	16.57

The largest yield for the quantity of dextrose used was obtained from the medium containing 1 per cent dextrose. The average yield for the two cultures was 22.95 per cent for this medium as compared with 15.29 per cent for the cultures cultivated in the 0.5 per cent dextrose medium. In the 1.0 per cent solution the cultures fixed on an average 16.57 mgm. of nitrogen per gram of sugar; and in the media containing 0.5 per cent dextrose, an average of 8.72 mgm. of nitrogen per half gram of dextrose. In other words, the rate of fixation was practically the same in both solutions.

A similar experiment was made with media containing approximately 0.5 per cent, 1.0 per cent, and 1.5 per cent of dextrose, respectively. The yield with each percentage of dextrose used, the quantity of nitrogen fixed, and the rate of dextrose fermentation were determined every two days for a period of six days. The cultures were vigorously aerated at 30° C. The dextrose content of the control media by analysis was 0.60 per cent, 1.1 per cent, and 1.5 per cent, respectively. The results are summarized in Table IV. The percentage of yield is based upon the quantity of dextrose originally present in the media.

TABLE IV.—*Comparison of various quantities of dextrose in culture No. 19 upon yield, nitrogen fixation, and dextrose fermentation.*

	0.6 per cent dextrose.			1.1 per cent dextrose.			1.5 per cent dextrose.		
	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.	2 days.	4 days.	6 days.
Weight (gm.)	0.0354 .0328	0.0399	0.0692 .0669	0.0766 .0574	0.1506 .1280	0.1245 .1317	0.1190 .1354	0.2383 .2713	0.2036 .1928
Yield (per cent)	11.3	13.3	22.6	12.1	25.3	23.3	16.9	33.9	27.0
Dextrose (gm.)	.20	Trace	0	.68	Trace	0	.98	Trace	0
Nitrogen (mgm.)	6.37	9.75	9.70	7.41	17.29	18.72	9.1	17.68	26.0

The results show that the yield increases with an increase in the concentration of dextrose in the media. An average of the three periodical analyses for each medium gives a yield of 15.7 per cent for the 0.6 per cent dextrose solution, 20.2 per cent for the 1.1 per cent solution, and 25.9 per cent for the 1.5 per cent solution.

The total nitrogen content of each medium at the completion of the experiment reveals the fact that nitrogen fixation occurred at the rate of 16.1 mgm. of nitrogen per gram of dextrose in the 0.6 per cent dextrose solution, 17.0 mgm. of nitrogen per gram of dextrose in the 1.1 per cent dextrose solution and 17.3 mgm. of nitrogen per gram of dextrose in the 1.5 per cent dextrose medium.

The dextrose disappeared rapidly from all solutions, being entirely consumed by the sixth day.

UTILIZATION OF MOLASSES

The waste molasses from a sugar factory should offer an available source of energy for Azotobacter. Hence an experiment was conducted in which molasses was substituted for dextrose in the medium, and the azofication ability of cultures was determined in this medium. The medium contained 1 per cent molasses, or by analysis 0.51 per cent of invert sugar. The quantity of nitrogen fixed per 100 cc. of medium for five cultures aerated for six days was as follows:

	Mgm.
1 B	1.5
2 B	3.3
5 B	3.3
12 B	3.4
232	3.6

The results show an average net gain of 3.03 mgm. of nitrogen per gram of molasses. A gram of molasses represents only 0.51 per cent of invert sugar, giving, therefore, a rate of fixation of 6.06 mgm. of nitrogen per gram of invert sugar.

To determine the yield from molasses, 250 cc. of the 1 per cent molasses medium was inoculated with culture No. 19 and aerated for four days at 30° C. The medium was analyzed for sugar, total nitrogen, and yield. The results are given in Table V.

TABLE V.—*Yield, nitrogen fixed, and sugar fermented from molasses*

Sugar per 100 cc.		Nitrogen per 100 cc.			Weight per 50 cc.			Yield.
Control.	Culture.	Gross.	Control.	Net.	Gross.	Control.	Net.	
Gm.		*Mgm.*	*Mgm.*	*Mgm.*	*Gm.*	*Gm.*	*Gm.*	*Per cent.*
0.50	Trace.	10.53	8.15	2.38	0.0799 0.0935	0.0063 0.0149	0.0761	30.44

The total nitrogen content of different samples of molasses has varied from about 6 to 12 mgm. per gram of molasses. This nitrogen appears to be in a soluble form, since lead acetate fails to precipitate it. On the other hand, if a lead acetate solution is added to an Azotobacter culture cultivated in this molasses medium, a heavy membraneous precipitate forms. An experiment, therefore, was arranged to ascertain whether

this nitrogen had been synthesized into a more complex nitrogenous substance by the growth of Azotobacter.

Eight flasks containing 250 cc. of molasses medium were inoculated, four with culture No. 19 and four with culture No. 232, and aerated thoroughly for eight days.

The contents of the four flasks of each culture were mixed, and total nitrogen determinations were made. The remaining portion was precipitated with a lead-acetate solution and filtered. This filtrate was perfectly clear. Total nitrogen determinations were likewise made on this filtrate. The control medium was treated in a similar manner.

The average results for all duplicate determinations are as follows:

Control.....{Medium 6.71 mgm. nitrogen per 100 cc.
{Filtrate, 6.40 mgm. nitrogen per 100 cc.

Azotobacter No. 19.....{Culture 9.52 mgm. nitrogen per 100 cc.
{Filtrate 0.8 mgm. nitrogen per 100 cc.

Azotobacter No. 232.....{Culture 10.30 mgm. nitrogen per 100 cc.
{Filtrate 0 mgm. nitrogen per 100 cc.

From these results it is evident that Azotobacter is capable of synthe sizing the nitrogen present in the molasses into more complex substances as well as assimilating nitrogen from the air.

UTILIZATION OF STRAW

Pringsheim and Lichtenstein (*13*) report an investigation the purpose of which was to enrich straw concentrate with protein by means of fungi. Hydrolyzed straw was spread out in thin layers and inoculated with an Aspergillus. The protein content of the straw increased from 0.9 per cent to 8.0 per cent within a week Digestion experiments with the food proved satisfactory.

The utilization of vegetable tissues as available sources of energy for Azotobacter has been reported by Murray (*11*) and Hutchinson (*12*).

Experiments were undertaken in the present case with a view to increasing the protein content of wheat straw by Azotobacter. To each 200 cc. of a dextrose, and also a molasses medium, there was added 1 per cent of a good grade of wheat straw which had been finely ground in a mill. This was seeded with Azotobacter cultures and aerated for varying lengths of time. Total nitrogen determinations were made in duplicate on the entire contents of the culture flasks. To note the effect of straw in the medium, similar determinations were made on cultures containing no straw.

The results are recorded in Table VI for three cultures aerated in the dextrose solution and in the straw dextrose medium for four days.

TABLE VI.—*Effect of straw on azofication*

Culture No.	Dextrose-Ashby medium (200 cc.).			Dextrose-Ashby and straw medium (200 cc.).		
	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1 B	1.4	7.80	6.4	9.4	20.01	106.1
10 B	1.4	8.08	6.68	9.4	19.86	10.46
232	1.4	9.97	8.57	9.4	22.36	12.96

The net gain of nitrogen fixed by all the cultures in the straw solution exceeded the net gain in the dextrose by an average of 4.13 mgm.

The experiment was repeated, and a daily analysis of the cultures was made. The cultures in this case were aerated for four days. The data are tabulated in Table VII.

TABLE VII.—*Effect of straw on azofication*

Culture No.	Medium.	Nitrogen in control.	1 day.		2 days.		3 days.		4 days.	
			Gross nitrogen.	Net nitrogen.	Gross nitrogen.	Net nitrogen.	Gross nitrogen.	Net nitrogen.	Gross nitrogen.	Net nitrogen.
		Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
10 B.	D. A. 200 cc. [1]	1.0	4.55	3.55	18.46	17.46	19.20	18.20	24.96	23.96
10 B.	D. A. S. 200 cc. [2]	10.07	10.40	.33	20.93	9.87	30.29	20.22	29.44	19.37
19...	D. A. 200 cc. [1]	1.0	8.38	7.38	15.14	14.14	24.70	23.70	25.02	24.02
19...	D. A. S. 200 cc. [2]	10.07	13.78	3.71	25.02	14.95	39.32	29.25	42.83	32.76

[1] Dextrose-Ashby 200 cc. [2] Dextrose-Ashby and straw 200 cc.

In general, the results are similar to those reported in the previous experiment. However, culture 10 B showed an approximate net gain of 2.0 mgm. at the end of the third day in the straw culture and a loss of over 4.0 mgm. of nitrogen on the fourth day. An average of the two cultures indicates a net gain of 4.15 mgm. for the straw medium for the entire period of the test.

As expected, a greater net gain of nitrogen was recorded for the dextrose solution than for the straw medium for the first two days. This indicates that the straw is not utilized until all of the greater part of the dextrose is consumed.

Calculating all the nitrogen in the wheat straw as protein nitrogen indicates a protein content of 3.14 per cent. In a similar manner, calculating the nitrogen in the straw solution as protein nitrogen indicates an average protein content of the straw from the two cultures of 11.29 per cent, thus giving a net gain of 8.15 per cent protein.

However, but little of this net gain of protein can be attributed to the influence of the straw, for an average net gain of only 2.07 mgm. of nitrogen per gram of straw was noted in the straw dextrose media.

A summary of both experiments indicates an average net gain of only 1.54 mgm. of nitrogen per gram of straw when added to dextrose medium.

In another experiment three cultures were aerated for four days in a molasses solution and three other cultures in a straw-molasses solution. The results are recorded in Table VIII.

TABLE VIII.—*Effect of straw and molasses on azofication*

Culture No.	Molasses medium (200 cc.).			Molasses and straw medium (200 cc.).		
	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.	Nitrogen in control.	Total gross nitrogen.	Net nitrogen.
	Mgm.	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*	*Mgm.*
1 B.....	17.54	19.69	2.15	25.37	28.98	3.61
10 B.....	17.54	21.50	3.96	25.37	33.88	8.51
232.....	17.54	18.57	1.03	25.37	28.55	3.18

The data show a net gain of nitrogen in favor of the straw medium for all cultures. The average net gain of nitrogen for all cultures grown in the molasses solution was 2.38 mgm. and for those grown in the straw solution 5.10 mgm. This gives an average of net balance in favor of the straw media of 2.72 mgm.

Another experiment was conducted in which two flasks containing 200 cc. of straw-molasses solution were seeded with two Azotobacter cultures and aerated for three days. Ferric-sulphate solution was added to precipitate the protein in each culture flask. The entire contents of each flask were then placed upon a filter, and the residue was collected. This precipitate was desiccated and total nitrogen determinations were made. A control medium was treated in a similar manner. The results are presented in Table IX.

TABLE IX.—*Effect of straw and molasses on azofication*

Culture No.	Total nitrogen per gram of straw.	Net nitrogen per gram of straw.
	Mgm.	*Mgm.*
Control	11.18	.....
232	15.41	4.23
1 B	15.84	4.66

The experiment shows an average net gain of 4.44 mgm. of nitrogen per gram of straw, or 2.77 per cent protein.

SUMMARY

(1) The protein content of Azotobacter growth obtained from a solid medium was found to be 11.81 per cent, while that collected from a liquid culture was 30.56 per cent.

(2) The yield of cells increased with the quantity of dextrose in the medium. An average yield of 15.7 per cent, 20.2 per cent, and 25.9 per cent of the sugar was obtained from medium containing 0.6 per cent, 1.1 per cent, and 1.5 per cent dextrose, respectively.

(3) The relative quantities of nitrogen fixed per gram of dextrose for the three different percentages of sugar were similar—namely, 16.1 mgm., 17.0 mgm., and 17.3 mgm., respectively.

(4) When molasses was used as a source of energy for Azotobacter development there was obtained a yield of cells equal to 30.44 per cent of the sugar in the molasses. This gives a higher percentage yield for the molasses sugar than for dextrose. If the moisture content of the molasses is considered, the percentage yield from the actual molasses exceeds the yield from dextrose.

(5) Azotobacter is able to convert the soluble nitrogenous substances present in molasses into more complex protein, as well as to utilize the molasses as a source of energy for the fixation of atmospheric nitrogen.

(6) The addition of straw to the dextrose or molasses medium did not cause any appreciable increase in the quantity of nitrogen fixed.

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STUDIES ON THE TEMPERATURE OF INDIVIDUAL INSECTS, WITH SPECIAL REFERENCE TO THE HONEY BEE [1]

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Minnesota Agricultural Experiment Station

INTRODUCTION

More observations have been made on the temperature of the bee colony than on the individual bee. Phillips and Demuth(*16*)[3] concluded that bees, essentially cold-blooded animals, were capable of regulating their temperature when in a colony by increasing the temperature of the colony when the air temperature went down and lowering the temperature of the colony when the air temperature went up. Since Phillips and Demuth have shown that the colony, which is composed of a large number of individuals, acts very much as a warm-blooded animal does, the temperature of the individual must be of great importance. If this were not true, it would be difficult to understand how the colony of individuals could control its temperatures. For this reason and because of the wide variation in the results obtained by previous workers this problem was chosen for study.

In a review of the literature on this subject it was found that previous workers had based the results of their observations on a few individuals. The results included in this paper are based on the readings of over 1,000 bees. The methods and results of the previous workers are given in the following review of the literature.

REVIEW OF LITERATURE

Hausmann (*10*) was the first to establish the temperature of an individual insect. He placed a Sphinx convolvuli together with a small thermometer in a glass receptacle, the air temperature of which was 17° R. (21.25° C.). After half an hour the temperature had risen to 19° R. (23.75° C.) and soon after fell to 17° R. Experiments with *Carabus hortensis* gave the same results.

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[2] The work on which this paper is based was done in the laboratories of the Division of Entomology of the University of Minnesota. To the members of the staff, and especially to Dr. R. N. Chapman, under whose immediate direction the study was conducted, acknowledgments are due for apparatus furnished and for constant helpful suggestions.

[3] Reference is made by number (italic) to "Literature cited," p. 286–287.

Davy (*4*) was the first to give the internal temperature of an insect. An incision was made in the body and the bulb of a very small mercurial thermometer was inserted. He obtained the following results:

	Air temperature.	Insect temperature.
	° C.	° C.
Blatta orientalis	28.3	23.9
Do	23.3	23.9
Gryllidae	16.7	22.5
Vespidae	23.9	24.4
Do	24.3	25.0
Lampyridae	22.8	23.0
Do	26.6	25.8

Nobili and Melloni (*15*) were the first to use electric methods in determining the temperature of insects. They used the electromotive force developed when the junction of wires of different metals of a common circuit are at different temperatures. They used bismuth and antimony wires. One of the thermocouples was in contact with the insect body; the other couple was free. They made a series of experiments on the temperature of larvae, pupae, and adult butterflies. Their conclusion was that the temperature of the insect was higher than that of the surrounding air.

Mussehl (*13*) ascertained that single bees became motionless at 5° R (6.25° C.) while they did not suffer from cold in the colony with the temperature of the hive at −1° R. (−1.25° C.).

Newport (*14*) used a thermometer of small caliber with a cylindrical bulb about one-half inch in length. The thermometer was placed beneath the insect and was as completely covered by the abdomen of the insect as possible. A second thermometer which had been carefully compared with the first was placed at the same level with and a short distance from the first one. The temperature of the insect was taken on the exterior and was always lower than that of the interior. He stated that the internal temperature was seldom if ever more than a degree and a half or at the most two degrees above the external temperature. The following are some of his results showing the difference between the air temperature and the external temperature of the insect:

	Temperature of air.	Body temperature.	Temperature difference.
	°F.	°F.	°F.
Bombus terrestris	66.9	73.4	6.5
Do	66.9	76.2	9.3
Do	66.9	73.4	6.5
Do	69.4	76.2	6.8
Do	68.0	77.5	9.5
Bombus lapidarius	68.0	71.5	3.5

At the time of taking the foregoing readings the insects were in an excited condition.

Dutrochet (7) used a thermocouple made by soldering an iron and copper wire together. He placed one couple in the body of the insect and the other couple in the body of a dead insect of the same species or in paper. He fastened several bees to a hive by means of a thread and took temperature readings while the bees were very active. One couple was placed 5 mm. deep in the body of the bee; the other couple was wrapped in paper to protect it from radiation. The air temperature was constant for four hours (19.2°C.). This experiment showed that the temperature of the insect was 0.18°C. lower than that of the surrounding air. The bees were next placed in a bell jar with the air dampened. They then had a temperature of 0.18°C. higher than the air temperature.

Experiments with *Bombus hortorum* were also performed. The thermocouple was not placed in the body but merely placed against it. The bee was then wrapped up in a piece of gauze which caused it to become very much excited. Under these conditions the temperature was 0.5°C. higher than that of the surrounding air. As the bee became quiet, its temperature was 0.03°C. lower than the air temperature. Dutrochet concluded that insects when active had a temperature higher than the surrounding air, when inactive a temperature that corresponded to that of the surrounding air.

Dzierzon (*8*) observed that bees became motionless at 5°R. (6.25°C.).

Dönhoff (*5*) placed 200 bees in a glass container the temperature of which was 22.5°C. In a short time the temperature had risen to 34.4°C. He also pressed an individual bee against a thermometer and found it to have a temperature of 15/16°C. higher than the air. He concluded that the difference in temperature between the bee and the surrounding air was greater when the air temperature was low and less when the air temperature was high.

Schönfeld (*17*) placed 100 bees in a water glass which was perfectly dry. A piece of old dried wax was attached to the cover of the glass in which there was a hole for the insertion of the thermometer. A small board was placed in the bottom of the glass on which the thermometer rested. The entire apparatus was placed on a cook stove. After 3 hours and 25 minutes the thermometer had risen from 10°R. (18.75°C.) to 31.5°R. (39.3°C.). All the bees, with the exception of 5, were found standing on the wax. One bee began to fan as the temperature reached 32.7°R. (40.8°C.) and at 36°R. (45.0°C.) 80 bees fell to the bottom of the glass and died. Sixteen bees were still living at 46°R. (57.5°C.), and when the cover was removed they flew out. The experiment was repeated and 2 bees withstood the temperature of 48.2°R. (60.2°C.) and flew away when the glass was opened.

Girard (*9*) carried on several experiments on insect temperature and found that the bumblebee had a lower temperature when in lack of honey. He concluded also that Hymenoptera had a higher temperature than the surrounding air but a significantly lower temperature than the Lepidoptera and Diptera.

Dönhoff (*6*) observed that bees soon died at −1.5°C. when placed in frozen ground.

Molin (*12*) found that bees became motionless at 5°R. (6.25°C.) and that at 7°R. (8.75°C.) they cleaned themselves and carried water. They left the hive and flew into the field at 12°R. (15°C.).

Koschewnikow (*11*) noted that bees kept at a temperature between 0° and 1°R. (0° to 1.25°C.) for 10½ hours lived. Other bees that were motionless for 30 hours at a temperature of −1°R. (−1.25°C.) also

survived. After the bees were kept at −2°R. (−2.5°C.) for 50 minutes they died.

In his work on maximum temperature he concluded that:

1. There was a range of 9° R. (11.25° C.) in the fatal temperature of bees.
2. Dryness and dampness had no effect on bees in a high temperature.
3. Workers and drones became very much excited when the temperature was above 30° R. (37.5° C.).
4. The highest minimum fatal temperature at which the workers died was 35° R. (43.75° C.), while the drones died at 30° R. (37.5° C.).
5. The highest temperature which the bees could withstand was 44° R. (55° C.).
6. The workers immediately upon hatching showed a sensitiveness to heat, none surviving more than 39° R. (48.75° C.).
7. Still younger bees with the chitin less hard but apparently well formed were in contrast to the ones mentioned above because they were more capable of withstanding a higher temperature. They died at 52° to 53° R. (65° C.). The explanation of this phenomenon appears to lie in the fact that their bodies contained and evaporated considerable moisture and that they did not move.
8. Upon bringing the bees immediatley into high temperature Koschewnikow observed that the period which passes between the time of inserting the bees and their death became shorter with increases in temperature.

In the following review of the experiment no statement was made as to whether or not the figures given were the actual internal temperatures of the insects.

Period of exposure.	Temperature to which bees were exposed.	Temperature of bees at death.
	°R.	°R.
2 minutes	44	45
2 minutes 10 seconds	45	46
2 minutes 5 seconds	46	46.75
2 minutes 15 seconds	47	48
1 minute 30 seconds	54	55
1 minute 10 seconds	55	55.5
1 minute 10 seconds	55.5	56
1 minute 10 seconds	56	56.25
45 seconds	57	57.5

Bachmetjew (1) performed several experiments on the temperature of insects, especially Lepidoptera. He used a thermocouple made by soldering steel and manganese wires together. Both wires were connected to a galvanometer, one directly and the second after it had passed through a commutator. The wires were insulated by passing them through small glass tubes. Butterflies were placed on the couple, and from many experiments Bachmetjew concluded that:

When the body temperature reached 39° C. the insect became very active and died at 46 to 47° C.

When the air temperature was lowered the body temperature of the insect was lowered to approximately −15° C.; there was then a rebound

in the body temperature from about −15° to about −1°. When the temperature of the body began to fall the insect died when the low point (−15° C.) was reached the second time.

Bachmetjew (*2*) made a general study of insect temperature in which he covered the work previously done on this subject.

Brunnich (*3*) made a study of the temperature of the bee body and the bee brood. He used a thermocouple made by soldering a copper and platinum wire together, forming the warm junction of the couple. To the free end of the platinum wire, which was only a few centimeters in length, he soldered another copper wire. This second union acted as the cold junction of the couple. He used the room temperature for his cold junction and made no allowance for changes in the temperature of the room. He also used a telescope reading galvanometer which he stated was not sensitive enough, since 15 seconds were required before the maximum reading was reached. The bees were greatly weakened by piercing with this rough thermocouple and soon died. He found that the body temperature of adult workers went as high as 39.6° C., while that of the drones went as high as 48.4°. The results, however, gave no indication of uniformity, because some individuals gave high temperatures while others, under the same conditions, gave low temperatures.

Since platinum is a good conductor of heat, there is great danger in having the warm and cold junction separated by a piece of wire only a few centimeters in length. In piercing the bee the temperature of its body or the handling of the wires, if held near the second couple, is likely to increase the temperature of that couple, thereby introducing an error in the readings. The increasing of the temperature of the second couple was undoubtedly the cause of the wide range in the body temperature cited by Brunnich in his paper.

METHODS

The piercing of the bees in the following experiment was done with a thermocouple (Pl. I, A), made by soldering No. 20 double cotton covered copper and constantin (a copper-nickel alloy) wire together. The wires were tapered to a fine point before soldering by inserting the ends in concentrated nitric acid and slowly withdrawing them. This process was repeated until the wires obtained the desired points. The tapered ends were then soldered together and the surplus solder filed off. A piece of cork was inserted between the two wires near their junction to strengthen the couple and aid in the handling of it.

The readings were made with the aid of a pyrovolter (Pl. I, C) and galvanometer (Pl. I, D), and the bee was pierced with a thermocouple of which the cold junction (Pl. I, B) was placed in a thermos bottle filled with ice and water to keep it at 0° C. A Northrup pyrovolter which has scales graduated into millivolts and degrees centigrade and a Leeds-Northrup outside galvanometer to aid in the setting of the zero point were used. By having one junction of the thermocouple in ice and water the resulting reading on the pyrovolter was the actual temperature of the bee in degrees centigrade.

One of the rooms in a cold-storage plant was used for the low temperature of 2.5°, 5.5°, 8°, and 9° C. For "room temperature," readings were made in the laboratory. Temperature readings for 27°, 30.5°, 35°, 39.5°, 43.5°, 52°, and 58° C. were made in a temperature box in

which the heat was regulated by a series of lights. The temperature box was made by placing insulite on the outside of the box 34 by 35 by 52 inches, the inside of which was lined with asbestos paper. There are two ventilating holes, one in the side near the bottom, the other in the top of the box.

At all temperatures the bees were allowed at least 10 minutes to become adapted to the surrounding air before the first of the group was pierced. When readings were made at 52° and 58° C. it was found that bees exposed to this high temperature for about 30 minutes were dead. The

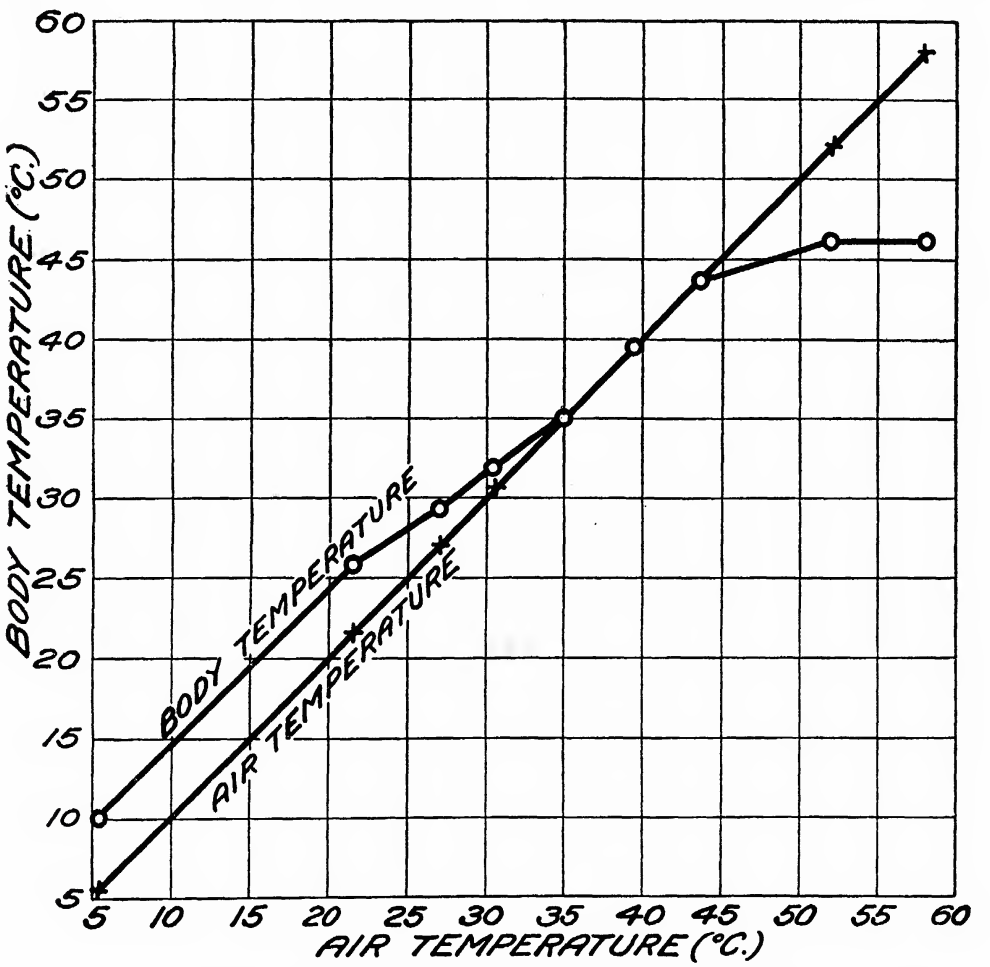

FIG. I.—Graph showing the relation of body temperatures of Italian bees with the surrounding air. The bees which were taken from one hive were under winter conditions.

bees were placed in the temperature box at this high temperature in groups varying from 5 to 8 bees per group and exposed to the temperature for 10 minutes before any were pierced. The groups were placed in the box while its temperature was about that of the room. The lights were then turned on, and after the temperature had reached 52° or 58° and remained constant for 10 minutes the temperature readings were made.

The data for the temperature curve (fig. 1) were obtained from Italian bees that were all taken from one hive and in winter condition. On November 25 this hive along with about 90 others was placed in a

room in a cold-storage plant which maintained a temperature of about 7° to 7.5° C. throughout the experiment. A control was made by taking bees from different hives and placing them in groups, no two bees from the same hive in a group. A different group was used for each of the points on the curve.

Because of the popular belief that the Carniolian bees can withstand a lower temperature than the Italian bees it was thought advisable to make temperature readings on the Carniolians. Readings were taken at the temperatures of 2.5°, 5.5°, 8°, 9°, 30°, 35°, and 52° C. A group of 10 Carniolians was placed in rooms having temperatures of 2.5°, 5.5°, and 9° C. along with a group of 10 Italian bees. In piercing the bees an Italian was pierced first and then a Carniolian until all the bees were pierced. At 8°, 30°, 35°, and 52° the Carniolians were handled in the same manner as described for the Italians.

When taken from the hive the bees were placed in individual cages which allowed each bee the space of ¼ by ¼ by ⅝ inch. The cage was made from a block of wood ¾ by ¾ by 2 inches with a ¼ by ¼ inch groove in one surface. Cotton thread was used to wind around the block to prevent the escape of the bee. The thread also aided in piercing, as it permitted full view of the bee and eliminated the possibility of cross currents which might occur when using a wire screen to cover the groove. All piercing was done in the thorax, since there are no large air sacs in this region. It did not make any difference whether the dorsal or ventral surface was pierced, as is shown in Table I. By using a fine thermocouple there was no noticeable bad effect on the bee such as Brunnich (*3*) described in his paper. Several bees which were pierced, placed in a cage and fed, lived as long as bees not pierced and kept under the same conditions. Upon piercing these bees the second time, the same differences between the body temperature and that of the air were obtained as at the first piercing.

All thermocouples used in the experiment were carefully compared with the mercurial thermometer used by taking the readings of the temperature of the air before any of the bees were pierced. The following is an example of the results obtained in such comparison:

Air temperature (mercurial thermometer) 24° C.

Air temperature (thermocouple) 23.5°, 24°, 24°, 24°, 24°, 24°, 23.5°, 24°, 24°, 24° C.

TABLE 1.—*Body temperatures of bees at air temperature 5.5° C.*

Bee No.	Body temperature.	Surface pierced.	Condition of bee.	Bee No.	Body temperature.	Surface pierced.	Condition of bee.
	°C.				°C.		
1	11	Ventral	Active.	51	10	Ventral	Inactive.
2	11	Dorsal	Do.	52	9	do	Do.
3	12	do	Do.	53	9	Dorsal	Do.
4	11	do	Do.	54	9	do	Do.
5	10.5	do	Do.	55	9.5	do	Do.
6	11	do	Inactive.	56	10	do	Do.
7	10	Ventral	Do.	57	10.5	do	Do.
8	12	Dorsal	Do.	58	10	do	Do.
9	11	Ventral	Do.	59	10.5	do	Do.
10	11	do	Do.	60	10	do	Do.
11	11.5	Dorsal	Do.	61	10.5	do	Active.
12	14	do	Do.	62	13.5	do	Do.
13	12	Ventral	Do.	63	10.5	do	Do.
14	11	Dorsal	Do.	64	10	Ventral	Do.
15	11	Ventral	Do.	65	9	Dorsal	Do.
16	10.5	do	Active.	66	9.5	Ventral	Do.
17	11	Dorsal	Do.	67	9.5	do	Inactive.
18	11	do	Do.	68	10	do	Do.
19	10.5	do	Do.	69	9	Dorsal	Do.
20	10.5	Ventral	Do.	70	9.5	Ventral	Active.
21	10	Dorsal	Do.	71	13.5	do	Do.
22	9	do	Inactive.	72	11	do	Inactive.
23	9.5	do	Do.	73	10	do	Do.
24	9.5	Ventral	Do.	74	10	Dorsal	Do.
25	10	Dorsal	Do.	75	9	do	Do.
26	10	Ventral	Do.	76	10	do	Active.
27	10	Dorsal	Do.	77	10	do	Do.
28	10	Ventral	Do.	78	9	do	Do.
29	11	Dorsal	Do.	79	10	do	Do.
30	9.5	do	Do.	80	10	do	Do.
31	8.5	do	Active.	81	10	Ventral	Do.
32	9	Ventral	Do.	82	9.5	do	Inactive.
33	9	do	Do.	83	10.5	do	Do.
34	9.5	do	Do.	84	10.5	do	Do.
35	9.5	do	Do.	85	10	do	Do.
36	9	Dorsal	Do.	86	11.5	Dorsal	Do.
37	9	do	Do.	87	11	do	Do.
38	9	do	Inactive.	88	11	do	Do.
39	9.5	Ventral	Do.	89	10.5	Ventral	Do.
40	9	Dorsal	Do.	90	10.5	Dorsal	Do.
41	10	Ventral	Do.	91	9.5	do	Active.
42	9	do	Do.	92	10	Ventral	Do.
43	9.5	Dorsal	Do.	93	9	Dorsal	Do.
44	9.5	do	Do.	94	9.5	do	Do.
45	9.5	Ventral	Do.	95	9	do	Do.
46	10	do	Active.	96	9.5	Ventral	Do.
47	10.5	do	Do.	97	9.5	Dorsal	Inactive.
48	9.5	do	Do.	98	9	do	Do.
49	9	do	Do.	99	9.5	do	Do.
50	10.5	do	Do.	100	9.5	Ventral	Do.

Average body temperature 10.2° C. Difference between average body temperature and air temperature 4.7 °C.

TEMPERATURE OF THE ITALIAN WORKERS

A striking result noted in the tables was the small range in the differences between the maximum and minimum temperature readings at the different temperatures, as is shown in Table II. This is especially noticeable at the higher temperatures. In Table I there is a range of 5.5° C. between the maximum and minimum temperatures in the readings of 100 bees. We find in Table II a variation in the differences from 9° at 21.4° to 1.5° at 27° and 43.5° with the difference of 2.5° at the temperatures of 30.5°, 35°, 52°, and 58°.

TABLE II.—*Body temperatures of bees at various air temperatures*

Air temperature.	Average body temperature.	Difference between body and air temperature.	Maximum body temperature.	Minimum body temperature.	Number of bees pierced.
5.5	10.2	4.7	14	8.5	100
21.4	25.8	4.4	31	22.0	100
27.0	29.1	2.1	30	28.5	54
30.5	32.0	1.5	34	31.5	100
35.0	35.1	.1	37	34.5	100
39.5	39.5	.0	42	38.0	100
43.5	43.6	.1	44	42.5	100
52.0	46.0	−6.0	48	45.5	100
58.0	46.4	−11.6	48	45.5	11

That bees are capable of regulating their temperature for a short period of time was shown by the fact that when bees were placed in the temperature box at 52° and 58° C. their body temperature did not at once correspond to that of the air, as it did from 35° to 44°, but was lower. After the bees were exposed to this temperature for about 25 minutes, their body temperature approached nearer that of the air temperature. After they were in the temperature box for 30 minutes, they were dead and had the same temperature as that of the air. A few bees were tried at a low temperature −10° to +21°, and it was noted that the temperature of the body fell with that of the environment.

By handling the bees in individual cages they were not excited before piercing. The space allotted to each bee was large enough to permit it some movement, but not large enough for it to make use of its wings. Throughout the entire experiment only two bees, No. 62 and 71, in Table I, were observed to be fanning. They were immediately pierced and found to give a reading of 8° C. above that of the room, while those not fanning had a temperature about 4.6° above the air temperature. The bees were pierced at a low temperature and in the individual cages, so that the heat produced by the fanning was conserved, thereby raising their own temperature.

When bees were exposed to the temperature of 5.5° C., they were taken into the room in groups of 15. A period of three minutes elapsed before the piercing of each successive bee to permit the thermocouple to return to room temperature. After the piercing of about the sixth bee the remainder of them became motionless but were easily revived by holding them in the hand for a few minutes. After a group of 10 bees was kept in the room for a period of two hours they were pierced, and they gave an average temperature of 2° above the room temperature. After the bees were kept at this low temperature for 48 hours they were dead and recorded the same temperature as the surrounding air. The bees may have died from either starvation or from cold.

When readings were taken at "room temperature" the work was done in the laboratory and the temperature varied from 19.5° to 24° C., with an average of 21.5°. The bees were brought into the laboratory in groups of 10. At all other temperatures, with the exceptions of 52° and 58°, the bees were used in groups of 25.

As the air temperature was increased, the difference between the body temperature of the bee and the temperature of the air decreased until at

35°, or brood-rearing temperature, the two corresponded. As the air temperature went up from 35° to 44° the temperature of the bee and that of the surrounding air were the same, as shown in Table II.

When readings were taken at 52° and 58° C. the time factor entered, as the bees were at the point of their fatal temperature (about 46° to 48°). A bee was placed in the temperature box for 20 minutes before piercing. The thermocouple was placed in the thorax and kept there for seven minutes, readings being recorded every minute, during which time all movement ceased. Seven minutes later, when the couple was again inserted in the thorax, the body temperature of the bee was identical with that of the surrounding air. For the 100 bees used at 52° there was an average body temperature of 46°. If left at this temperature for 30 minutes the bees died, which indicated that the fatal temperature was around 46° to 48°.

About 250 bees were placed in a half pint (250-cc.) bottle. A thermocouple inserted near the top of the bottle gave a reading of 32° C. The temperature of the air in the room was at 25°. When the thermocouple was inserted farther into the bottle, so that the bees clustered on the wires, the thermocouple registered 34°. Later, when the bees were quiet, the couple was reinserted and the temperature was at 29°. The bees were agitated and the temperature went up to 32°. Temperature readings were taken of individual bees, while the cluster was at 32°, and they gave an average temperature reading of 34°.

Ten of these bees placed in a 10-cc. vial gave a temperature reading of 32° C. When the bees were quiet, they had a temperature of 29°. Agitating the bees caused their temperature to rise slowly until it reached 32° and remained there as long as the bees were active. This demonstrated that the bees can effect the temperature of their environment. If the heat radiated by the bee can be conserved, the temperature of the environment will go up. This in turn will permit the bee to raise its own temperature correspondingly, as there is a constant relationship between the body temperature of the bee and that of the environment it is in.

As is shown by the temperature curve (fig. 1) the supply of oxygen was a large factor in the amount of radiation that was set up by the bees. If the oxygen is limited, as may be done by corking the bees up tight in a bottle, the bees become inactive and the temperature goes down. If, on the other hand, the cork is removed and fresh oxygen is admitted, the bees give an immediate response.

TEMPERATURE OF ITALIAN DRONES

A few temperature readings were made on the drones which were taken from the hive early in the morning before they had left the hive to fly. At room temperature, 23° C., the body temperature of the drones corresponded to that of the workers, while at 52° the drones, average temperature was 42.4° compared with 46° for the workers.

TEMPERATURE OF CARNIOLIAN WORKERS

In work on the Carniolians more emphasis was placed on the lower temperature than on the higher ones. In Table III we find that there is no difference between the Carniolian and Italian bees for the temperatures of 2.5°, 5.5°, and 9° C. A comparison of Tables II and III for the temperatures of 30°, 35°, and 52° gives the same results.

TABLE III.—*Comparison of body temperatures of Carniolian and Italian bees at various air temperatures*

Number of bees pierced.		Air temperature.	Average body temperature.		Difference between body and air temperatures.		Maximum body temperature.		Minimum body temperature.	
Carniolians.	Italians.		Carniolians.	Italians.	Carniolians.	Italians.	Carniolians.	Italians.	Carniolians.	Italians.
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
10	10	2.5	4.9	5.1	2.4	2.6	7	6	4	4.5
8	8	5.5	8.5	8.5	3.0	3.0	9	9	8	8.0
.....	.....	8.0	13.2	.....	5.2	.....	16	.....	11.5	25.0
10	10	9.0	13.8	13.6	4.8	4.6	16.5	16.0	12.5	12.5
25	.....	30.0	32.1	.....	2.1	.....	33.0	.....	31.5	.....
25	.....	35.0	35.0	.....	0.0	.....	35.0	.....	34.5	.....
25	.....	52.0	46.3	.....	−5.7	.....	48.0	.....	45.5	.....

FREEZING POINT

A study of the freezing point and the phenomenon of supercooling of the bee was made. An ether bath and a potentiometer were used. The ether bath was more easily controlled than an ice bath, and the temperature dropped about 0.5° C. per minute. Care was taken not to move the bee or have it come in contact with anything, as the least amount of movement will bring about the freezing of the insect. If the insect is moved or shaken about the time the rebound from supercooling to the actual freezing point is to take place, the bee will freeze and the rebound is not evident. It was also found that if the temperature of the bee when nearing its freezing point went down slowly, the freezing would take place and there would be no supercooling with a rebound to the freezing point. However, if the temperature went down rapidly as it neared the freezing point, the bee was supercooled and the rebound occurred. Three individual bees were taken down to −2.3°, when there was a rebound to −0.8°, or the actual freezing point. Another bee was supercooled to −4.3°, when the rebound occurred, the temperature going up to −2°., the actual freezing point. This conforms with the results obtained by Bachmetjew. The variation in the results is probably due to the fact that individuals do not have the same freezing point.

SUMMARY

The large variation in the results obtained by the early workers was due to the differences in methods of performing their experiments. Many of the old workers used mercurial thermometers, or thermocouples, which were then just coming into use. Nobili and Melloni (*15*) were the first to ever use this method. Because of the wide variation and unrefinement of the methods used by the early workers and the results they obtained their work remains only of historical value.

Bachmetjew (*2*) has contributed considerably to the general work on insect temperature by bringing together and giving a summary of the older works. In addition to this, he has performed many original experiments on the temperature of individual insects.

Brunnich (*3*) is the most recent worker on the temperature of bees. The lack of uniformity in his results is undoubtedly due to the fact that

he used the room temperature for the cold junction of his thermocouple. This cold junction was not controlled at a constant temperature, but was subject to fluctuations due to changes in the temperature of the room and also to the heat conducted along the short platinum wire from the bee which was pierced and from the hand with which the thermocouple was held while piercing the bees and taking the readings. Thus fluctuations in readings which Brunnich attributed to fluctuations of the temperature of the bee were more probably due to the fluctuations in the temperature of the cold junctions.

The author obtained very uniform results by using refined thermocouple methods and by having the cold junction in ice and water, keeping it constantly at 0° C.

CONCLUSIONS

From the foregoing results the following conclusions were made:

The average body temperature of the bee is 4.7° C. above that of the surrounding air when the air temperature is 5.5° and coincides with the air temperature when that is between 35° and 44°.

At 52° C. or above the temperature of the bee's body is lower than that of the air if not exposed to the high temperature for a long period of time.

The maximum fatal temperature of bees is about 46° to 48° C., and the freezing point is about −1°.

There is no appreciable difference between the body temperature of the Carniolian and Italian bees.

Bees are not wholly subject to the temperature of their environment, but are capable within certain limits of regulating their body temperature.

The ability of a colony to regulate its temperature is undoubtedly due to the ability of the individual to regulate its body temperature plus the ability to regulate and conserve the heat produced.

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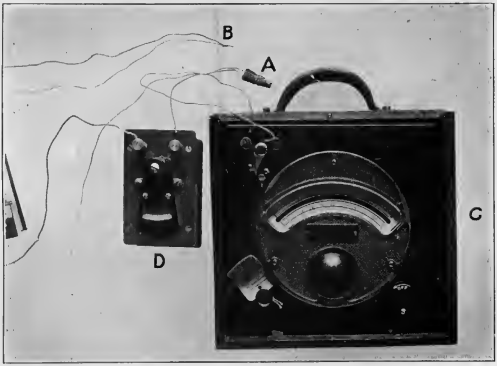
PLATE I

A.—Warm junction of the thermocouple.
B.—Cold junction of the thermocouple.
C.—Pyrovolter.
D.—Galvanometer.


B
A
C
D

A STUDY OF THE EFFECT OF CHANGING THE ABSOLUTE REACTION OF SOILS UPON THEIR AZOTOBACTER CONTENT [1]

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INTRODUCTION

In studying the apparent correlation between the absolute reaction of soils and the presence of Azotobacter it occurred to the writer that if such a correlation existed one ought to be able, by varying the reaction, to control the Azotobacter content of any soil. To test the correctness of this view three lines of investigation were suggested.

(1) If two soils, one with a hydrogen-ion concentration favorable to the growth of Azotobacter, the other unfavorable, are mixed in varying proportions the hydrogen-ion concentration of some mixtures should be favorable and others unfavorable to the growth or existence of this group of organisms. All mixtures giving a hydrogen-ion concentration less than the maximum tolerated by Azotobacter should, upon subsequent analyses, show their presence. On the other hand, all mixtures giving a hydrogen-ion concentration greater than the maximum should not show the presence of Azotobacter, provided a sufficient incubation period elapsed to bring about their destruction.

(2) If the absence of Azotobacter in any soil is due to its high hydrogen-ion concentration, one should be able to decrease the acidity by the addition of increasing quantities of nontoxic basic materials, to a point where, once introduced, Azotobacter would survive.

(3) If a high hydrogen-ion concentration is inimical to the presence of Azotobacter one should be able, by the addition of acid, to increase the acidity of any soil containing Azotobacter to a point beyond the maximum tolerated by these organisms and thereby cause their disappearance.

The purpose of this paper is to report a few typical examples of a large number of experiments that have been carried out along these lines.

METHODS

The methods employed in the experiments here reported were similar to those previously reported.[2] Tests for Azotobacter were made by inoculating a mannite cultural solution with 10 per cent of soil, or the equivalent, as a suspension. Incubation was at room temperature. At frequent intervals during incubation the cultures were examined macroscopically to ascertain whether or not a film characteristic of Azotobacter was present. If the film was not typical and there were any indications of Azotobacter being present a microscopic examination was made. If the evidence thus obtained showed the presence of Azotobacter, it has been indicated by a plus sign in the following tables. A minus sign

[1] Accepted for publication June 29, 1922. Contribution No. 48, Department of Bacteriology, Kansas Agricultural Experiment Station.

[2] GAINEY, P. L. SOIL REACTION AND THE GROWTH OF AZOTOBACTER. *In* Jour. Agr. Research, v. 14, p. 265–271. 1918.

signifies the absence of Azotobacter. Where a question mark has been inserted it was impossible to tell whether Azotobacter were present.

The hydrogen-ion concentration determinations were made either colorimetrically after Gillespies method,[3] or electrometrically upon suspension of the soil. For the latter purpose a Leeds and Northrup potentiometer outfit was used in connection with saturated K Cl—calomel and hydrogen electrodes similar to that described by Hildebrand.[4] In general the two methods agreed very closely.

The mixtures of soils, or soils with various additions, were placed in 500-cc. wide-mouthed bottles plugged with cotton, and the moisture content was brought to a favorable point. Incubation was at room temperature. At frequent intervals the moisture lost through evaporation was restored. A more detailed description of the methods employed will be presented when the data are published in full.

RESULTS

MIXING SOILS

In the two experiments reported in Tables I and II, two soils (A and E), containing a vigorous Azotobacter flora, and one soil (B), in which Azotobacter have never been found, were studied. Soil A is approximately neutral, P_H 6.94. Soil E contains a high percentage of limestone and is alkaline, P_H 7.73. Soil B is strongly acid, P_H 3.65. Soils A and B would, under normal conditions, be expected to have approximately the same reaction, since the two are from similar locations and were taken only a few yards apart. However, the soil where B was taken was planted to pine trees a number of years ago and the high acidity is undoubtedly due to the decomposition of the highly acid pine needles. Extensive use has been made of this soil because it is the only strongly acid soil yet located in this immediate vicinity.

Previously reported experiments,[5] indicated that the maximum hydrogen-ion concentration tolerated by Azotobacter in soils is near 1×10^{-6}, or P_H 6.0. If P_H 6.0 represents the maximum acidity tolerated by this group of organisms, then all mixtures of soils A and B or E and B less acid than P_H 6.0 should, upon subsequent analyses, show Azotobacter, while all mixtures in which the acidity was very much greater than P_H 6.0 should not show their presence. It is probable that Azotobacter can exist for some time in a hydrogen-ion concentration that would not permit growth. This being true, some of the less acid samples in which Azotobacter can not grow might be able to initiate the growth of Azotobacter in a cultural solution. It would be expected that the higher the hydrogen-ion concentration the more rapidly the Azotobacter would be destroyed. From the relative reactions of the three soils it would also be expected that the quantity of soil A necessary to add to soil B in order to reduce the acidity of the mixture to P_H 6.0 would be larger than would be required in soil E. A glance at the second, third, and fourth columns of Tables I and II will show that in mixtures of soils A and B the ratio of A to B required to give an acidity less than P_H 6.0 lies somewhat between 9 to 1 and 4 to 1. In mixtures of soils E and B the ratio of E to B required to give the same reaction is 1 to 3.

[3] GILLESPIE, L. J. THE REACTION OF SOIL AND MEASUREMENTS OF HYDROGEN-ION CONCENTRATION. *In* Jour. Wash. Acad. Sci., v. 6, p. 7–16, 2 fig. 1916.

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[5] GAINEY, P. L. OP. CIT.

TABLE I.—*Effect of mixing an alkaline and an acid soil upon presence of Azotobacter. Mixtures of soils A and B* [a]

Sample No.	Grams of soil A.	Grams of soil B.	PH.	Azotobacter cultures					
				No $CaCO_3$ added to soil.			$CaCO_3$ added to soil.		
				Jan. 30.	May 22.	Dec. 11.	Jan. 30.	May 22.	Dec. 11.
1	300	0	7.02	+	+	+	+	+	+
2	300	0	6.86	+	+	+	+	+	+
3	270	30	6.42	+	+	+	+	+	+
4	270	30	6.32	+	+	+	+	+	+
5	240	60	5.76	+	+	+	+	+	+
6	240	60	5.82	+	+	+	+	+	+
7	150	150	4.60	+	+	−	+	+	+
8	150	150	4.61	+	−	−	+	+	+
9	60	240	4.11	+	−	−	+	+	+
10	60	240	4.09	+	−	−	+	+	+
11	30	270	3.79	+	−	−	+	+	+
12	30	270	3.90	+	−	−	+	+	+
13	15	285	3.77	+	−	−	+	+	+
14	15	285	3.75	+	−	−	+	+	+
15	3	297	3.75	+	−	−	+	+	+
16	3	297	3.68	+	−	−	+	+	+
17	1	299	3.66	+	−	−	+	+	+
18	1	299	3.65	+	−	−	+	+	+
19	0	300	3.68	−	−	−	−	−	−
20	0	300	3.62	−	−	−	−	−	−

[a] +=Presence of Azotobacter
−=Absence of Azotobacter
Experiment set up Jan. 30. Moisture content optimum. Acidity determined electrometrically.

TABLE II.—*Effect of mixing an alkaline and an acid soil upon presence of Azotobacter. Mixtures of soils E and B* [a]

Sample No.	Grams of soil E.	Grams of soil B.	PH.	Azotobacter cultures		
				May 1.	May 18.	June 21.
1	200	0	7.73	+	+	+
2	199	1	7.64	+	+	+
3	195	5	7.67	+	+	+
4	190	10	7.67	+	+	+
5	175	25	7.56	+	+	+
6	150	50	7.61	+	+	+
7	125	75	7.52	+	+	+
8	100	100	7.37	+	+	+
9	75	125	7.06	+	+	+
10	50	150	6.02	+	+	+
11	25	175	4.87	+	+	−
12	10	190	3.99	+	?	−
13	5	195	3.94	+	+	−
14	1	199	3.74	−	−	−
15	0	200	3.64	−	−	−

[a] ?=Azotobacter indefinite.
+=Presence of Azotobacter.
−=Absence of Azotobacter.
Experiment set up May 1, Moisture content optimum. Acidity determined electrometrically.

A further examination of the two tables will show that, when cultured immediately after mixing, all mixtures of A and B and E and B, except 1 part of E to 199 parts of B, contained Azotobacter. Subsequent cultures, made after varying periods of incubation, show a gradual disappearance of Azotobacter in the more acid mixtures until only those samples contain them in which the acidity approaches very close to or is less than P_H 6.0. Numerous experiments have shown that the more acid a soil is the quicker will Azotobacter disappear therefrom when introduced, and that they can exist for some time in soils only slightly more acid than P_H 6.0. All the evidence accumulated so far, however, indicates that they can not remain indefinitely in a soil very much more acid than P_H 6.0.

ADDITION OF CALCIUM CARBONATE

In these experiments the aim was to find out whether lowering the hydrogen-ion concentration of a soil more acid than P_H 6.0, and not containing Azotobacter, would render the soil capable of supporting such a flora when introduced. The method followed was to add increasing quantities of various basic materials, particularly calcium carbonate, to acid soils containing no Azotobacter, inoculate the soil with Azotobacter, and after varying incubation periods culture to see whether or not the introduced organisms survived.

TABLE III.—*Effect of $CaCO_3$ upon the longevity of Azotobacter in an acid soil (B)* [a]

Sample No.	Percentage of $CaCO_3$.	Pounds $CaCO_3$ per acre.[b]	Azotobacter cultures.			
			Sept. 1.	Oct. 21	Jan. 4.	Feb. 18.
1	0.0	0	+	+	−	−
2	.1	3,000	+	+	−	−
3	.2	6,000	+	+	−	−
4	.3	9,000	+	+	−	−
5	.4	12,000	+	+	−	−
6	.5	15,000	+	+	−	−
7	.6	18,000	+	+	+	+
8	.7	21,000	+	+	+	+
9	.8	24,000	+	+	+	+
10	.9	27,000	+	+	+	+
11	1.0	30,000	+	+	+	+
12	1.1	33,000	+	+	+	+
13	1.2	36,000	+	+	+	+
14	1.3	39,000	+	+	+	+
15	1.4	42,000	+	+	+	+
16	1.5	45,000	+	+	+	+

[a] +=Presence of Azotobacter. −=Absence of Azotobacter. Experiment set up Aug. 20. Moisture content optimum.
[b] 3,000,000 pounds soil.

In Table III it will be observed that a minimum of 18,000 pounds per acre (3,000,000 pounds), or 0.6 per cent calcium carbonate, were required to render soil B capable of supporting an Azotobacter flora. Unfortunately the hydrogen-ion concentration of the samples of soil of these experiments was not determined.

TABLE IV.—*Effect of basic compounds upon longevity of Azotobacter in an acid soil (B)*[a]

Sample No.	Basic compounds added.	Pounds per acre.[b]	P_H.	Azotobacter cultures.		
				Feb. 9.	Mar. 18.	May 7.
1	0.....	.....	4.3	−	−	−
2	0.....	.....	4.3	−	−	−
3	0.01 per cent $CaCO_3$.....	300	4.3	−	−	−
4	.05 per cent $CaCO_3$.....	1,500	4.4	−	−	−
5	.10 per cent $CaCO_3$.....	3,000	4.7	−	−	−
6	.50 per cent $CaCO_3$.....	15,000	5.3	+	−	−
7	1.00 per cent $CaCO_3$.....	30,000	6.5	+	+	+
8	2.50 per cent $CaCO_3$.....	75,000	7.0	+	+	+
9	1.01 per cent Na_2CO_3.....	300	4.3	−	−	−
10	.05 per cent Na_2CO_3.....	1,500	4.8	−	−	−
11	.10 per cent Na_2CO_3.....	3,000	5.2	−	−	−
12	.50 per cent Na_2CO_3.....	15,000	5.8	−	−	−
13	1.00 per cent Na_2CO_3.....	30,000	6.5	−	−	−
14	2.50 per cent Na_2CO_3.....	75,000	[c]8.6	−	−	−
15	.01 per cent $Mg.CO_3$.....	300	4.3	−	−	−
16	.05 per cent $Mg.CO_3$.....	1,500	4.3	−	−	−
17	.10 per cent $Mg.CO_3$.....	3,000	4.3	−	−	−
18	.50 per cent $Mg.CO_3$.....	15,000	5.4	+	−	−
19	1.00 per cent $Mg.CO_3$.....	30,000	6.6	+	+	+
20	2.50 per cent $Mg.CO_3$.....	75,000	7.8	+	−	−

[a] + = Presence of Azotobacter. − = Absence of Azotobacter. Experiment set up Jan. 21, Moisture content optimum.
[b] 3,000,000 pounds soil.
[c] Greater than 8.6.

The data reported in Table IV were secured from the same soil, but the variations in the quantity of basic material added were much greater. Here again 15,000 pounds of calcium, magnesium, or sodium carbonate failed to reduce the acidity to P_H 6.0 or to make conditions favorable for the existence of Azotobacter. The P_H where 15,000 pounds of calcium carbonate were added was 5.3. Where 30,000 pounds, the next largest quantity, were added the P_H was 6.5 and the sample contained Azotobacter at all subsequent analyses.

Soil G with a reaction very close to P_H 5.8 (colorimetrically) was used in the experiments reported in Table V. The quantity of calcium, sodium, or magnesium carbonate necessary to reduce the acidity to P_H 6.0, or less, was not as great as was required by soil B. Two months after incubation started all samples except No. 12 (Table V) contained Azotobacter. In this particular case the high concentration of magnesium carbonate had apparently destroyed all organisms of this group. At the next analyses, seven weeks later, Azotobacter had disappeared from all samples more acid than P_H 6.0 and still remained in all those with a less high hydrogen-ion concentration, except when apparently destroyed by the high concentration of magnesium and sodium carbonate.

When excessive quantities of sodium and magnesium carbonate were added they apparently became toxic to the Azotobacter. The quantity necessary to produce such a condition was greater in soil G than in soil B. Lipman [6] has reported a toxic effect upon Azotobacter from both sodium and magnesium carbonate.

[6] LIPMAN, Charles B., and SHARP, L. T. TOXIC EFFECTS OF "ALKALI SALTS" IN SOILS ON SOIL BACTERIA. III. NITROGEN FIXATION. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 35, p. 647–655, 1 fig. 1912.
LIPMAN, Charles B., and BURGESS, Paul S. THE PROTECTIVE ACTION, AGAINST $MgCO_3$ OF $CaCO_3$ FOR A. CHROOCOCCUM. *In* Jour. Agr. Sci., v. 6, p. 484–498. 1914. Bibliographical footnotes.

TABLE V.—*Effect of basic compounds upon the longevity of Azotobacter in an acid soil (J)*[a]

Sample No.	Basic compounds added.	Pounds per acre.[b]	P_H.	Azotobacter cultures.		
				Feb. 10.	Mar. 19.	May 8.
1	0.01 per cent $CaCO_3$	300	5.9	+	+	−
2	.05 per cent $CaCO_3$	1,500	6.0	+	+	−
3	.10 per cent $CaCO_3$	3,000	6.2	+	+	+
4	.50 per cent $CaCO_3$	15,000	7.4	+	+	+
5	1.00 per cent $CaCO_3$	30,000	7.4	+	+	+
6	2.50 per cent $CaCO_3$	75,000	7.4	+	+	+
7	.01 per cent Na_2CO_3	300	5.8	+	+	−
8	.05 per cent Na_2CO_3	1,500	6.0	+	+	+
9	.10 per cent Na_2CO_3	3,000	6.2	+	+	+
10	.50 per cent Na_2CO_3	15,000	7.6	+	+	+
11	1.00 per cent Na_2CO_3	30,000	[c]8.6	+	+	+
12	2.50 per cent Na_2CO_3	75,000	[c]8.6	+	−	−
13	.01 per cent $Mg.CO_3$	300	5.8	+	+	−
14	.05 per cent $Mg.CO_3$	1,500	6.0	+	+	+
15	.10 per cent $Mg.CO_3$	3,000	6.4	+	+	+
16	.50 per cent $Mg.CO_3$	15,000	7.6	+	+	+
17	1.00 per cent $Mg.CO_3$	30,000	8.0	+	+	+
18	2.50 per cent $Mg.CO_3$	75,000	8.6	+	+	−

[a] + = Presence of Azotobacter. − = Absence of Azotobacter. Experiment set up Jan. 21, Moisture content optimum. Acidity determined colorimetrically.
[b] 3,000,000 pounds soil.
[c] Greater than 8.6.

It is evident from the data presented in Tables III, IV, and V that the addition of calcium carbonate to an acid soil, not containing Azotobacter, if in sufficient quantity to reduce the hydrogen-ion concentration to approximately P_H 6.0 or lower, is all that is necessary to render the soil capable of supporting an Azotobacter flora. In no case, however, have Azotobacter been observed to appear in a soil so treated unless accompanied by inoculation either natural or artificial. In other words, Azotobacter are actually not present and must be introduced or the addition of calcium carbonate can have no effect upon nitrogen fixation by this group of organisms.

EFFECT OF ADDING ACID

In the experiments reported in Tables VI and VII, increasing quantities of various acids were added to soils A and C. Both these soils contained abundant Azotobacter. The reaction of soil A was very near P_H 7.0 and of soil C was near P_H 6.5.

Sulphuric, hydrochloric, acetic, and butyric acids were added to soil C reported in Table VI. Sulphuric and hydrochloric acid increased the hydrogen-ion concentration of the soil as the quantity of acid increased. As soon as the acidity passed much beyond P_H 6.0 Azotobacter were unable to survive prolonged incubation. When acetic or butyric acid was added it apparently had no effect upon the reaction, unless to reduce the hydrogen-ion concentration. This is contrary to the data presented in Table VII and is probably due to the time at which the hydrogen-ion concentration determinations were made. In Table VI the reaction was determined at the end of the period of incubation, while in Table VII the acidity determinations were made immediately

after being added or at the beginning of incubation. There are in soils organisms capable of utilizing many organic acids or their salts as food. This would cause the disappearance of the acid.

TABLE VI.—*Effect of adding acid to a soil containing Azotobacter (soil C)* [a]

Sample No.	Acid added to 200 gm. soil.	P_H.	Azotobacter cultures.	
			Mar. 2.	June 23.
1	0.2 cc. *N*/1 sulphuric	6.53	+	+
2	1.0 cc. *N*/1 sulphuric	6.73	+	+
3	5.0 cc. *N*/1 sulphuric	5.88	+	+
4	10.0 cc. *N*/1 sulphuric	5.29	+	(?)
5	20.0 cc. *N*/1 sulphuric	4.74	+	−
6	.2 cc. *N*/1 acetic	6.42	+	+
7	1.0 cc. *N*/1 acetic	6.61	+	+
8	5.0 cc. *N*/1 acetic	6.64	+	+
9	10.0 cc. *N*/1 acetic	6.74	+	(?)
10	20.0 cc. *N*/1 acetic	7.10	−	(?)
11	.2 cc. *N*/1 butyric	6.44	+	+
12	1.0 cc. *N*/1 butyric	6.58	+	+
13	5.0 cc. *N*/1 butyric	6.69	+	+
14	10.0 cc. *N*/1 butyric	6.70	−	+
15	20.0 cc. *N*/1 butyric	7.05	−	+
16	.2 cc. *N*/1 hydrochloric	6.41	+	+
17	1.0 cc. *N*/1 hydrochloric	6.51	+	+
18	5.0 cc. *N*/1 hydrochloric	5.58	+	−
19	10.0 cc. *N*/1 hydrochloric	5.51	+	−
20	20.0 cc. *N*/1 hydrochloric	4.51	+	−
21	0	6.50	+	+

[a] + = Presence of Azotobacter.
− = Absence of Azotobacter.
? = Azotobacter indefinite.
Experiment set up Feb. 16. Moisture content optimum.
Acidity determined electrometrically.

The increased growth of such organisms would bring about the accumulation of protein in their bodies which, upon decomposition, would result in the formation of more ammonia and hence a possible decrease in the hydrogen-ion concentration. The initial high acidity might result in either the partial or total destruction of the Azotobacter flora. If only a few organisms survived they might escape detection by the methods used. As soon, however, as the reaction again became favorable they would become abundant and be easily detected. This is probably what occurred in samples 14 and 15 of Table VI. On the other hand, if all the Azotobacter were killed they would never reappear unless inoculation took place, and subsequent analyses would fail to reveal their presence even though the reaction were favorable. The rapidity with which the Azotobacter are destroyed probably depends upon the degree of acidity, while the completeness with which they disappear from an acid soil depends upon the length of time the organisms are in contact with the acid condition. In the examples just given their complete destruction would depend upon the initial acidity and the period that elapsed before the hydrogen-ion concentration was again reduced below the maximum tolerated.

The data in Table VII show that with increasing quantities of organic as well as mineral acids the hydrogen-ion concentration increases. From

the reaction of samples 6 and 18 in Table VII Azotobacter would not be expected to be present. For reasons just discussed, it is probable that their presence in these samples is due to the fact that the initial hydrogen-ion concentration did not exist sufficiently long to destroy them.

TABLE VII.—*Effect of adding acid to soil containing Azotobacter (soil A)* [a]

Sample No.	Acid added to 200 gm. soil.	P_H.	Azotobacter cultures.	
			Aug. 22.	Oct. 10.
1	0	6.81	+	+
2	10 cc. 2*N* sulphuric	2.52	−	−
3	20 cc. 2*N* sulphuric	2.26	−	−
4	50 cc. 2*N* sulphuric	2.01	−	−
5	50 cc. 2*N* sulphuric+10 gm. $CaCO_3$	1.83	−	−
6	10 cc. 2*N* lactic	5.70	+	+
7	20 cc. 2*N* lactic	4.50	−	−
8	50 cc. 2*N* lactic	4.06	−	−
9	50 cc. 2*N* lactic+10 gm. $CaCO_3$	7.03	+	+
10	10 cc. 2*N* lactic	6.46	+	+
11	20 cc. 2*N* lactic	5.09	+	+
12	50 cc. 2*N* lactic	3.73	−	−
13	50 cc. 2*N* lactic+10 gm. $CaCO_3$	6.00	+	−
14	10 cc. 2*N* formic	6.20	+	+
15	20 cc. 2*N* formic	4.09	−	−
16	50 cc. 2*N* formic	3.43	−	−
17	50 cc. 2*N* formic+10 gm. $CaCO_3$	7.78	+	−
18	10 cc. 2*N* citric	4.60	+	+
19	20 cc. 2*N* citric	2.62	−	−
20	50 cc. 2*N* citric	2.10	−	−
21	50 cc. 2*N* citric+10 gm. $CaCO_3$	2.55	−	−

[a] +=Presence of Azotobacter.
−=Absence of Azotobacter.
Experiment set up Aug. 6. Moisture content optimum.
Acidity determined electrometrically.

The data presented in Table VII indicate that if sufficient calcium carbonate is added to neutralize the added acid the Azotobacter will not be affected. However, large quantities of calcium carbonate are ineffective unless the quantity is sufficient to maintain a favorable reaction.

CONCLUSIONS

(1) If two soils, one more acid than P_H 6.0 and containing no Azotobacter and the other less acid than P_H 6.0 and containing Azotobacter, are mixed in varying proportions, incubated for some time, and cultured for Azotobacter, all mixtures giving an acidity less than P_H 6.0 will show the presence of Azotobacter, while all cultures very much more acid than P_H 6.0 will fail to show Azotobacter.

(2) If sufficient calcium carbonate is added to a soil more acid than P_H 6.0 and not containing Azotobacter to reduce the hydrogen-ion concentration to less than P_H 6.0, the soil will support Azotobacter.

(3) If sufficient acid is added to a soil less acid than P_H 6.0 and containing Azotobacter to increase the acidity to a point very much greater than P_H 6.0, Azotobacter will disappear from the soil, provided this acidity exists for sufficient time to complete their destruction.

OXIDATION OF SULPHUR BY MICROORGANISMS IN BLACK ALKALI SOILS [1]

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The accumulation of sodium carbonate in the soil (black alkali soil) brings about a condition in which the soil practically has to be abandoned as far as utilization for the growth of plants is concerned. Irrigation, whereby the carbonates are washed out from the soil, brings about a temporary and unsatisfactory relief. If the sodium carbonate could be converted into sodium sulphate (white alkali), much more satisfactory results could be ontained, since, as pointed out recently by Vinson and associates (*13*),[2] white alkali is readily and completely leached out from soil, while black alkali resists leaching. The use of sulphuric acid on alkali soil has been suggested by C. B. Lipman and Sharp (*6*); this acid was found to exert a favorable influence upon the soil by neutralizing the carbonate and improving the physical condition of the soil through flocculating the colloids. However, the sudden introduction of large quantities of acid into the soil may have an injurious influence upon the soil microflora. Elementary sulphur would prove, in this respect, of greater benefit, since, not only would the injury to the soil microflora be less but the addition of small quantities of sulphur even may stimulate bacterial activities.

J. G. Lipman (*7*) was the first to suggest the use of sulphur for alkali soil, in order that the acid formed from the oxidation of sulphur may reduce the alkalinity of the soil and transform the sodium carbonate into sodium sulphate. O'Gara (*9*), when applying sulphur and sulphuric acid to soils, observed that there was a reduction of the carbonate and an increase in the sulphate content of the soil and a decided increase in the crop yield. Hibbard (*3*) demonstrated that by adding sulphur to alkali soil the alkalinity is neutralized, this effect being of great value in the reclamation of alkali land. Rudolfs (*10*) found on adding elemental sulphur, at the rate of 1,000 pounds per acre, that the reaction of the soil was reduced from P_H between 9.6 and 9.8 to P_H 9.3; with 2,000 pounds per acre, to P_H 9.2; with 3,000, to P_H 8.9; and with 3,500, to P_H 8.2. However, if black alkali soil is first leached, the use of 3,000 pounds of sulphur per acre will change the reaction of the soil from P_H 9.2 to P_H 7.7.

Although some of the sulphur may be oxidized in the soil without the intervention of life, as pointed out by Kappen and Quensell (*5*) and others, it is primarily as a result of activities of certain microorganisms that the rapid oxidation of the elemental sulphur takes place in both

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[2] Reference is made by number (italic) to "Literature cited," p. 305.

acid and alkaline soils. Two organisms have been isolated, which are responsible for this process. These two organisms are different in nature, in both their morphological and physiological characters: (1) *Thiobacillus thiooxidans*, isolated at this station from sulphur-soil composts and described in detail elsewhere (*15*), was found to be very active in oxidizing sulphur practically quantitatively in acid soils; and (2) *Thiobacillus B* was isolated from alkaline soils on which the sulphur had been actively oxidized. The organism resembles in general morphological and physiological characteristics *T. thioparus*, studied by Nathanson (*8*) Beijerinck (*1*), and others, and occurs commonly in the soil. It grows readily in alkaline media having a reaction equivalent to P_H 9.8 and oxidizes readily thiosulphate with an abundant formation of elementary sulphur. A detailed description of methods for the study of this organism and its relation to the oxidation of sulphur in the soil are published elsewhere (*14*). No definite proof can, however, be given that *T. B* is absolutely pure and not contaminated with *T. thiooxidans* or a related strain.

In the experiment reported below, a black alkali soil from the University of California Ranch near Fresno, Calif., was used. Dr. Hoagland, of the University of California, who kindly supplied the soil, stated that "the physical condition of the soil was bad, the soil was extremely alkaline, and no ordinary growth of plants is supported." The reaction of the soil was about P_H 9.6 to 9.8.

The soil was placed in 100-gm. portions in tumblers and the proper amount of sulphur was added and thoroughly mixed with the soil. Fifty mgm. of sulphur in 100 gm. of soil is equivalent to 1,000 pounds of sulphur per 1 acre of soil (2,000,000 pounds per acre basis). The proper quantity of water was added and the tumblers were incubated at 25° to 28° C. in the dark.

The reaction of the soil was determined by shaking thoroughly 5 gm. of soil with 10 cc. of water, centrifuging, then determining the hydrogen-ion concentration (P_H value) colorimetrically and in some cases electrometrically. The sulphates were determined by shaking the soil with distilled water (*2*) in a shaking machine for 2 to 6 hours, then filtering clear and precipitating as barium sulphate. The carbonates and bicarbonates were determined by the method outlined by Schreiner and Failyer (*11*).

Tables I and II show the course of reaction resulting from the oxidation of sulphur in alkali soil and the chemical transformations that have taken place as a result of these changes.

TABLE I.—*Course of sulphur oxidation in alkali soil*

Period of incubation.	No sulphur added.	50 mgm. sulphur per 100 gm. soil.	100 mgm. sulphur per 100 gm. soil.	200 mgm. sulphur per 100 gm. soil.	500 mgm. sulphur per 100 gm. soil.	1,000 mgm. sulphur per 100 gm. soil.
	P_H	P_H	P_H	P_H	P_H	P_H
20 days	9.6	9.6	9.1	9.0	8.9	8.7
42 days	9.6	9.4	9.0	8.5	7.1	6.7
62 days	9.6	9.4	8.5	7.8	7.6	7.0
86 days	9.6	9.3	8.6	8.0	7.2	6.8
105 days	9.6	9.1	7.9	7.2	6.0	4.4
167 days	9.6	7.9	7.4	6.7	5.7	3.5

TABLE II.—*Transformation of a black alkali soil into a white alkali* [1]

	No sulphur added.		50 mgm. sulphur per 100 gm. soil.		100 mgm. sulphur per 100 gm. soil.		200 mgm. sulphur per 100 gm. soil.		500 mgm. sulphur per 100 gm. soil.		1,000 mgm. sulphur per 100 gm. soil.	
	Individual tumblers.	Average.	Individual tumblers.	Average.	Individual tumblers.	Average.	Individual tumblers.	Average.	Individual tumblers.	Average.	Individual tumblers.	Average.
P_H	9.6	9.6	7.8 8.0 7.9	7.9	7.6 7.1 7.4	7.4	6.4 7.0 6.6	6.7	5.5 5.5 6.2	5.7	2.8 3.0 4.6	3.5
Sulphates (milligrams of S in 100 gm. of soil)	26 26 29.5	27.2	77.6 72.4 77.5	75.8	95.5 95.5 93.6	94.9	158.0 172.8 162.4	164.4	229.3 225.5 188.6	211.1	636.3 436.7 270.0	447.7
Percentage of sulphur oxidized	.....	.....	.....	97.2	.....	67.7	.....	67.6	.....	36.8	.....	42.05
Carbonates (milligrams of CO_3 in 100 gm. of soil)	47.2 51.2 49.2	49.2	7.9 5.9 5.9	6.6	.....	0	.....	0	0	.....	0	0
Bicarbonates (milligrams of HCO_3 in 100 gm. of soil)	182 178 172	177.3	102 104 104	103.3	70 72 74	72.0	38 32 34	34.7	20 15 24	20.0	0 0 10	3.3

[1] Cultures incubated 167 days at 25° to 27° C.

The results brought out in Tables I and II point out definitely that not only is elemental sulphur oxidized readily to sulphuric acid, which results in the neutralization of the carbonates of the soil, but the reaction of the soil can be brought down to any desired point, depending entirely upon the quantity of sulphur added and the length of time during which the sulphur is allowed to be in contact with the soil.

The next experiment deals with the oxidation of sulphur by pure and crude cultures of the sulphur-oxidizing bacteria in alkaline soil under sterile conditions. The soil was placed in 100-gm. portions in 250 cc. Erlenmeyer flasks; 100, 200, and 500 mgm. portions of sulphur were added to the soil portions, which were then thoroughly mixed, then the optimum amount of water was added and the flasks were sterilized for 1½ hours at 15 pounds pressure. The flasks were then inoculated by means of a sterile pipette, with 2 drops of the vigorously growing cultures and incubated at 25° to 28° C. Alkaline soils in which active sulphur oxidation had taken place were used as the crude alkaline compost. The results are given in Table III.

TABLE III.—*Oxidation of sulphur in alkaline soil by different bacteria*

Quantity of sulphur used.	Type of culture.	Incubated 14 days.	Incubated 30 days.			Incubated 60 days.			
		P_H	P_H	Carbonates in 100 gm. of soil (mgm. of CO_3).	Bicarbonates in 100 gm. of soil (mgm. of HCO_3).	P_H	Carbonates in 100 gm. of soil (mgm. of CO_3).	Bicarbonates in 100 gm. of soil (mgm. of HCO_3).	Sulphates in 100 gm. of soil (mgm. of S).
None	Control	9.6	9.6	49.0	159	9.6	52.5	143	30.1
100 mgm.	Crude alkaline compost.	7.7	7.5	0	50.0	7.7	0	43.5	115.5
200 mgm.	do.	7.4	6.3	0	31.0	5.6	0	8.5	201.0
500 mgm.	do.	7.0	4.9	0	15.0	4.7	0	6.5	254.8
100 mgm.	*Thiobacillus thiooxidans.*	9.2	8.8	8.0	142.0	8.6	0	86.0	109.4
200 mgm.	do.	8.6	9.0	14.7	127.0	7.5	0	53.0	145.3
500 mgm.	do.	8.6	9.0	5.8	118.0	7.4	0	45.0	160.7
100 mgm.	*Thiobacillus B.*	8.8	9.0	29.4	132.0	8.8	0	80.0	78.2
200 mgm.	do.	8.2	8.0	18.5	138.0	8.0	0	55.5	118.4
500 mgm.	do.	8.0	7.8	15.6	136.0	7.6	0	48.0	123.3

The crude culture proved to be most efficient in oxidizing sulphur in alkaline soil to such an extent that the reaction has been reduced, where sufficient sulphur has been used, from most alkaline to distinctly acid. This was accompanied by a complete disappearance of the carbonates, an almost complete disappearance of the bicarbonates, and an increase in the quantity of sulphates. With 100 mgm. of sulphur per 100 gm. of soil, equivalent to 1 ton of sulphur per acre (on the basis of the upper 6⅔ inches of soil), 85 per cent of the sulphur has been oxidized by the crude culture to sulphates within 60 days, and the reaction reduced from P_H 9.6 to P_H 7.7. When the quantity of sulphur was doubled, practically the same percentage of sulphur (85.5) was oxidized in 60 days and the P_H changed to 5.6 Where a large excess of sulphur was used, or 500 mgm. per 100 gm. of soil, only 44 per cent of the sulphur was oxidized to sulphate, and the reaction of the soil changed to P_H 4.7, which would already prove injurious to certain crops because of the excess acidity. The pure cultures did not prove quite as effective as the crude culture. This may be due to the fact that the oxidation of sulphur in alkaline soil is carried out not by one organism but by several organisms taking part in the process, as is shown by the experiments reported in Table IV, where a mixture of the two organisms is used. The acid soil used in these experiments is a Sassafras sandy loam, slightly acid in reaction. These experiments were carried out both under sterile and nonsterile conditions in tumblers.

TABLE IV.—*Oxidation of sulphur in acid and alkaline soils by pure and crude cultures of sulphur-oxidizing bacteria*

Soil type.	Sterilization of soil.[1]	Organism.	Incubated 21 days.	Incubated 48 days.
			P_H	P_H
Acid; 100 gm. of soil + 100 mgm. of sulphur.	+	Control	6.0	5.8
	−	do	5.2	4.4
	+	Crude, acid compost	5.7	3.8
	−	do	4.8	3.8
	+	Crude, alkaline compost	5.4	5.0
	−	do	5.4	4.0
	+	*Thiobacillus thiooxidans*	5.6	5.0
	−	do	5.0	3.8
	+	*Thiobacillus B*	5.8	5.4
	−	do	5.4	4.0
	+	*Thiobacillus thiooxidans* + *Thiobacillus B*	5.4	3.4
	−	do	4.8	3.6
Alkali; 100 gm. of soil + 200 mgm. of sulphur.	+	Control	9.6	9.2
	−	do	8.8	8.2
	+	Crude, acid compost	9.0	8.2
	−	do	9.0	8.4
	+	Crude, alkali compost	7.5	7.1
	−	do	7.6	7.3
	+	*Thiobacillus thiooxidans*	8.8	8.6
	−	do	8.2	8.0
	+	*Thiobacillus B*	8.2	7.8
	−	do	8.0	7.6
	+	*Thiobacillus thiooxidans* + *Thiobacillus B*	7.8	7.6
	−	do	7.6	7.4

[1] + indicates that the flasks containing soil and sulphur were sterilized, at 15 pounds pressure, for 1½ hours: − = soil unsterilized, in tumblers.

In the acid soil, oxidation of sulphur took place in the unsterilized containers whether inoculated or uninoculated, more so in the inoculated cultures, particularly where the *Thiobacillus thiooxidans* was introduced. This is due to the fact that the sulphur added, in nonsterile condition, probably has been inoculated previously from the laboratory air. However, when the soil and the sulphur are previously sterilized, oxidation takes place only to a very unappreciable extent, unless the proper sulphur-oxidizing organisms are introduced. In the alkaline soil, the most efficient results were obtained from inoculation with the crude alkaline culture, or alkaline soil, in which sulphur oxidation had taken place previously. The pure cultures, particularly the *Thiobacillus B* culture, which was almost inactive in the acid (sterilized) soil, proved more efficacious than the *T. thiooxidans;* but a mixture of the two cultures proved nearly as efficacious as the crude alkaline composts.

The fact that the change of the soil carbonates to sulphates would also favor plant growth, which is found to be so in actual field results, was demonstrated in these experiments by the increase in the number of bacteria developing in the soil. The soils used in the experiment reported in Tables I and II were at the end of the experiment air dried, then again moistened and, after 5 to 6 days, the bacterial numbers were determined by the ordinary plate method. The alkali soil, to which no sulphur had been added, contained 320,000 bacteria per gram of soil; where 50 mgm. of sulphur had been added per 100 gm. of soil and the reaction changed to P_H 7.9, there were 665,000 bacteria per gram; 100 mgm. of sulphur, reaction P_H 7.4, 875,000; 200 mgm. of sulphur, reaction P_H 6.7, 1,275,000; 500 mgm. of sulphur, reaction P_H 5.7, 3,650,000; 1,000 mgm. of sulphur, reaction P_H 3.5, no bacteria, only a few occasional fungi. In this case, the soil reaction has been made so acid as to completely kill off the bacteria. This danger, however, could hardly be expected in the field, because no such large quantities of sulphur (1 per cent) would ever be employed.

In the following experiment, the reaction of the soil was adjusted by the addition of sulphuric acid and sodium carbonate. The following amounts of acid and alkali were used to adjust the reaction of the soil:

	P_H.
Untreated soil	5.6
5 cc. *N/2* H_2SO_4 per 100 gm. of soil	5.3
0.5 cc. *M/2* Na_2CO_3 per 100 gm. of soil	5.8
1.0 cc. *M/2* Na_2CO_3 per 100 gm. of soil	6.0
3.0 cc. *M/2* Na_2CO_3 per 100 gm. of soil	6.2
7.0 cc. *M/2* Na_2CO_3 per 100 gm. of soil	6.4
20.0 cc. *M/2* Na_2CO_3 per 100 gm. of soil	8.4

One-gm. portions of powdered sulphur were added to each 100-gm. portion of soil in Erlenmeyer flasks; the flasks were well shaken, plugged with cotton, sterilized at 15 pounds pressure for 1½ hours, then inoculated with pure cultures of the organisms and incubated at 25°C. At the end of definite intervals of time the moisture content was brought up by the addition of sterile distilled water. The results are presented in Table V.

TABLE V.—*Oxidation of sulphur by pure cultures of bacteria at various reactions*

Type of culture.	P_H value.				Soluble sulphates (sulphur) in 100 gm. of soil after 50 days' incubation.
	Initial reaction of soil.	16 days after.	30 days after.	50 days after.	
					Mgm.
Control	5.2	5.2	5.3	5.4	19.2
	5.6	5.6	5.6	5.6	13.7
	5.8	5.8	5.8	5.7	11.0
	6.0	6.0	5.8	5.8	13.7
	6.2	6.2	6.2	6.2	13.7
	8.4	8.4	8.2	8.2	21.9
Thiobacillus thiooxidans	5.2	5.2	3.4	2.8	315.1
	5.6	5.4	3.0	2.8	314.6
	5.8	5.6	5.5	5.5	20.6(?)
	6.0	5.8	3.0	2.6	358.0
	6.2	5.4	2.8	2.8	380.0
	6.6	6.6	6.6	3.0	304.4
	8.4	8.4	8.2	8.0	27.4
Thiobacillus B	5.2	5.3	5.2	5.4	39.7
	5.6	5.3	5.2	5.2	20.6
	5.8	5.5	5.5	5.4	21.9
	6.0	6.0	5.5	5.5	15.2
	6.2	6.0	5.8	5.4	19.2
	6.6	6.6	6.6	6.4	24.7
	8.4	8.2	8.0	7.8	.....
T. thiooxidans+*T. B*	5.2	5.2	3.4	2.8	302.5
	5.6	3.1	2.8	2.6	287.7
	5.8	2.8	2.8	2.6	311.1
	6.0	2.8	2.6	2.6	308.3
	6.2	2.8	2.8	2.6	367.0
	6.6	3.1	2.8	2.8	274.0
	8.4	8.2	8.0	7.4	.....

Both the reaction and the sulphur content of the control indicate that no sulphur or only traces of it were oxidized under sterile conditions. However, in the presence of the sulphur-oxidizing bacteria, the oxidation of the sulphur took place very rapidly, with the transformation of large quantities of sulphur to sulphuric acid. When the reaction of the soil was acid, the oxidation of the sulphur in 50 days was carried on entirely by *Thiobacillus thiooxidans*. *Thiobacillus B* oxidized only very small quantities of sulphur; but, in the presence of both organisms, the speed of the reaction was hastened, as indicated by the measurements of the P_H values obtained in 16 days. This may be explained by either of two assumptions: (1) If we suppose a so-called "lag phase" in bacterial development, *Thiobacillus B* produces substances which appreciably shorten the "lag phase" of *T. thiooxidans;* (2) *Thiobacillus B* may oxidize the sulphur not directly to sulphates but to other compounds of sulphur, which are then rapidly oxidized to sulphates by *T. thiooxidans*. This is made clear in the study of the pure cultures of *Thiobacillus B*, with sodium thiosulphate as a source of energy, when a large part of the thiosulphate is transformed to persulphates, a part to elementery sulphur, and only a part to sulphates. This preparatory function of the *Thiobacillus B* may explain a number of hitherto unexplained difficulties observed in the study of sulphur oxidation by microorganisms in the soil. Of these we need mention only a few.

Nathanson (*8*), who first studied the Thiobacillus group, of which *Thiobacillus B* (not *T. thiooxidans*) is a representative, stated that thiosulphate is oxidized to sulphate and tetrathionate, with the production of free sulphur; however, on sodium sulphid agar plates, when free sulphur is formed abundantly in a nonbiological way, this sulphur is oxidized by the bacteria. The reaction proposed by Nathanson ($3Na_2S_2O_3 + 5O = Na_2S_4O_6 + 2Na_2SO_4$) could hardly be justified, particularly in view of the fact that the sulphur was supposed to be produced in a purely nonbiological way, by the interaction of the tetrathionate with the remaining thiosulphate, and was not supposed to play any part in the reaction. Beijerinck (*1*), who identified Nathanson's organism with his *T. thioparus*, then suggested the following reaction:

$$Na_2S_2O_3 + O = Na_2SO_4 + S.$$

But Jacobsen (*4*), who worked in Beijerinck's laboratory, found that the same organism oxidized elemental sulphur quantitatively to sulphate, which would again hardly justify Beijerinck's formula, if the organism was the same. Trautwein (*12*), however, obtained the separation of elemental sulphur from thiosulphate only in crude cultures of the organisms; but, in pure culture, no separation of elemental sulphur was found. At the same time a large part of the thiosulphate was transformed to sulphate. At first he thought the following reaction to be justified:

$$Na_2S_2O_3 + 4\ O + H_2O = Na_2SO_4 + H_2SO_4.$$

In view of the fact that the reaction of the medium did not turn acid and that a large quantity of persulphate was formed, he finally concluded that the reaction takes place as follows:

$$\text{I.}\quad 4Na_2S_2O_3 + 12\ O = 4Na_2S_2O_6.$$
$$\text{II.}\quad 3Na_2S_2O_3 + 5\ O = Na_2S_2O_6 + 2Na_2SO_4.$$

In the investigations carried on in this laboratory with pure cultures of *Thiobacillus B*, it was found that this organism transforms thiosulphate into persulphate, sulphate, and elemental sulphur, with only a limited production of acid (P_H changed in culture solution from 9.8 to 6.4 or 7.0). When *T. thiooxidans* is added to the culture solution, the elemental sulphur separated by the *Thiobatillus B* is rapidly oxidized to sulphuric acid and the final reaction may go down from P_H 9.8 to P_H 1.2 as a result of the action of the two organisms. The culture solution used in this case consists of 5 gm. sodium thiosulphate, 1 gm. sodium bicarbonate, 0.2 gm. dipotassium phosphate, 0.1 gm. magnesium chlorid, 0.1 gm. ammonium chlorid, 0.25 gm. calcium chlorid, and 1,000 cc. of tap water.

These discrepancies in the type of reaction that takes place when thiosulphate is acted upon by sulphur-oxidizing bacteria can be explained by the fact that different workers used different forms of a closely related group of organisms; in some cases the culture used possibly contained not one organism but a mixture of two or more organisms. *Thiobacillus B*, isolated in this laboratory and closely related, in its morphology and physiology, to *T. thioparus* and allied forms studied by Nathanson (*8*), Beijerinck (*1*), Trautwein (*12*), and others (*4*), was possibly contaminated in some cases by *T. thiooxidans*, which oxidizes actively elemental sulphur and is described by Waksman and Joffe (*15*). *T. thioparus* will act upon sulphur compounds under distinctly alkaline conditions, as in

culture media containing soluble carbonates or bicarbonates or black alkali soil; the elemental sulphur will be oxidized only comparatively slowly, but, in the presence of *T. thiooxidans* this oxidation will be rapidly hastened and the reaction quickly changed to any degree of acidity, depending merely on the quantity of sulphur used.

The possibility of the participation of other organisms oxidizing sulphur or sulphur compounds in the soil, or in any way influencing the process, in addition to the two mentioned, is not excluded. Neither is the possibility excluded that the oxidation of sulphur in alkaline soil is carried on chiefly by a strain of *Thiobacillus thiooxidans* which has adapted itself to alkaline conditions. While *T. thiooxidans* grows vigorously on artificial media and does not deteriorate with age, *Thiobacillus B* rapidly deteriorates under cultural conditions and the very slow oxidation of sulphur by this organism, particularly in the latter part of the work, may be due to this phenomenon.

SUMMARY

(1) The use of sulphur in the presence of the proper sulphur-oxidizing bacteria will result in the transformation of black alkali soil to white alkali soil.

(2) The final reaction of the soil depends on the quantity of sulphur used and the length of time which the sulphur is allowed to be in contact with the soil.

(3) The oxidation of sulphur in black alkali soil is probably carried on by the agency of more than one sulphur-oxidizing bacterium.

(4) In the presence of two bacteria, one of which can act upon sulphur under distinctly alkaline conditions while the other rapidly oxidizes sulphur under acid conditions, the speed of the reaction is greatly hastened.

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PEACH ROSETTE, AN INFECTIOUS MOSAIC[1]

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HISTORY OF THE DISEASE

According to Smith[2] peach rosette was first noted in Georgia in 1881. By 1891 this disease had been reported from 22 counties in Georgia, from South Carolina, and from Kansas. Rosette was also reported as occurring on plums in Georgia and Kansas, and on almonds in Kansas.

Since 1903, reports of the presence of rosette have come to the Plant Disease Survey, of the United States Department of Agriculture, from 19 counties in Georgia, 1 county in Alabama, 5 counties in South Carolina, 4 counties in Tennessee, 1 county in West Virginia, 19 counties in Missouri, and 2 counties in Oklahoma. These records indicate that for the past 40 years rosette has taken its toll of trees, and that it has spread over a considerable area.

IDENTITY OF THE DISEASE

In 1890–91, Smith[2] conducted experiments in middle Georgia in which he showed that peach rosette is an infectious disease. Of 125 seedling peach trees into which he inserted buds from a rosetted peach tree, 121 developed rosette.

In June, 1891, Smith[3] inoculated 37 Elberta peach trees with buds from a rosetted Kelsey plum. Two trees developed rosette and died in August, 1892. The other inoculated trees remained healthy. As a result of this experiment Smith said:

> The small per cent of cases to unions makes it necessary to repeat this experiment before it can be stated positively that the plum disease is identical with that of the peach and transmissible to it, as seems very probable from its appearance.

In June, 1891, Smith[2] inoculated 104 Marianna plum trees by inserting buds from rosetted peach trees. After 16 months not a single case of rosette had developed on the Marianna plums, so he concluded:

> There is, therefore, good reason to believe that the Marianna plum is not subject to this disease.

In June, 1891, 12 Marianna plum trees were inoculated by Smith[2] with buds from a rosetted Kelsey plum. On final examination in November, 1892, the Marianna plums showed no signs of rosette.

The presence of rosette in orchards at the Georgia Experiment Station, and in a number of commercial orchards in various sections of the State led to further study of this disease by the writer, beginning in 1919. As data presented by Smith[4] indicated that fungi and microscopic bacteria were not the cause of rosette, the writer did not attempt to repeat this phase of the work.

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[1] Accepted for publication Aug. 18, 1922.

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PEACH TO PEACH, PLUM, AND APRICOT

EXPERIMENT 1.—In the spring of 1919, rosette appeared on one side of a six-year-old seedling peach tree in an experimental orchard at the station. The other side of this tree appeared normal throughout the summer of 1919. During this time buds from healthy peach, plum, and apricot trees were inserted into the new growth of the normal appearing branches. The plum buds failed to unite with the peach stock. The peach and apricot buds united with the peach stock, but remained dormant throughout the summer. In the spring of 1920, the branches of the seedling peach which showed rosette in 1919, did not produce leaves, and examination showed that they were dead. The branches which appeared normal in 1919 produced rosetted growth in the spring of 1920. The peach and apricot buds inserted in 1919 produced typical rosetted shoots showing that the causal entity had passed from the diseased peach stock to the buds. The apricot buds produced shoots from two to three inches in length and the leaves had the mottled appearance of a mosaic disease. The mottled appearance was not so striking on the peach leaves. The shortened internodal growth, together with mottling of the leaves of some hosts and the absence of microscpic bacteria, put peach rosette in the class of virus or mosaic diseases. This experiment indicated that both the peach and the apricot are hosts for rosette.

PEACH TO PEACH

EXPERIMENT 2.—On June 10, 1919, buds from a healthy Elberta peach tree about 20 years of age were put in the new growth of two seedling peach trees about two years of age to serve as controls. On the same date buds from rosetted shoots of the seedling peach described in experiment 1 were put in the new growth of two peach seedlings the same age as the controls. On August 29, 1919, it was observed that the Elberta buds on the control trees had grown into healthy shoots. In one of the inoculated trees a diseased bud had developed a rosette of leaves, and below the point where this bud was inserted some of the buds of the stock had developed rosettes characteristic of this disease. In the other inoculated peach seedling the bud had remained dormant, but the buds of the stock immediately below the point of inoculation had developed typical rosettes. In May, 1920, it was observed that the two control trees were healthy and growing vigorously, while the inoculated trees had become completely rosetted (Pl. 1, A).

PEACH TO APRICOT TO PEACH

EXPERIMENT 3.—On August 15, 1919, two buds from a rosetted twig of the peach tree described in experiment 1 were put into the new growth of a healthy Royal apricot, which was in its second year of growth. The peach buds remained dormant until the spring of 1920 when they developed into rosetted shoots. The lateral buds on the apricot branch below the point of inoculation also developed weak shoots, but the leaves did not have the typical rosetted appearance. The internodal growth, although longer than that of the rosetted peach shoot (Pl. 2, A), was much less than that of healthy apricot trees growing near. Plate 2, B, shows the stunted growth of this Royal apricot 12 months after inoculation with peach rosette. While the growth is not typical of rosette as

it appears in the peach, the shortened internodal growth, and the mottling of the leaves indicate that the causal entity had been transferred from the peach to the apricot.

On September 2, 1920, two buds were taken from the rosetted Royal apricot and inserted in a peach seedling, growing in a pot in the greenhouse. These buds remained dormant until April, 1921, when they began to develop mottled leaves. The new growth of the peach seedling developed typical rosettes (Pl. 1, B). Both the potted rosetted peach seedling and the rosetted Royal apricot tree died during the summer of 1921.

Experiment 3 shows that the causal entity of rosette may be transferred from peach to apricot, and from apricot to peach, proving that apricot and peach rosette are identical as far as causal entity is concerned, but somewhat different in external manifestations of the disease.

STUNTING OF APRICOT GROWTH BY ROSETTE

EXPERIMENT 4.—In the spring of 1920 another case of natural infection of peach rosette (Pl. 3, A) developed in a 6-year-old seedling peach tree growing in the same orchard about 150 feet from the rosetted seedling described in experiment 1.

On June 19, 1920, buds from this second rosetted seedling peach were inserted into a branch of a one-year-old Moorpark apricot about a foot above the ground. By August 20, 1920, both of the peach buds had grown into rosetted shoots (Pl. 5, B), but the stock showed symptoms of rosette only in the growth immediately below the inserted peach buds, where the apricot leaves became mottled. An apricot limb adjoining the one which was inoculated, made a growth of 4 feet and 4 inches during the summer of 1920, and bore normal green leaves. There was no growth of lateral buds. On April 11, 1921, the lateral as well as the terminal buds on all the apricot branches had produced mottled, greenish yellow leaves, in marked contrast to the normal green leaves of the near-by healthy apricot. This indicated that the causal entity of rosette had spread throughout the apricot tree. During the summer of 1921 this Moorpark apricot tree grew very slowly, the maximum growth of any one branch being 5 inches. During the same time the near-by healthy apricot tree made a growth of 4 feet and 9 inches. Plate 3, B (taken in the fall of 1921) shows the inoculated apricot tree with a background, at the left, in contrast to the healthy apricot tree at the right. An indication of the stunting effect of rosette is shown by the inoculated tree in which the maximum vertical growth of 4 feet and 4 inches made during the summer of 1920 represents normal growth; while the maximum horizontal growth, 5 inches, represents development made during the summer of 1921 under the retarding influence of rosette.

PEACH TO CULTIVATED PLUM

EXPERIMENT 5.—On August 6, 1919, a bud from a healthy Blue Damson plum was placed in the trunk of a healthy peach seedling which came up in the spring of 1919. This plum bud made a few inches of growth during the summer of 1919, and in the summer of 1920, made a growth of more than 2 feet. Neither the plum branch nor the peach stock showed any symptoms of disease up to June 19, 1920, when two buds from the rosetted peach tree described in experiment 4, were inserted in

the Damson plum branch. The diseased peach buds started growth within two weeks, producing typical rosetted shoots, but with somewhat larger leaves and more internodal growth than did similar rosetted buds on the diseased peach stock from which the buds were taken. During the summer of 1920, the plum buds just below the place where the peach buds were inserted, developed small rosettes of mottled leaves (Pl. 4, A) indicating that the causal entity had passed from the diseased peach buds into the healthy Damson plum branch. None of the branches of the peach stock on which the plum was budded showed symptoms of rosette during the summer of 1920. The growth of the peach stock was so vigorous that a small copper wire, by which a label had been attached to the trunk in 1919, became embedded in the tissues of the stock, a few inches above the point where the Damson plum bud was inserted. In the spring of 1921, rosette developed in all growth of the Damson plum branch, and in all branches of the peach stock which grew from the trunk at points below where the copper wire was embedded in the tissues. None of these rosetted branches set fruit, though a few produced weak blossoms. All of the branches which grew from the trunk above where the wire was embedded produced normal leaves and blossoms which set numerous fruits. By July, 1921, the leaves on this tree began to wither, and by August 20 the tree was practically dead (Pl. 4, B). No symptoms of rosette appeared on the tree above the embedded wire, and the leaves and fruits shriveled and clung to these branches for some time after the tree was dead. The fact that no symptoms of rosette appeared above the embedded wire supplies additional data as to the tissues through which the causal entity progresses, and is being further investigated.

In this experiment rosette was transferred from the peach to the plum, and back to the peach, indicating that peach and plum rosette are identical.

Rosette has also been transferred from peach to Red June plum by means of infected buds.

PEACH TO WILD PLUM TO PEACH

EXPERIMENT 6.—On September 22, 1920, buds from a rosetted Mayflower peach, 7 years old, which developed as a natural infection in one of the station orchards in the spring of 1920, were put in two wild Chickasaw plum trees, growing in a fence row on the station. These buds remained dormant until the spring of 1921, when both the peach buds and the plum stock developed rosetted shoots. The inoculated plums (Pl. 4, C) grew more slowly than the surrounding healthy plum trees during the summer of 1921. The appearance of the rosetted wild plum is not so striking as that of a rosetted peach, because the plum is naturally of dwarfed growth. The wild plum, being of no economic importance, grows in waste places without coming under the close observation of man; therefore one or more rosetted wild plum trees might easily be an unobserved source of infestation to surrounding orchards.

On May 23, 1921, buds from the rosetted wild Chickasaw plum were inserted in the new growth of a 2-year-old seedling peach. During the summer one of these plum buds produced a rosetted shoot about an inch in length, but no symptoms of rosette appeared in the peach stock up to the time it was defoliated by frost. In the spring of 1922 this

peach seedling showed rosette in all new growth (Pl. 5, A). Peach seedlings into which healthy plum buds were inserted in 1921 showed no symptoms of rosette in the new growth of 1922.

This indicates that the casual entity of rosette may readily be transmitted from peach to wild plum, and from wild plum to peach.

Rosette has also been transmitted from the wild Chickasaw plum to the Red June plum by means of infected buds.

PLUM TO PEACH

EXPERIMENT 7.—A natural infection of a Maynard plum developed in an orchard on the station in the spring of 1920. By June 15, 1920, most of the lateral buds had grown into rosetted shoots from 1 to 3 inches long; and by August, 1920, this tree (Pl. 6, B) had made very little new growth as compared with a near-by healthy plum tree (Pl. 6, A). On June 18, 1920, buds from this rosetted plum tree were put into a healthy seedling peach tree in its second season's growth. Buds from a healthy plum were put into another peach seedling to serve as a control. On August 18, 1920, it was observed that some of the rosetted Maynard plum buds put into the peach seedling had produced shoots several inches long. Below the point where the diseased buds were inserted the peach buds had developed rosetted shoots (Pl. 6, C). The disease continued to spread in this peach seedling during the rest of the season of 1920, and when new growth started in the spring of 1921 this tree showed rosette in all parts. It died before midsummer. One of the control buds produced normal leaves (Pl. 7, A) and the peach stock on which it was growing was alive and healthy in the fall of 1921 when it was removed to make room for other experimental work.

This experiment indicates that rosette originating in the cultivated plum may be transmitted to the peach.

PEACH TO MARIANNA PLUM

EXPERIMENT 8.—On June 18, 1920, buds from the rosetted peach seedling described in experiment 4 were put into a healthy Marianna plum branch, near the base of the tree. One bud started growth within two weeks and produced a rosetted shoot (Pl. 7, B) with larger leaves and longer internodal growth than rosetted shoots on peach stocks. No signs of rosette appeared on the Marianna stock during the summer of 1920. After becoming dormant in the fall of 1920, this Marianna plum was transplanted to a large pot and placed in the greenhouse. In the spring of 1921 the peach shoot developed rosetted leaves, but continued to grow throughout the summer. The Marianna plum stock developed normal leaves on all of its branches, which grew rapidly throughout the summer. This Marianna plum (Pl. 7, C) had been under observation in the greenhouse during the winter, and up to May 1, 1922, it showed no symptoms of rosette. The rosetted peach shoot continued to grow slowly. When rosetted peach buds were put into a susceptible host, as peach, apricot, or ordinary cultivated plums, they died within 12 months. On the resistant Marianna plum stock the rosetted peach bud grew into a shoot which at the time this paper was written had lived for 22 months. This indicated that the resistant stock exerted a marked influence on the virulence of the causal entity of rosette in the peach scion.

PLUM TO MARIANNA PLUM

EXPERIMENT 9.—On June 18, 1920, buds from the rosetted Maynard plum described in experiment 7 were put into a Marianna plum branch, near the base of the tree. By September 18, 1920, one of the diseased buds had produced a rosetted shoot 8 inches in length with three branches from 3 to 5 inches in length (Pl. 8, A). This rosetted Maynard plum branch made considerably more growth on the Marianna stock than similar buds made on the susceptible Maynard stock, but the growth was decidedly rosetted and the leaves were mottled yellowish green. The Marianna stock grew vigorously throughout the summer of 1920 and showed no external symptoms of rosette.

Buds from a healthy Mayflower peach tree were put into the new growth of the Marianna stock on which the rosetted Maynard plum shoot was growing on September 18, 1920. These peach buds remained dormant until the spring of 1921, when two buds grew into healthy peach shoots. The Marianna plum branches and the Mayflower peach shoots made a vigorous growth during the summer of 1921, which showed no symptoms of rosette. The Maynard plum shoot made some growth during the summer of 1921, but at all times it had the characteristic symptoms of rosette. In the spring of 1922 the Marianna plum stock and the two Mayflower peach shoots (Pl. 8, B) developed normal leaves in contrast to the rosetted Maynard plum branch.

This experiment gave additional evidence that the Marianna plum is not susceptible to rosette. It also indicated that the causal entity of rosette does not pass from a host, such as the Maynard plum, through the tissues of the resistant Marianna plum stock to another susceptible host, as the Mayflower peach.

PEACH TO MAZZARD CHERRY

EXPERIMENT 10.—On June 19, 1920, buds from the rosetted peach tree described in experiment 4 were put into a healthy Mazzard cherry seedling about 1 year of age. On the same date buds from a healthy Elberta peach tree were put into a near-by Mazzard cherry tree of the same age to serve as a control. One of the rosetted buds united with the cherry stock and made a very feeble growth of rosetted leaves. The healthy buds united with the cherry stock but remained dormant. During the summer of 1920 the inoculated cherry stock grew slowly as compared with the control tree. The leaves of the inoculated tree became yellowish green and the new growth was small and in tufts similar to rosettes of peach leaves. The inoculated cherry stock developed leaves from both lateral and terminal buds in the spring of 1921, giving the new growth a decidedly rosetted appearance, especially at the tips of the branches. The control cherry stock produced vigorous new growth from the terminal buds. Very little growth was made by the diseased cherry tree during the summer of 1921, as shown by the smaller and more rolled leaves compared to those of the healthy control tree. The healthy Mazzard cherry control tree matured its buds and became dormant during the fall of 1921, while the diseased cherry tree attempted to make new growth from the terminal buds throughout the winter. By April 25, 1922, the inoculated tree (Pl. 9, A) was much stunted and had the appearance of being in an advanced stage of rosette, while the control

tree (Pl. 9, B) showed no symptoms of rosette and had made a vigorous growth.

This experiment indicates that the causal entity of peach rosette may be transmitted to Mazzard cherry and may produce symptoms similar to but not exactly the same as rosette of the peach. In the case of the Mazzard cherry there is evidently some resistance to the causal entity of rosette, for the infected cherry tree was alive June 1, 1922 (when this paper was written), 23 months after showing symptoms of rosette. Rosette has also been transmitted to two additional Mazzard cherry trees by means of infected peach buds.

WILD PLUM TO BITTER ALMOND

EXPERIMENT 11.—Through the courtesy of members of the California Agricultural Experiment Station, fresh seed of Bitter almond, and Texas Seedling almond were obtained and planted in the greenhouse on November 24, 1920. During April, 1921, some of the young almond trees were transplanted to the nursery. On May 23, 1921, buds from a rosetted wild Chickasaw plum (used in experiment 6) were put into two Bitter almond seedlings in the nursery. Three uninoculated trees of the same variety served as control. The plum buds united with the almond stocks and during the summer of 1921, one grew into a rosetted shoot about 6 inches long. Almond buds on the stock below the point where the rosette plum buds were inserted grew into small rosetted shoots which died during the winter. By May 24, 1922, the new growth of this inoculated tree was stunted (Pl. 10, A), and the leaves were yellowish green. The other inoculated tree had shown no marked symptoms of rosette at the time this paper was written. The adjoining uninoculated trees made a vigorous growth in the spring of 1922 and showed no symptoms of rosette.

This experiment indicates that the Bitter almond is susceptible to rosette.

APRICOT TO BITTER ALMOND AND TEXAS SEEDLING ALMOND

EXPERIMENT 12.—On April 18, 1921, buds from the rosetted Royal apricot of experiment 3 were put into one Bitter almond and one Texas Seedling almond growing in pots in the greenhouse. The buds united with the almond stocks, but made very little growth during the summer of 1921. Lateral buds on the two almond stocks, below the points of inoculation, developed small rosetted shoots indicating that the causal entity had been transferred from the rosetted apricot to both Bitter almond and Texas Seedling almond. Uninoculated almond trees of the two varieties growing in near-by pots remained healthy. In the spring of 1922 the inoculated trees became rosetted in all parts; the growth was stunted and the leaves were yellowish green. The uninoculated trees made a vigorous growth, however, and bore healthy green leaves.

This experiment proves that both the Bitter almond and the Texas Seedling almond are susceptible to rosette.

ALMOND TO PEACH

EXPERIMENT 13.—On February 2, 1922, buds were taken from the rosetted Bitter almond and Texas Seedling almond of experiment 12 and inserted in healthy peach seedlings growing in pots in the greenhouse.

One of the Bitter almond buds had produced a much branched shoot about 16 inches long by June 1, 1922, the leaves of which were more tufted in growth than those of healthy Bitter almonds. The peach shoots which grew from the stock below the point of inoculation had developed an upward rolling of their older leaves and most of the lateral buds had produced small rosettes of yellowish green leaves (Pl. 10, B).

The Texas Seedling almond buds remained dormant and up to June, 1922, the peach stock into which they were inserted had shown no symptoms of rosette. Peach seedlings budded to healthy almonds have remained healthy up to June 1, 1922.

This, together with the foregoing experiments, indicates that peach, apricot, plum, and almond are susceptible to rosette and that in all cases the causal entity is the same.

SOIL TRANSMISSION OF ROSETTE

EXPERIMENT 14.—Natural infection of rosette has been observed by the writer in Georgia on peach trees from 2 to 8 years of age. Where rosette develops in an orchard of young trees the question arises as to the advisability of setting a healthy tree in the place from which a diseased tree has been removed. It has been proved by Smith[5] that infection may be produced through inoculation of peach roots, so it seemed advisable therefore to test soil transmission.

Two 6-year-old peach trees, which had developed rosette in the spring of 1919, were dug up September 5 of the same year and removed from the orchard. Early in January, 1920, a healthy 1-year-old peach tree on peach stock was set in each hole. No attempt was made to remove fragments of roots left from the rosetted trees. The two transplanted trees made satisfactory growth during the summers of 1920 and 1921 showing no symptoms of rosette. This indicates that one may safely set a healthy tree in a place from which a rosetted tree has been removed.

THE TRANSMISSION OF ROSETTE BY MEANS OF SAP FROM DISEASED TREES

In rosette of the several species of Prunus there is a shortening of internodal growth and in some cases mottling of the leaves similar to mosaic diseases of vegetable and field crops. Numerous inoculations have been made in various parts of susceptible species of Prunus using methods known to be successful in transmitting mosaics of other plants. In no case has rosette been produced by transfers of sap from rosetted to healthy trees, but experiments along this line are being continued. The results obtained thus far confirm data presented by Smith[6] to the effect that under artificial conditions rosette is transmitted only when an organic union takes place between infected tissues, and tissues of a susceptible host. Thus in experiments conducted to date, rosette differs from other mosaics in the method of artificial transmission, indicating that the causal entity is somewhat different from that of other mosaics.

[5] Smith, Erwin F. ADDITIONAL NOTES ON PEACH ROSETTE. In Jour. Mycol., v. 7, p. 226–232. 1893.
[6] Smith, Erwin F. THE PEACH ROSETTE. *In* Jour. Mycol., v. 6, p. 143–148, pl. 8–13. 1891.

NATURAL TRANSMISSION OF ROSETTE

The development of rosette in trees, often at a considerable distance from any known source of infection, indicates that winged insects or birds may be associated with natural transmission of this disease.

Various insects are found associated with rosetted trees, the most abundant being the black peach aphis, *Anuraphis persicae niger* Smith. Numerous tests have been made by removing insects, including several species of beetles and leafhoppers from various parts of rosetted trees, and caging them on healthy peach and plum trees. In no case has rosette developed. At various times throughout the growing season for the past two years, numerous black peach aphids have been transferred from rosetted peach and plum trees to healthy peach, plum, cherry, apricot, and wild plum trees growing in cages. The colonies of aphids increased rapidly in size showing that they were under favorable conditions. In no case did rosette develop as a result of these aphid transfers. These tests indicate that the causal entity of rosette is not readily transferred by the types of insects which are known to carry mosaic virus of other plants.

The writer is of the opinion that further study will disclose the fact that an animal, other than man, is responsible for the dissemination of the causal entity of rosette. Therefore observations along this line are being continued.

SUMMARY

The data obtained from the foregoing experiments verify the findings of Smith to the effect that rosette is readily transmitted from peach to peach by infected buds.

Rosette has also been transmitted to two varieties of apricots, two varieties of cultivated plums, one wild plum, one cherry, and two varieties of almonds by means of infected buds.

On some hosts rosette produces a mottling of the leaves similar to mosaics.

The Marianna plum is immune to rosette.

Limited tests indicate that rosette is not soil-transmitted.

Numerous attempts to transmit rosette by means of sap from diseased trees has proved unsuccessful.

In a large number of transfers of various types of insects from rosetted trees to healthy susceptible hosts, not a single case of rosette was transmitted.

PLATE 1

A.—A 2-year-old peach tree into which buds from a rosetted peach were inserted June 10, 1919. Note the appearance in May, 1920, with all growth rosetted.

B.—The peach seedling into which buds from a rosetted Royal apricot were inserted September 2, 1920. Note the appearance in the summer of 1921, with all growth rosetted.


B


A


B


A

PLATE 2

A.—A Royal apricot tree into which buds from a rosetted peach were inserted August 15, 1919. Note the rosetted peach shoot, and the stunted growth of the apricot shoots, made in 1920.

B.—The Royal apricot tree inoculated with peach rosette August 15, 1919. Note the stunted appearance of the whole tree on September 2, 1920.

PLATE 3

A.—A 6-year-old peach tree which developed a natural infection of rosette in the spring of 1920. Note the rosetted growth on the left in comparison with the apparently normal growth of two limbs on the right. During the summer of 1920 this tree developed symptoms of rosette in all parts.

B.—To the left, with a background, the Moorpark apricot shown in Plate 5, B, which was inoculated with peach rosette, June 19, 1920. Note the stunted growth of this tree in the fall of 1921, in comparison with the healthy apricot tree, to the right, without a background.


B


A


C


B


A

PLATE 4

A.—The Blue Damson plum branch into which rosetted peach buds were inserted June 19, 1920. Note the rosetted and mottled plum leaves just below the rosetted peach shoots.

B.—The peach seedling which was infected with rosette through a Blue Damson plum branch inoculated with buds from a rosetted peach. The arrow at *a*, shows the point on the peach stock from which the plum branch grew from a healthy bud inserted in 1919. The arrow at *b*, shows the location of the embedded copper wire above which the causal entity of rosette did not go. Note the condition August 20, 1920, with the leaves and fruits shriveled and dying.

C.—One of the wild Chickasaw plum trees into which rosetted Mayflower peach buds were inserted September 22, 1920. Note the stunted growth and the rosetted condition of this plum tree on September 2, 1921.

PLATE 5

A.—A seedling peach into which buds from a rosetted wild Chickasaw plum were inserted May 23, 1921. Note the completely rosetted condition of the peach seedling in May, 1922.

B.—A Moorpark apricot into which buds from a rosetted peach were inserted June 19, 1920. Note the rosetted shoots produced by the peach buds at *b*, and the apparently healthy growth of the apricot.


A
B
b
b
USDA

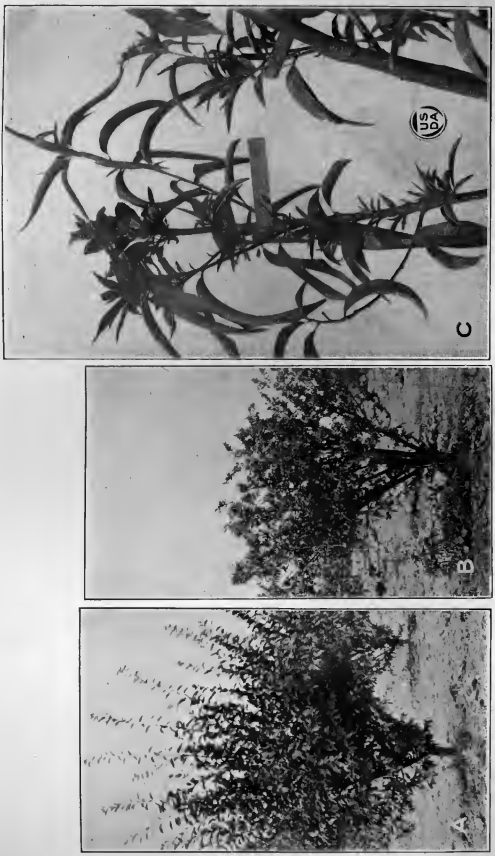
C
B
A

PLATE 6

A.—A healthy plum tree showing the vigorous growth made by August, 1920.

B.—The Maynard plum which developed rosette in the spring of 1920. Note the stunted growth and rosetted leaves which developed by August, 1920.

C.—The peach seedling into which rosetted Maynard plum buds were inserted June 18, 1920. Note the rosetted plum shoot which developed from one of the buds, and the rosetted peach shoots which developed as a result of inoculation with infected plum buds.

PLATE 7.

A.—The peach tree into which healthy plum buds were inserted June 18, 1920, to serve as a control. Note the healthy growth of the peach stock, and of one plum bud (indicated by the arrow).

B.—The Marianna plum tree into which buds from a rosetted peach seedling were inserted June 18, 1920. Note the rosetted shoot produced by one of the peach buds.

C.—The Marianna plum tree, shown in B, after another season's growth. Note the vigorous, healthy growth of the Marianna stock in contrast to the stunted growth of the peach shoot at *a*, with small rosettes at the tips of the branches.


C
B
A


B
a
b


A

PLATE 8

A.—The Marianna plum tree into which buds from the rosetted Maynard plum were inserted June 18, 1920. Note the vigorous growth of the Marianna branches in contrast to the rosetted shoot, at the right, produced by one Maynard bud after three months' growth.

B.—The Marianna plum stock (same as in A but from the opposite side of the tree), showing the healthy growth of the two Mayflower peach shoots at *a*, in contrast to the stunted, rosetted Maynard plum shoot at *b*.

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PLATE 9

A.—The Mazzard cherry stock into which buds from a rosetted peach seedling were inserted June 19, 1920. Note the stunted growth and rosettes of leaves.

B.—The Mazzard cherry stock of the same age as A, into which healthy Elberta buds were inserted as a control.


A
B
USDA


A
B
U.S.
D.A.

PLATE 10

A.—Bitter almond seedlings growing in the nursery. Note the stunted growth of the tree to the left with a rosetted plum shoot near the base, on May 24, 1922. This tree was inoculated May 23, 1921, with infected wild Chickasaw plum buds. The uninoculated seedlings to the right are healthy, and growing vigorously.

B.—A seedling peach tree into which a bud from a rosetted Bitter almond was inserted February 2, 1922. Note the much branched and tufted growth of the almond shoot which developed from the diseased bud. All the peach shoots which have developed below the point of inoculation have upward rolled leaves, and the lateral and terminal buds have produced small rosettes of yellowish green leaves.

TOXICITY AND ANTAGONISM OF VARIOUS ALKALI SALTS IN THE SOIL [1]

By F. S. HARRIS, *formerly Director*, M. D. THOMAS, *Associate in Agronomy*, and D. W. PITTMAN, *Instructor in Agronomy, Utah Agricultural Experiment Station* [2]

In the studies of soil alkali which have been carried out at the Utah Station (*4*, *5*) [3] during the past 10 years, a large number of the factors which influence the toxicity of most of the commonly occurring alkali salts have been correlated. It has been frequently noticed, however, that the toxicity of a mixture of salts in a soil seems to be the sum of the separate toxicities of the constituents of the mixture, and since these observations are at variance with the marked antagonistic action of the same salts in solution cultures a more detailed study of this question has been undertaken.

The experiments described in this paper were planned to show the influence on plant growth of adding other salts, as well as acids and manure, to a soil already impregnated with sodium carbonate. The possibility of finding a marked antagonism between some of these added substances and "black alkali" was an incentive to make the scope of the investigation as broad as possible. Since the results from this point of view, however, are largely negative the data are presented as a contribution to the literature on the toxicity of mixtures of alkali salts. Some preliminary work has also been done on soils impregnated with sodium chlorid and sodium nitrate instead of sodium carbonate.

REVIEW OF THE LITERATURE

Experiments conducted by Kearney and Cameron (*6*) were among the first to show the ameliorating effect of adding a second alkali salt to a solution which was already toxic to plants. In their work the plants were germinated while not in contact with the alkali salts, and the roots were then held in the solution for 24 hours. The toxicity of the solution, was determined by slight injury to the root tips. The toxic concentrations were not proportional to the concentrations which absolutely prevented growth of the plants, and the toxicity of the salts exchanged places somewhat when the absolute rather than the minimum check in growth was considered. Very small quantities of salts caused slight injury to the roots. The addition of a second cation to a toxic solution was found to reduce the toxicity of the solution more than the addition of a second anion. Sodium in most combinations greatly weakened the toxic action of magnesium. Calcium, especially in the form of the sulphate, markedly counteracted the injurious effect of either sodium or magnesium ions. Calcium sulphate was much more beneficial to the sulphates of sodium and magnesium than to their chlorids. A very

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[2] The authors wish to express their appreciation to Mr. N. I. Butt for his help in calculating and tabulating the data presented in this paper.
[3] Reference is made by number (italic) to "Literature cited," p. 337–338

effective neutralization of sodium carbonate injury was found when calcium chlorid was added to the solution.

The work of Miyake(*11*) with rice in cultures of magnesium sulphate, magnesium chlorid, calcium chlorid, sodium sulphate, and sodium chlorid showed that while *N/10* solutions of the individual salts were toxic, when two of these solutions were mixed in certain proportions the toxic effect was more or less neutralized. The greatest neutralization of toxicity was observed when calcium chlorid was added to magnesium sulphate or chlorid. Some antagonism was also noticed between chlorids and sulphates of sodium and magnesium, as well as between potassium chlorid and magnesium or calcium chlorid. In a study of the antagonism between sodium and potassium, the salts—sodium nitrate, potassium chlorid, potassium nitrate, sodium chlorid, potassium sulphate, and sodium sulphate—were used. The antagonistic action of the cations on each other was much greater than that of the anions. The greatest antagonism between sodium and potassium usually occurred when one part of one *N/10* solution was mixed with four parts of another.

Osterhout(*12*) found that antagonism between sodium chlorid and potassium chlorid was greater when one of the salts predominated in the solution than when both were present in nearly equal quantities. Small quantities of ammonium chlorid, magnesium chlorid, or calcium chlorid reduced the toxicity of either potassium chlorid or sodium chlorid, but with calcium chlorid even larger quantities were beneficial.

Kearney and Harter(*7*) tested the tolerance of eight different kinds of plants to solutions of sodium and magnesium salts and found that calcium sulphate greatly diminished the toxicity of the salts, especially magnesium. Calcium sulphate changed the order of toxicity of the solutions.

Hansteen(*3*), working with wheat seedlings, has found that calcium compounds exert a beneficial effect on the toxicity of solutions of alkali salts.

A comparison of soils and solutions by Harris(*4*) showed the antagonism between the common alkali salts to be more pronounced in solution cultures of wheat seedlings than in loam soils. In fact the only consistent case of antagonism observed in soil seems to have been with the nitrates of potassium, sodium, and magnesium at 4,000 parts per million (*4, p. 46*).

Some of the most positive antagonistic results in soils have been secured by measuring the activity of soil bacteria. By this means it has been shown by different experimenters that there is antagonism between anions as well as between cations of the salts common in alkali soils. This work has been so well summarized in a paper by Greaves(*2*) that the reader is referred to this publication for a review of this phase of the subject.

Experimenting with barley growing on a clay-adobe soil, Lipman and Gericke(*8*) found antagonism between sodium chlorid and sodium sulphate and between sodium chlorid and sodium carbonate in the second crop, though there was no antagonism shown between these salts in the crop grown soon after the salts had been added. A slight antagonism was noticed between sodium carbonate and sodium sulphate in the first crop. A marked antagonism between sodium sulphate and calcium sulphate was apparent in both the first and second crops.

To discover the possibilities of applying the principles of antagonism to the correction of alkali as found in field soils, Lipman and Sharp(*10*) secured natural alkali soil containing 6,400 parts per million of water-soluble salts of which 4,590 parts per million were sodium chlorid, 980 parts per million sodium sulphate, and 830 parts per million sodium carbonate. Adding 119 parts per million of sulphuric acid to this soil was found to be especially beneficial to the growth of barley; and up to 451 parts per million, the highest quantity tried, this acid was helpful. Calcium sulphate at the rate of 670 parts per million, ferrous sulphate at 324 parts per million, and manure at 3,240 parts per million all materially improved the crop-producing power of the soil. Copper and sodium sulphates at the rate of 65 and 130 parts per million, respectively, were harmful to the crop.

Lipman and Gericke(*9*), growing barley on a clay-adobe soil, found that copper and zinc reduced the toxicity of sodium chlorid, sodium sulphate, and sodium carbonate. Marked antagonism was also noticed between these salts, especially between copper sulphate and sodium chlorid when applied to a sand soil.

Caldwell(*1*) found no antagonism between sodium chlorid and any one of the chlorids of calcium, magnesium, potassium, copper, or ammonium, with the possible exception of ammonium and magnesium in certain proportions, when he grew corn in quartz sand. Potassium and sodium were always more toxic together than when only one was present at a given strength. Adding either calcium chlorid or copper chlorid to sodium chlorid appeared to ameliorate the injurious effect of the latter by diluting the solution rather than by counteracting the harmful effects of the sodium salt.

EXPERIMENTAL WORK

METHOD

The experiments here reported were conducted in glass tumblers containing the equivalent of 200 gm. of dry soil and the optimum amount of moisture. Wheat was the crop grown. The requisite quantity of sodium carbonate in 10 per cent solution was added to five 7-kilo portions of air-dry soil, and each portion was mixed separately by forcing it through a fine sieve twice. They were then placed together in a large can and shaken thoroughly at intervals for several days before being used. This method of mixing gave a very satisfactory distribution of the carbonate, as was shown by analyses of a large number of samples taken from different parts of the can. To the soil for each tumbler the other materials were then added, in solution whenever possible, together with the necessary water, and the soil was thoroughly mixed on a piece of oilcloth. Ten kernels of wheat were planted in each vessel. The surface of the soil was covered with a thin layer of a mixture of 50 per cent paraffin and 50 per cent petrolatum to prevent surface evaporation. A short glass tube extending to the center of the soil mass from the surface permitted the addition of water as it was needed to keep the weight of the tumbler constant. The mulch was very effective, and it was therefore possible to avoid altering the uniformity of the distribution of the soluble material through irrigation—at least until the plants were fairly large—as the weight of the tumbler did not change appreciably

at first. The plants which came up were counted daily and every four or five days their height was recorded also. At the end of 21 days they were measured for the last time, cut off at the surface of the ground, and dried to constant weight at 100° F. Data were thus secured on the days to germinate, the average height, and the dry weight of the plants. Most of the discussion in this paper, however, will be based on the dry weight which is perhaps the most reliable index of growth.

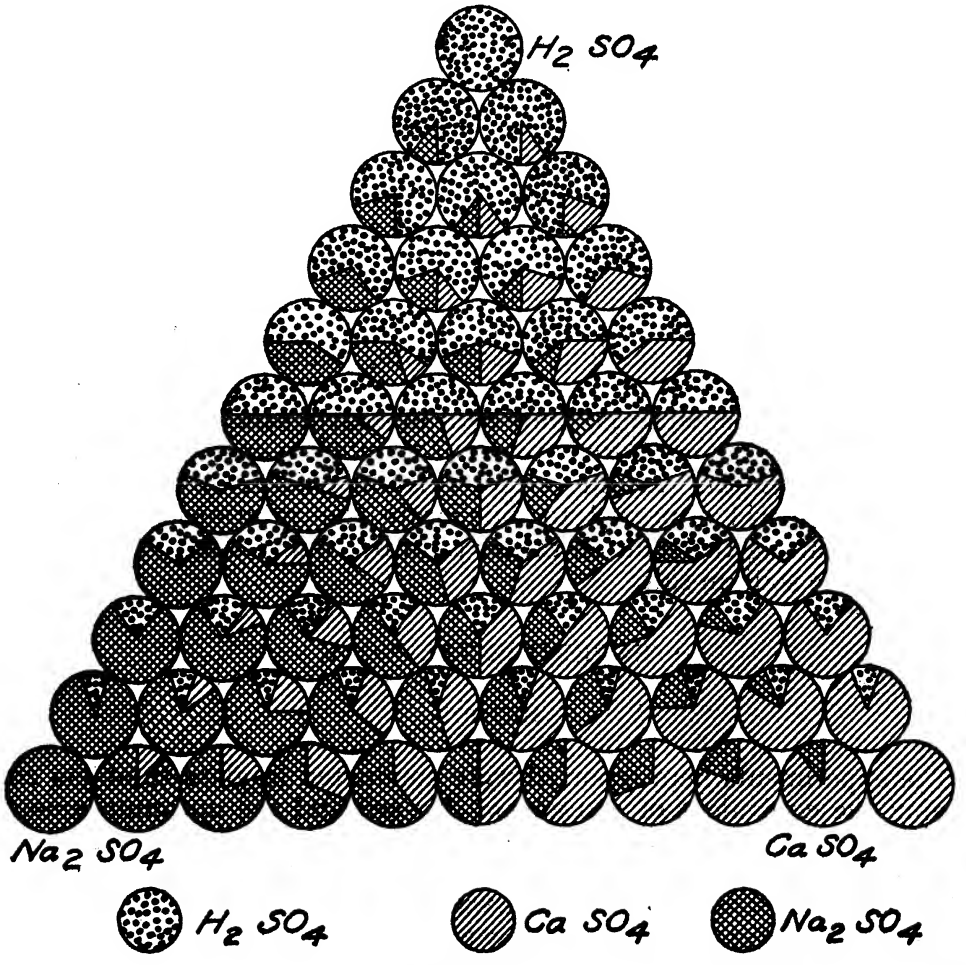

FIG. 1.—Diagram showing the arrangement of the tumblers in the preliminary experiments. A uniform concentration of sodium carbonate was present in all the tumblers, and an equal total quantity of the sulphates was added to each in the relative proportion indicated by the shading.

PRELIMINARY EXPERIMENTS

During the summer of 1919 some preliminary work was done on this problem. The technic of the experiment was essentially the same as that described above, except that no mulch was used and the preliminary mixing of the carbonate with the soil was not quite so thoroughly done. The tumblers were arranged in a triangular formation consisting of 66 glasses. All contained a single concentration of sodium carbonate together with sodium sulphate, calcium sulphate, and sulphuric acid in the different concentrations represented in figure 1. The total concentration in parts per million of the added sulphates was always the

same as that of the carbonate, but there were 10 different quantities of each individual sulphate in the triangular grouping. In addition, there were 12 extra tumblers containing the four added substances alone, at their maximum concentrations. A "trial" consisted of 10 of these treatments in which the quantity of carbonate and sulphates added ranged from 1,000 to 10,000 parts per million. Three trials were first carried out with these materials in sand, Greenville loam, and West Logan clay loam. Then two more trials were made with Greenville loam and the same sulphates but with the substitution of sodium chlorid in one case and sodium nitrate in the other in place of sodium carbonate. In the last two trials the concentrations of the salts and also the sulphates ranged from 500 to 5,000 parts per million.

Mechanical and partial chemical analyses of the soils used in these experiments have already been published by one of the writers (*13*). All the soils contained a large quantity of calcium carbonate. The sand was coarse and low in organic matter. The two heavier soils were nearly identical in texture, but the Greenville soil contained a little more organic matter.

A critical study of the results of this preliminary test has shown that the experimental error in the individual tumblers may frequently be large enough to destroy the regularity of the variations due to the gradual interchanging of the three sulphates so that a presentation of the data in full detail would be confusing. The triangular formation has therefore been divided into seven regions, namely, the center, the three corners, and the middles of the three sides. The value for each region has been found by averaging three to six tumblers. It is fully realized that no two tumblers had exactly the same "alkali" treatment, and, accordingly, this mode of presentation may not seem strictly justifiable, but since it has been observed that the average for three glasses in a given area is usually rather close to the average of six or more tumblers at that place, it is felt that this objection is of minor importance. The results are reported on the basis of the average dry weight per plant. The use of this basis neglects the fact that the percentage of germination in the higher concentrations of alkali is very much reduced and therefore the curves do not fall as rapidly as they would on the basis of total dry-weight production. The results seem quite comparable, however, and, as the relations in the higher concentrations are thus more clearly brought out, it has seemed best to adopt this basis. The data are given graphically in figures 2 to 6, each of which consists of four charts. Chart A shows the results of the sulphuric acid treatment with and without sodium carbonate, while B and C give the corresponding data for calcium sulphate and sodium sulphate, respectively. Finally, the two and three component mixtures added to the carbonate soil are shown in Chart D. The data for the untreated carbonate soil is reproduced in all the charts as a heavy unbroken line.

The results of the experiment with sand are given in figure 2. The beneficial action of calcium sulphate (fig. 2, B) and sulphuric acid (fig. 2, A) on the carbonate soil is forcibly shown. The acid is somewhat more efficacious than the calcium sulphate, as would be expected from the fact that the gypsum is too insoluble to be added in solution and hence would react more slowly. Since the sand contained a large quantity of calcium carbonate, some calcium sulphate was doubtless formed on the addition of the acid, and it therefore seems likely that both correc-

tives functioned in the same way. Sodium sulphate showed a very slight ameliorating action at 1,000 parts per million, at which concentration it appears to be decidedly stimulating to plant growth when added alone, but at higher concentrations it increased the toxicity of the carbonate (fig. 5, C). The mixtures of equal parts of two or three sulphates were all somewhat beneficial, but the presence of sodium sulphate noticeably reduced the corrective power of the mixture in proportion to its concentration (fig. 5, D). All the observations that have been made with the sandy soil can be readily explained by the simple ionic reactions between the added substances, and, accordingly, this case is not essentially different from a solution culture.

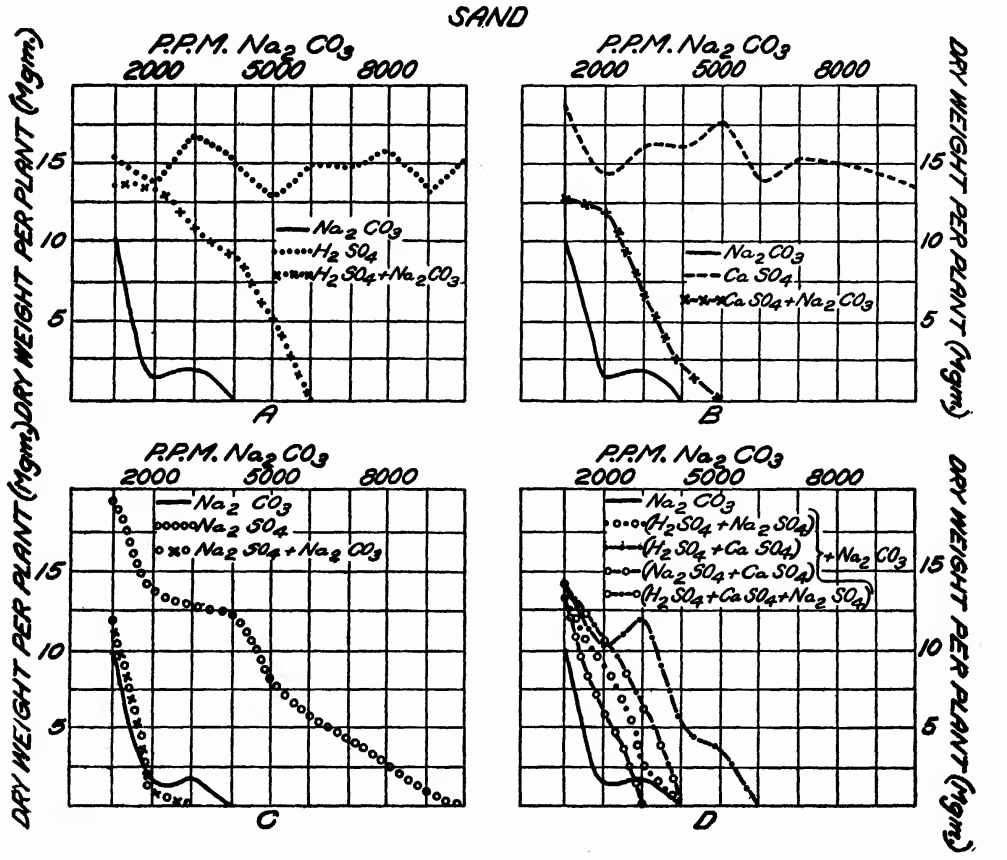

FIG. 2.—Diagram showing the effect on the growth of wheat plants of adding sulphates to sand impregnated with an equal total quantity of sodium carbonate.

In figures 3 and 4 are shown the results obtained when the previously mentioned materials were added to Greenville loam and West Logan clay loam, respectively. Both soils behave similarly and may be conveniently discussed together. As in the case of sand, neither the sulphuric acid nor the calcium sulphate is toxic at any of the concentrations used. The sodium sulphate begins to reduce the growth of the plants appreciably at about 5,000 parts per million, and the mixtures of this salt with sodium carbonate show decided additive toxicity except possibly at the lowest concentrations. For example, the dry weight per plant in Greenville soil is reduced to one-half normal (6.7 mgm.)

by sodium carbonate alone at 7,500 parts per million, by sodium sulphate at 9,500 parts per million, and by a mixture of the two containing 5,000 parts per million each (fig. 3, C). Considering the fact that there is some chemical removal of these salts from the soil solution by the soil material (*13, p. 431*) so that their concentration is really lower than the amount added indicates, these toxicity relations appear to be very nearly additive.

All the other mixtures follow the untreated sodium carbonate curve much more closely, indicating that they have very little, if any, influence on the toxicity of this salt, though the presence of sodium sulphate in

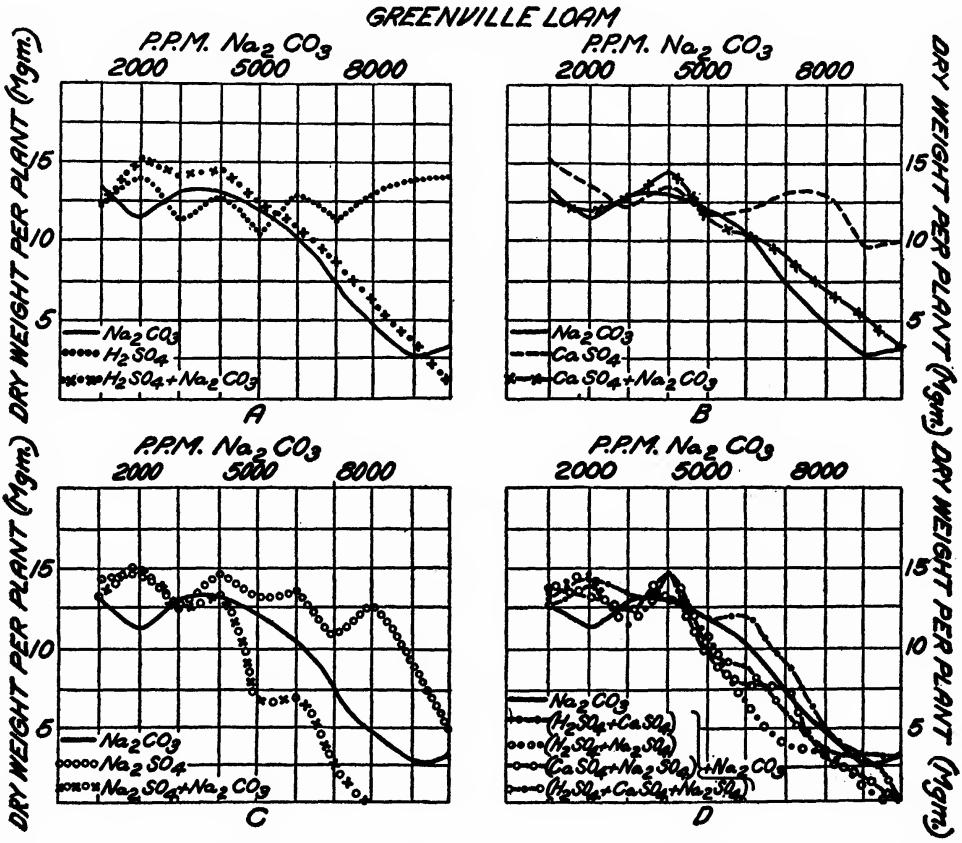

FIG. 3.—Diagram showing the effect on the growth of wheat plants of adding sulphates to Greenville loam already impregnated with an equal total quantity of sodium carbonate.

them is nearly always made manifest by the somewhat lower position of the curve. It is interesting to note that the calcium sulphate when mixed with sodium carbonate does not react in the same way in both soils. In the Greenville soil it shows no harmful effects at all in conjunction with the carbonate, whereas in the West Logan soil it seems to lower the yield slightly. There is certainly no striking evidence of antagonism between any of these substances in either of the two heavier soils.

Figure 5 represents the data for the dry weight per plant obtained by treating the Greenville soil, already impregnated with sodium chlorid, with sulphuric acid, calcium sulphate, and sodium sulphate. The con-

centrations of all these substances ranged from 500 to 5,000 parts per million. The general relations are nearly the same as with the carbonate shown in figures 3 and 4. The simple mixture of sodium chlorid and sodium sulphate shows additive toxicity, while the other mixtures add a much smaller amount or nothing at all to the harmful effects of the chlorid. The three and four component mixtures follow the simpler chlorid curve closely (fig. 5, D). No antagonism for sodium chlorid is evident.

The arrangement of the experiment represented in figure 6 differs from that of figure 5 only in having sodium nitrate substituted for

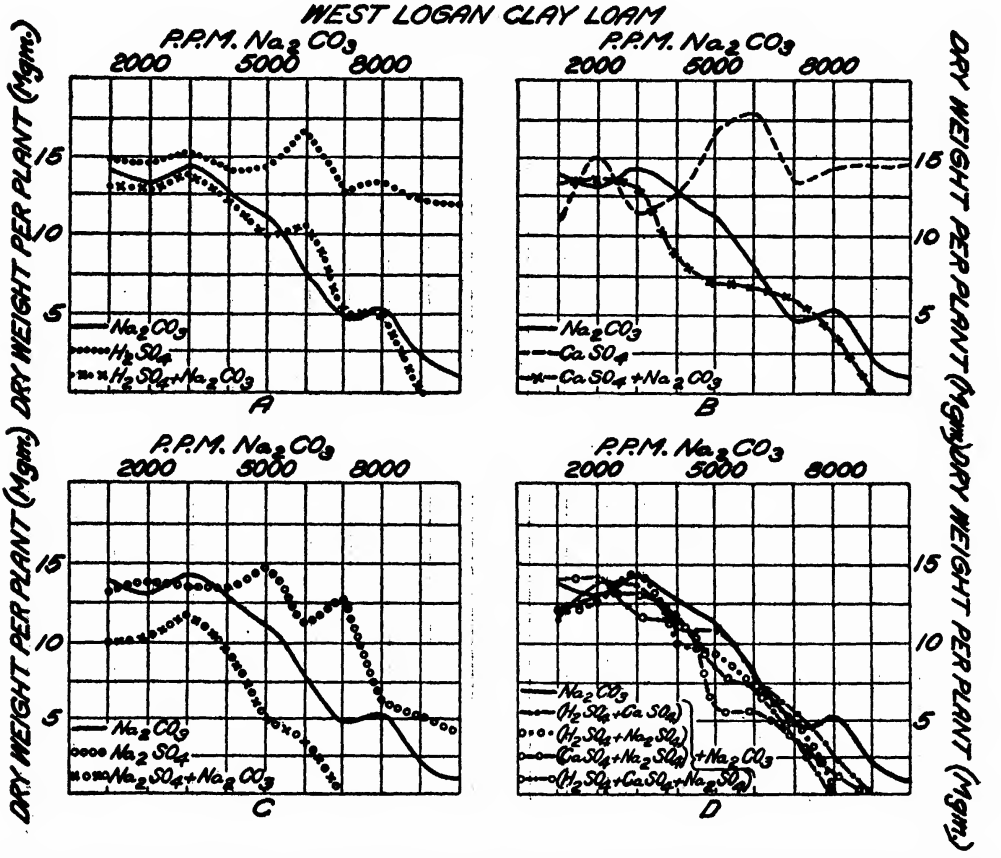

FIG. 4.—Diagram showing the effect on the growth of wheat plants of adding sulphates to West Logan clay loam already impregnated with an equal total quantity of sodium carbonate.

sodium chlorid. The relative shapes and positions of all the curves are so exactly analogous to those in figure 5 that the remarks and conclusions given above apply in this case also.

LATER WORK

With the experience gained in the experiments already described, the investigation of this problem was continued in the summer of 1921. It was decided to concentrate effort on one soil, containing one salt as a base, and to vary the subsequent treatment as much as possible. Accordingly, six different trials were carried out with Greenville loam

as the soil and sodium carbonate as the common salt in all the mixtures. In each trial three different substances were added, so that the effects of 15 materials in conjunction with sodium carbonate have been studied.

The triangular arrangement of the experiment was adhered to, but the number of individual treatments was reduced from 66 to 15, as shown in figure 7. In addition there were included in each triangular arrangement five control treatments in which each of the four individual materials and also distilled water were added to the soil alone. The whole experiment was done in duplicate. The same sodium carbonate treatment was present in five of these triangular groupings in which the concentrations

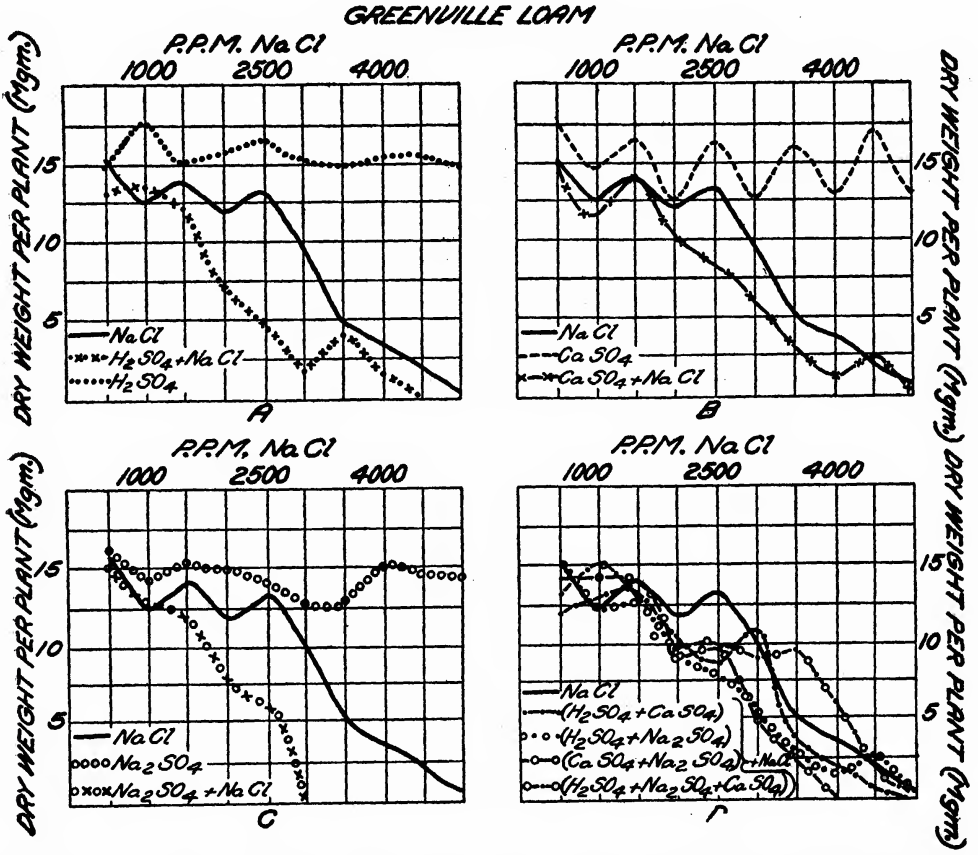

FIG. 5—Diagram showing the effect on the growth of wheat plants of adding sulphates to Greenville loam already impregnated with an equal total quantity of sodium chlorid.

of the other added substances, when they were toxic in themselves, were made to range as nearly as possible from stimulating amounts to decidedly toxic quantities. Four concentrations of sodium carbonate ranging from 1,000 to 7,500 parts per million made up a trial, which thus consisted of 20 units containing 40 tumblers each.

In figures 8 to 13 the results of six trials are presented graphically on the basis of the dry weight per plant. The straight dotted line across all the charts gives the yield secured from the untreated soil, as derived from 40 tumblers. The heavy unbroken line shows the yield from the soil treated with sodium carbonate alone and is the average of 10 tumblers.

It will be noticed that every concentration of sodium carbonate is represented in the figure by three charts containing six curves in each. On

the left is shown the results of mixing the three added substances at their five maximum concentrations with the untreated soil and also with the carbonate soil (fig. 7, tumblers 1, 11, and 15). The center chart represents tumblers 2, 3, 7, 10, 12, and 14 (fig. 7). These contain sodium carbonate and a binary mixture with one of the added substances distinctly predominating. On the right are given the results for tumblers 4, 5, 6, 8, 9, and 13 (fig. 7). These contain the carbonate and both binary and ternary mixtures in which the concentrations are more nearly equal. It should be emphasized that the fractions occurring in the legends to

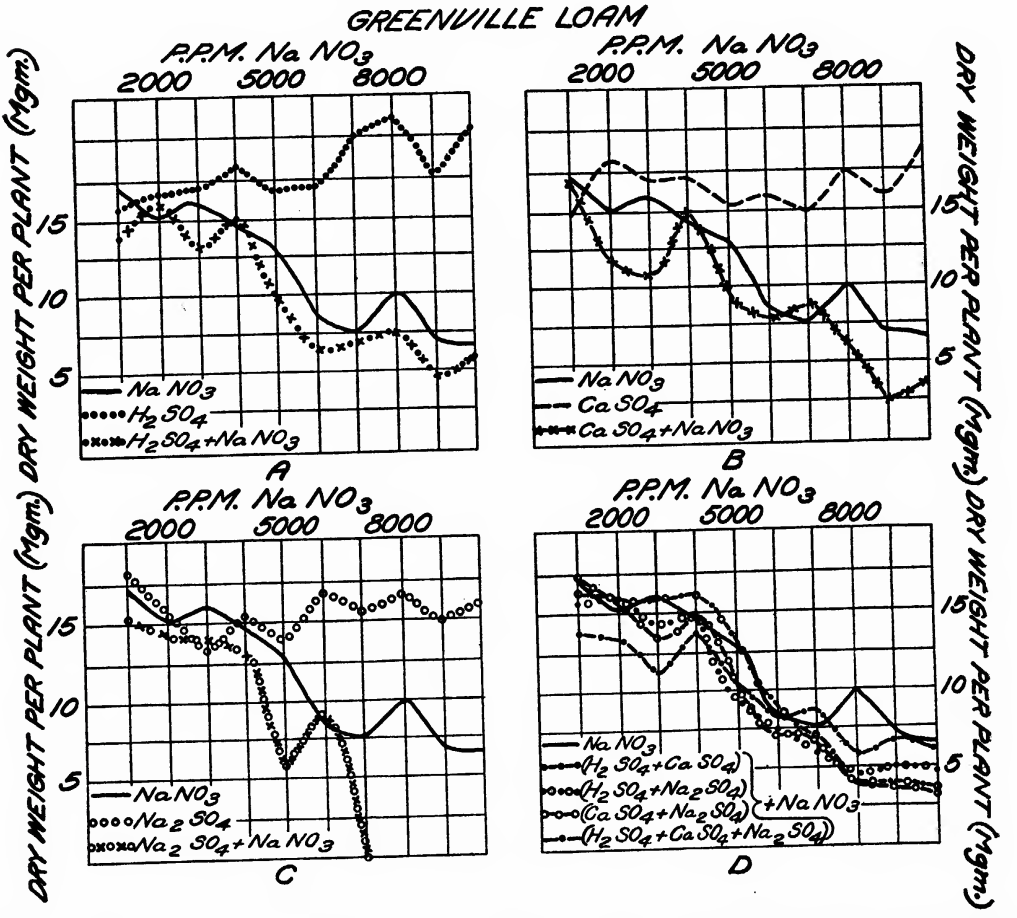

FIG. 6.—Diagram showing the effect on the growth of wheat plants of adding sulphates to Greenville loam already impregnated with an equal total quantity of sodium nitrate.

describe mixtures refer to a proportionate part of the maximum concentration of the unmixed substance and not to the total amount of the mixture. For example, in figure 8, in the second triangle, the maximum quantities of sulphuric acid, potassium sulphate, and sodium sulphate are 4,000, 3,000, and 3,000 parts per million, respectively. Then the mixture one-fourth sulphuric acid, one-half potassium sulphate, and one-forth sodium sulphate in this grouping contains 1,000 parts per million sulphuric acid, 1,500 parts per million potassium sulphate, and 750 parts per million sulphate, or a total of 3,250 parts per million, and it is this total which is plotted in the curves for this mixture.

SULPHURIC ACID, POTASSIUM SULPHATE, AND SODIUM SULPHATE

The concentrations used in this trial (fig. 8) are as follows:

	Parts per million at concentration—				
	1	2	3	4	5
H_2SO_4	2, 000	4, 000	6, 000	8, 000	10, 000
K_2SO_4	1, 000	3, 000	5, 000	8, 000	10, 000
Na_2SO_4	1, 000	3, 000	5, 000	8, 000	10, 000
Na_2CO_3	1, 000	2, 000	4, 000	6, 000	.....

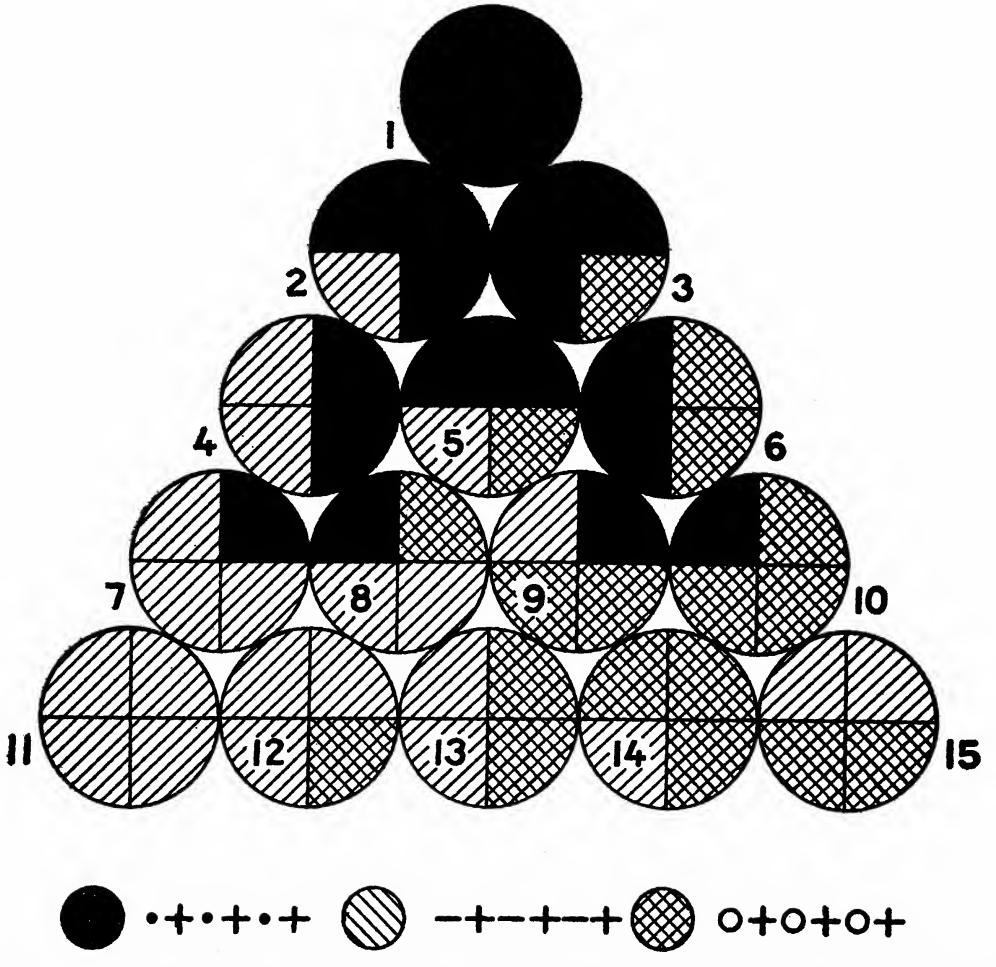

FIG. 7.—Diagram showing the arrangement of the tumblers in the final experiments. A uniform concentration of sodium carbonate was present in all the tumblers and the treatment was added as indicated by the shading. The maxium concentrations used are given in the text.

When added to the untreated soil, the potassium sulphate is only slightly toxic at 8,000 and 10,000 parts per million; the sodium sulphate is very detrimental to plant growth at 8,000 parts per million, and the acid is not harmful at all at 10,000 parts per million. This is indicated by the left section of figure 8, which also shows that the presence of sodium carbonate in the soil lowers the positions of these curves in proportion to its amount. The divergence of these pairs of curves due to

sodium carbonate also increases somewhat as the concentration of the sulphates increase. It is curious that this divergence should be appreciable when the quantity of carbonate present is not sufficient to show

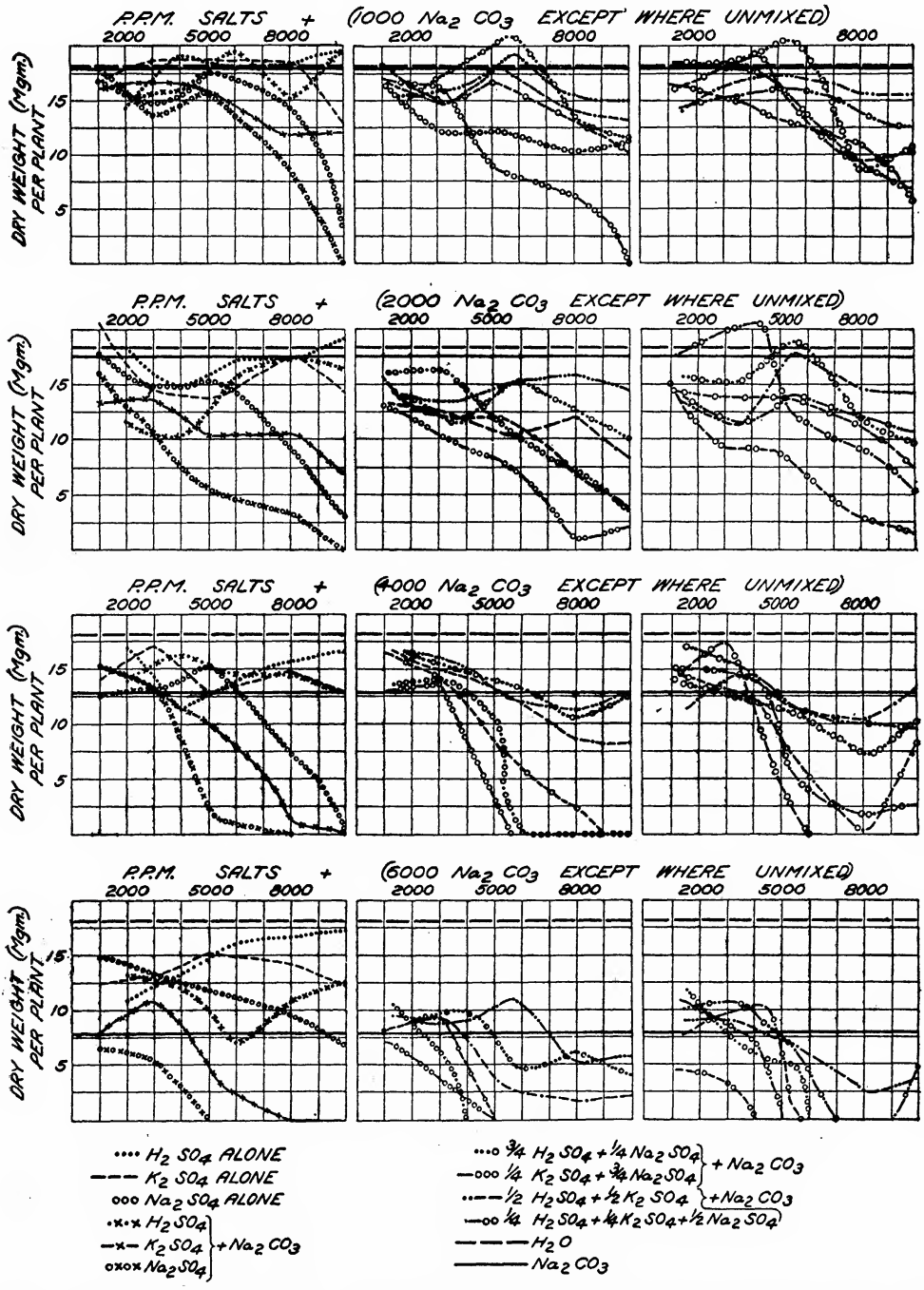

FIG. 8—Diagram showing the effect on the growth of wheat plants of adding sulphates in various proportions to Greenville loam already impregnated with sodium carbonate. The maximum concentrations of the individual sulphates are given in the text.

any toxicity, and as this condition does not obtain in the other trials it may be due to experimental error in this case. The data are not exact enough to permit quantitative relations to be drawn with certainty, but they seem to indicate that when 5,000 parts per million or more of

the sulphates are present the divergence of the curves is roughly equal to the toxicity of the carbonate.

The values for the binary and ternary mixtures given in the middle and right sections of figure 8 arrange themselves in fairly regular order

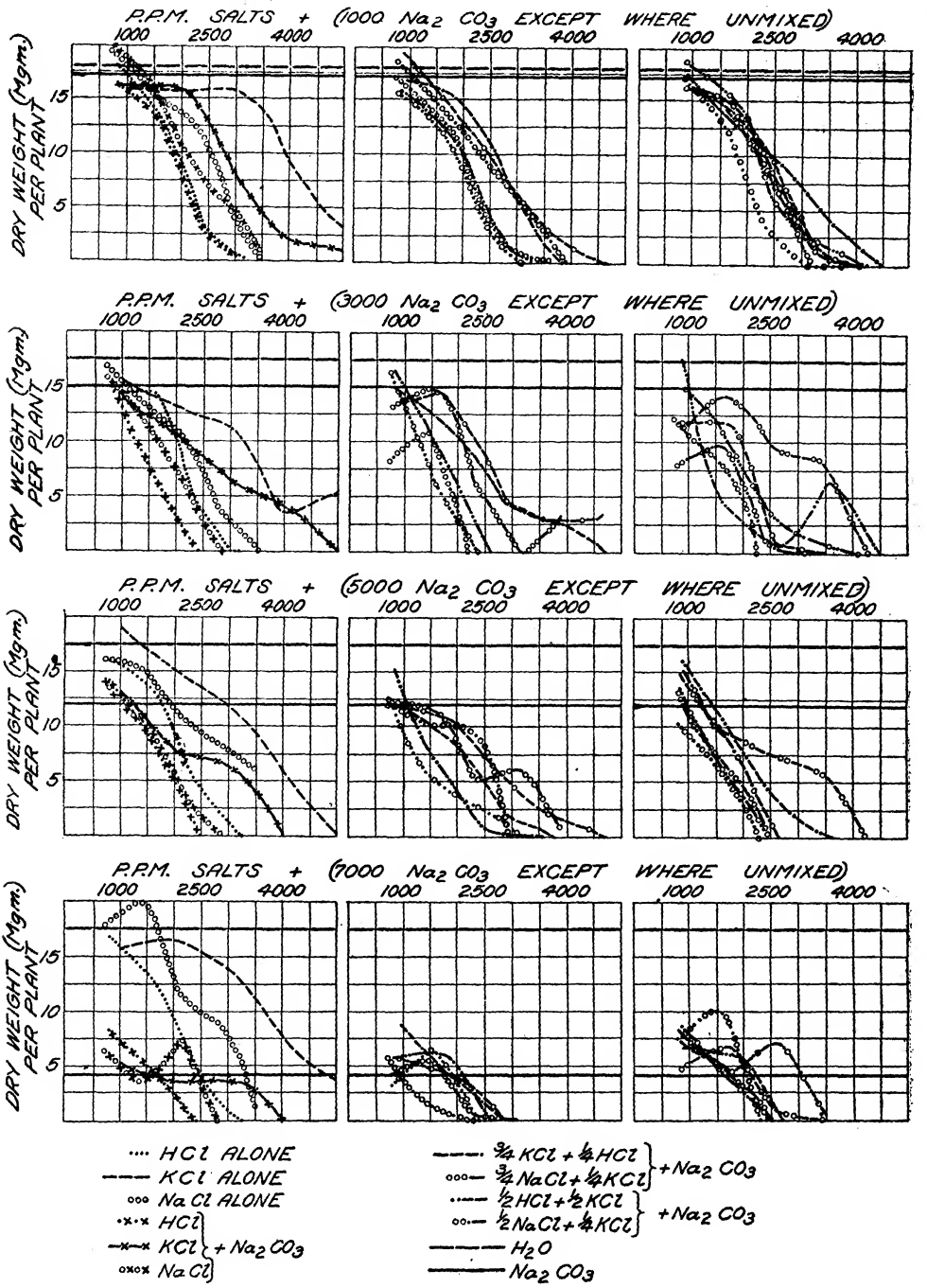

FIG. 9.—Diagram showing the effect on the growth of wheat plants of adding chlorids in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual chlorids are given in the text.

in accordance with the separate toxicities of the components, the individuality of these materials being essentially maintained even in the most complicated mixtures.

It should be particularly noted that the addition of nontoxic amounts of sulphates (up to 3,000 to 5,000 parts per million) to the soil containing appreciably harmful concentrations of carbonate almost invariably causes the growth of the plants to be somewhat better than with the carbonate alone. Three exceptions to this rule appear when sodium sulphate alone and also two mixtures of this salt with potassium sulphate are added to the most toxic black alkali soil. A distinct corrective action or antagonism for sodium carbonate is thus indicated, since no marked stimulation or corrective action is apparent when these substances are added to the untreated soil or to the practically nontoxic carbonate soils.

HYDROCHLORIC ACID, POTASSIUM CHLORID, AND SODIUM CHLORID

The concentrations used in this trial (fig. 9) are as follows:

	Parts per million at concentration—				
	1	2	3	4	5
HCl	800	1,600	2,400	3,200	4,000
KCl	1,000	2,000	3,000	4,000	5,000
NaCl	700	1,400	2,100	2,800	3,500
Na_2CO_3	1,000	3,000	5,000	7,000	.....

The order of increasing toxicity of these substances is potassium chlorid, sodium chlorid, and hydrochloric acid. This order is maintained quite consistently throughout all the mixtures in the trial. The acid probably exerts its harmful properties through being changed to calcium chlorid. This salt is nearly as toxic as sodium chlorid, weight for weight, and the deficiency is more than made up by the fact that two parts of acid produce three parts of calcium chlorid.

It is to be noted again that sodium carbonate at 3,000, 5,000, and 7,000 parts per million lowers the yield of the single chlorid treatments by its own toxicity. A slight correction of the harmful effects of both 5,000 and 7,000 parts per million of the carbonate has been brought about by the lowest concentrations of the chlorids; but this was very slight indeed in the former case, due to the fact that the sodium carbonate was less harmful than usual in this instance.

NITRIC ACID, POTASSIUM NITRATE, AND SODIUM NITRATE

This treatment (fig. 10) contains the following concentrations:

	Parts per million at concentration—				
	1	2	3	4	5
HNO_3	1,000	2,000	3,000	4,000	5,000
KNO_3	1,000	2,000	3,000	4,000	5,000
$NaNO_3$	1,000	2,000	3,000	4,000	5,000
Na_2CO_3	1,000	3,000	5,000	7,000	.....

The nitric acid is the most toxic material in this trial, as was the hydrochloric acid in figure 9, and the potassium salt is the least harmful. The general similarity of the nitrate and chlorid curves is evident, and

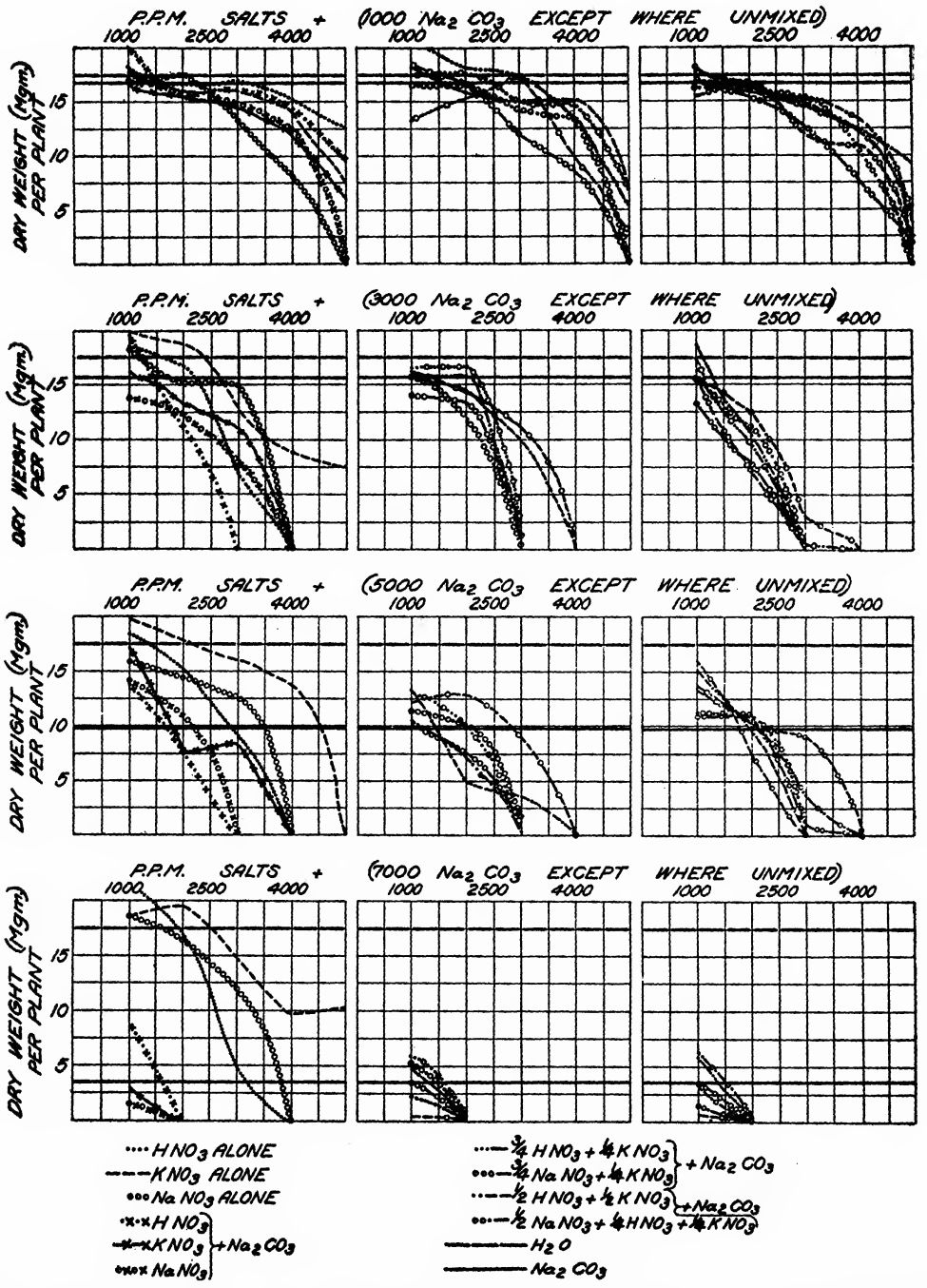

FIG. 10.—Diagram showing the effect on the growth of wheat plants of adding nitrates in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual nitrates are given in the text.

the same conclusions may be drawn concerning the additive toxicity of the carbonate and the corrective action of small quantities of the nitrates on this salt. In this case, however, the antagonistic action is fairly large in the third carbonate concentration and negligible in the fourth.

A special point of interest in this trial is the fact that both potassium nitrate and nitric acid are distinctly stimulating to plant growth at 1,000 parts per million. It should also be noted that their corrective action at 1,000 parts per million, as shown in the first and second rows of figure 10 as well as in the third row with 5,000 parts per million carbonate, is appreciably greater than in any of the other diagrams where a nontoxic concentration of an inorganic substance is used, calcium sulphate excepted. Accordingly, their beneficial action in this connection may be due, at least in part, to an increasing of the vigor of the plant growth rather than to the specific reduction of the toxicity of the carbonate which seems to take place in cases of antagonism in solution cultures. Nitric acid and potassium nitrate give promise of real utility in correcting small and moderate toxicities of sodium carbonate in soil.

SODIUM CHLORID, SODIUM NITRATE, AND SODIUM SULPHATE

The concentrations of the salts in this trial (fig. 11) are as follows:

	Parts per million at concentration—				
	1	2	3	4	5
$NaCl$	700	1,400	2,100	2,800	3,500
$NaNO_3$	1,000	2,000	3,000	4,000	5,000
Na_2SO_4	1,000	3,000	5,000	8,000	10,000
Na_2CO_3	1,000	3,000	5,000	7,500	.....

The salts are arranged in order of decreasing toxicity in the table. The results of the experiment are in agreement with the general observations which have already been made on the additive toxicity of the carbonate. Nontoxic quantities of all the salts, particularly sodium sulphate, increase the yield in the presence of 5,000 parts per million carbonate, but 7,500 parts per million seems to be so toxic that relief is impossible.

DISODIUM PHOSPHATE, SODIUM ARSENITE, AND BORAX

These miscellaneous substances (fig. 12) were added in the following quantities:

	Parts per million at concentration—				
	1	2	3	4	5
$Na_2B_4O_7$	140	280	420	560	700
Na_3AsO_3	194	388	582	776	970
Na_2HPO_4	1,500	3,000	4,500	6,000	7,500
Na_2CO_3	1,000	3,000	5,000	7,000	.....

The borax and arsenite are 10 times as toxic as the phosphate and they are therefore plotted in figure 12 with one-tenth the phosphate scale. Under these conditions the borax and phosphate curves are nearly coincident, but they are somewhat higher than the arsenite curve.

It is unfortunate that the initial quantity of the latter salt was so large, because no information is afforded concerning the nontoxic concentrations of sodium arsenite, except inferentially from the complex mixtures.

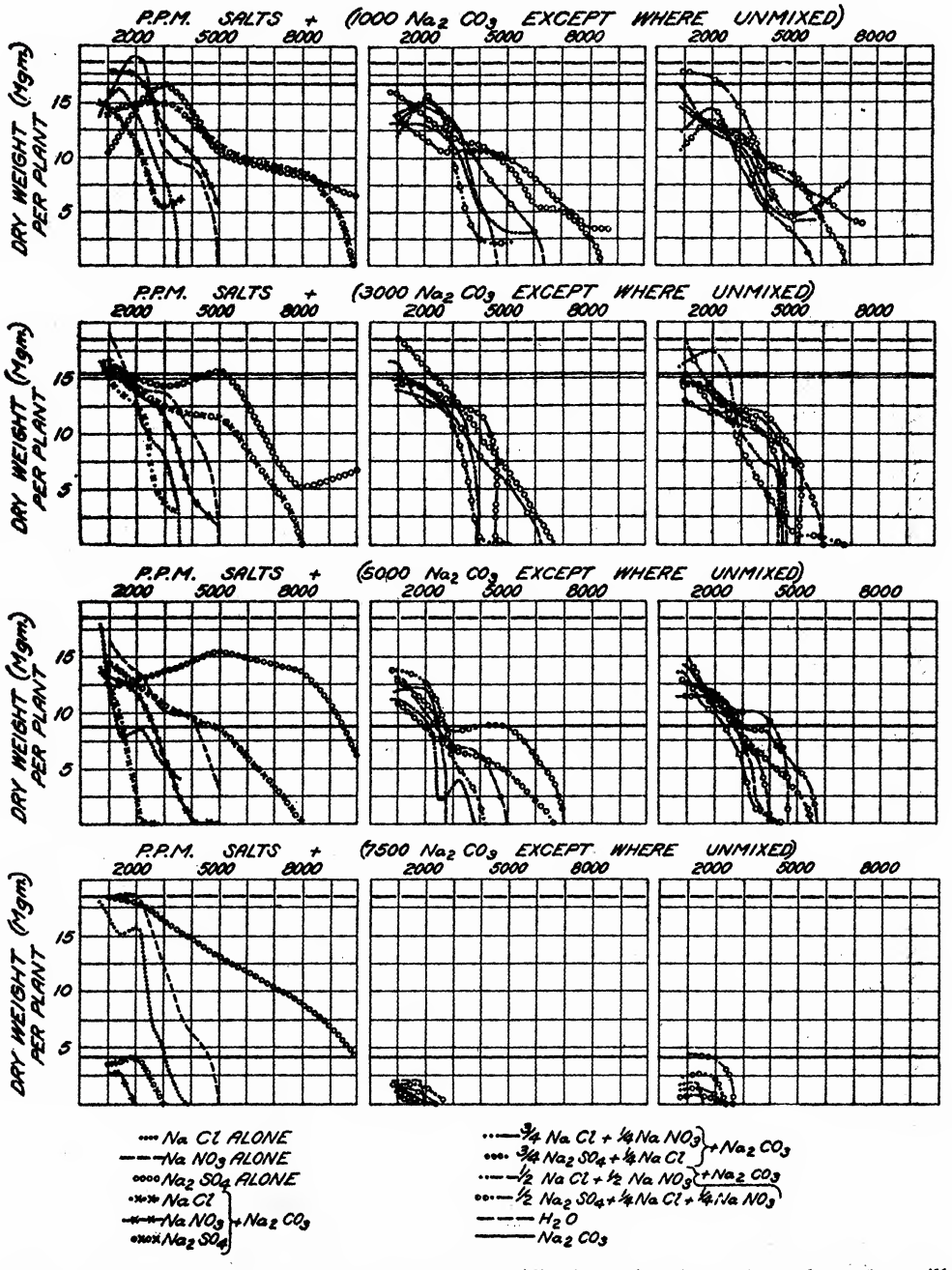

FIG. 11.—Diagram showing the effect on the growth of wheat plants of adding sodium salts to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual salts are given in the text.

The usual regular arrangement of the mixture curves and also the additive toxicity of the carbonate is evident in the diagram. No corrective action on black alkali, however, is shown in this experiment.

The physiological action of the borax and arsenite on the wheat plants was so striking and so characteristic that it should be mentioned here. Borax caused the leaves of the plants to remain closed up around

the stock, which then twisted itself into curious shapes, at the same time exhibiting a distinct chlorotic condition. The intensity of these symptoms varied with the concentration of the borax. They were dis-

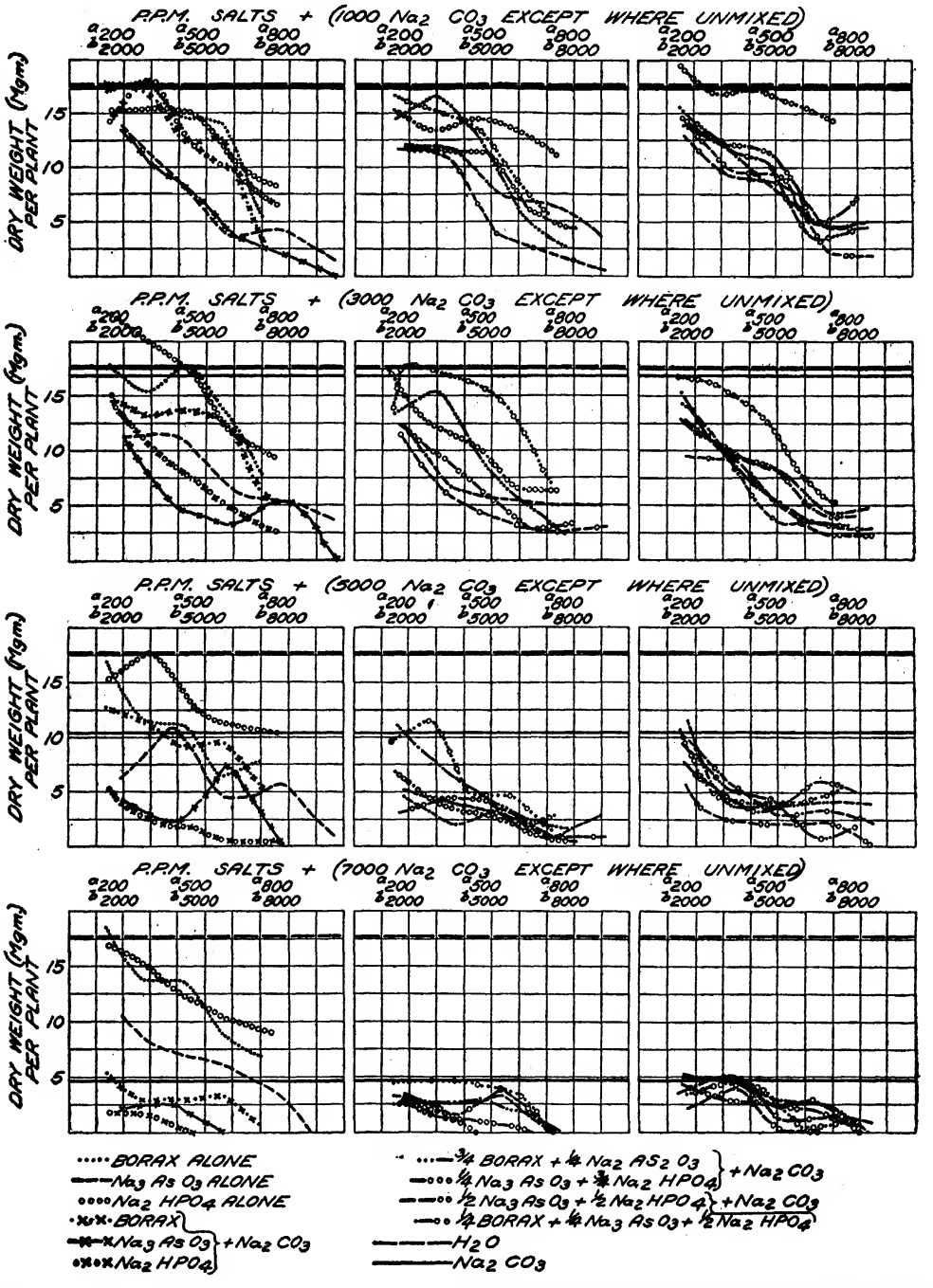

FIG. 12.—Diagram showing the effect on the growth of wheat plants of adding miscellaneous sodium salts in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual salts are given in the text.

[a] This reading applies for a sodium arsenite or borax mixture or where used alone.

[b] This reading applies for sodium phosphate mixtures or where used alone.

cernible with as little as 70 parts per million, though at the lowest concentrations they tended to disappear as the plant grew. The arsenical poisoning was characterized by a stunted growth, straight, leafless stock, and a dark, unhealthy color.

CALCIUM SULPHATE, SULPHUR, AND MANURE

For the sake of completeness three well-known black alkali amendments were included in the experiment. Since none of them were toxic, the concentrations were made to range beyond the limits set by agricultural practice:

	Parts per million at concentration—				
	1	2	3	4	5
$CaSO_4$	1,000	3,000	6,000	10,000	20,000
Sulphur	200	1,000	3,000	6,000	10,000
Barnyard manure	2,000	5,000	10,000	15,000	20,000
Na_2CO_3	1,000	2,000	5,000	7,000	.....

The materials were mixed with the soil in a dry, powdered condition before the water was added. With the larger quantities of manure the soil was somewhat deficient in moisture because of the larger absorptive power of this treatment for water. The results for manure can not, therefore, be taken as final, since under other moisture conditions a different behavior might be expected.

The experiment (fig. 13) shows that all three added substances are somewhat stimulating alone and that all have the power of counteracting partially the toxicity of sodium carbonate. This corrective action is accomplished in the lower carbonate treatments about equally well by all three materials, but with sodium carbonate at 7,500 parts per million calcium sulphate is the most effective and even seems to be a necessary component of the amending mixture. It should be noted that curves with 7,500 parts per million sodium carbonate represent average values for two entirely independent but closely concordant experiments.

SUMMARY

(1) The toxicity relations for wheat of certain alkali salts alone and in combination with each other have been investigated with special reference to the alleviation of black alkali trouble.

(2) It has been shown that when a given concentration of sodium carbonate in sand is treated with an equal quantity of calcium sulphate or sulphuric acid, an appreciable lowering of the carbonate's toxicity is evident. This relation, however, is not so manifest in heavier soils. Sodium sulphate increases the toxicity of the carbonate under the same circumstances.

(3) Either sodium chlorid or sodium nitrate could be substituted for sodium carbonate in the arrangement described above without changing the results. No antagonism between these salts and the sulphates could be detected in Greenville loam.

(4) During 1921 each one of four different concentrations of sodium carbonate, already incorporated in the Greenville soil, has been treated with five concentrations of the following substances, in groups of three:

(1) Sulphuric acid, potassium sulphate, and sodium sulphate.
(2) Hydrochloric acid, potassium chlorid, and sodium chlorid.
(3) Nitric acid, potassium nitrate, and sodium nitrate.
(4) Sodium chlorid, sodium sulphate, and sodium nitrate.
(5) Sodium arsenite, sodium phosphate, and borax.
(6) Calcium sulphate, sulphur, and barnyard manure.

(5) The toxicities of all the substances in the first five groups are increased by the specific toxicity of sodium carbonate when they are added to soil already impregnated with this salt.

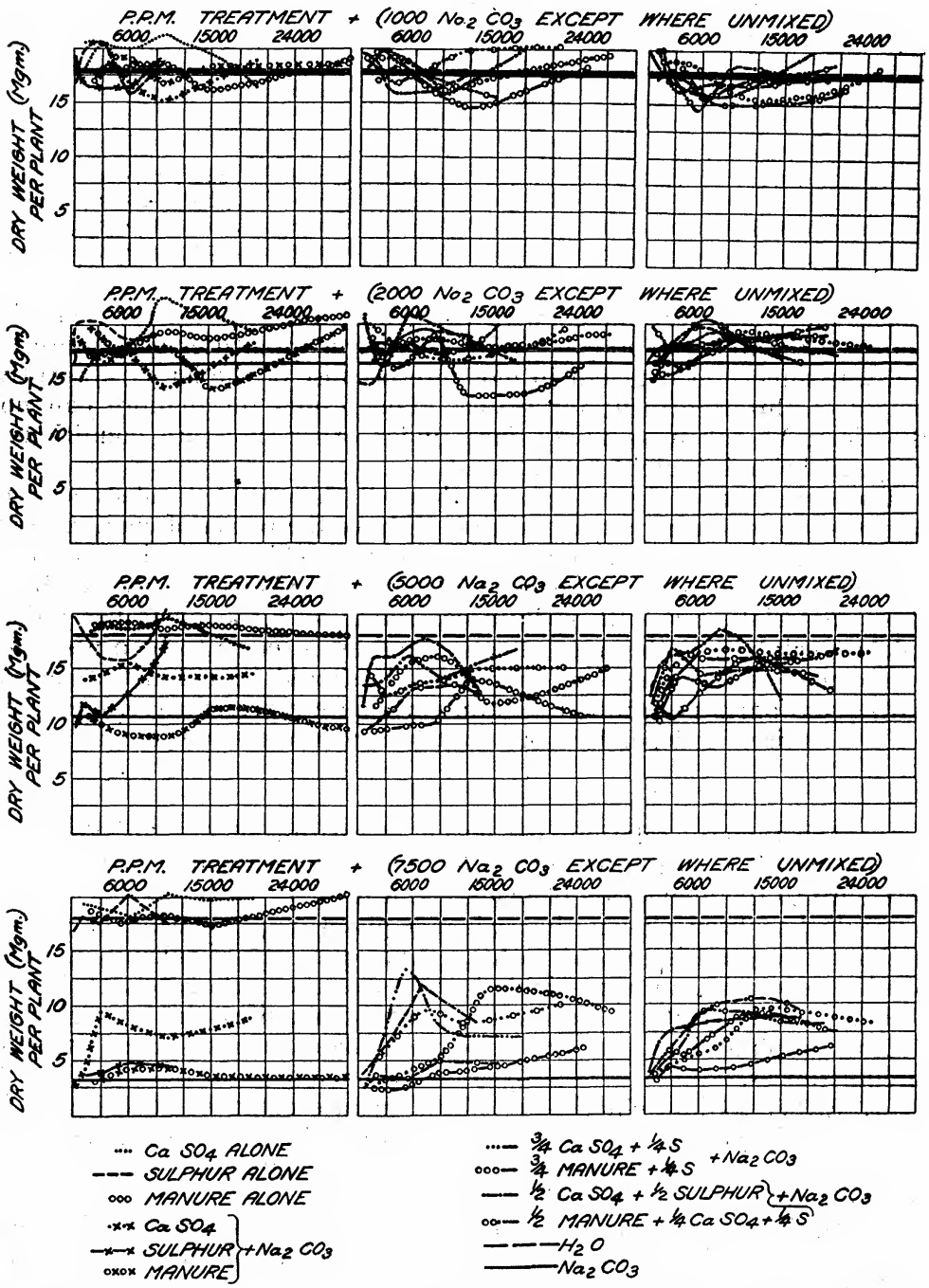

FIG. 13.—Diagram showing the effect on the growth of wheat plants of adding manure, sulphur, and gypsum in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual added substances are given in the text.

(6) Nontoxic quantities of all the substances in the first four groups have the power of correcting, to some extent at least, the harmful effects of moderate, and in some cases of fairly high, concentrations of sodium carbonate. The salts in the fifth group do not exhibit this property at

all. This phenomenon may be due in part at least to specific stimulation of plant growth by these substances rather than to any antagonistic action on the sodium carbonate which would lower the toxicity of that salt as is frequently observed in solution culture.

(7) Potassium nitrate and nitric acid are both distinctly stimulating to plant growth at 1,000 parts per million, and the addition of this quantity of these materials to soil containing 5,000 parts per million sodium carbonate or less was particularly beneficial.

(8) Under the conditions of the experiment, barnyard manure was an effective amendment for soil containing 2,000 parts per million sodium carbonate, but its corrective power for more toxic concentrations of black alkali was much less evident, probably because the optimum moisture content of the soil was not maintained when the larger amounts of manure were added.

(9) Calcium sulphate, alone and in conjunction with sulphur and manure, is the most effective corrective that was tried, particularly on the more toxic black alkali soils.

(10) The curious physiological effects of borax and sodium arsenite on the wheat plants have been noted.

(11) While the data herein presented throw some light on the antagonistic action of various alkali salts, it is evident that the problems of coping with black alkali is far from being solved.

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IDENTIFICATION OF CERTAIN SPECIES OF FUSARIUM ISOLATED FROM POTATO TUBERS IN MONTANA [1]

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INTRODUCTION

The economic importance to this State of Fusarium wilt and various storage and field rots of potatoes has been recognized for several years. Between 1914 and 1918 isolations were made from affected tubers until nearly 100 cultures had been obtained. These isolations have been cultured on artificial media, being transferred about twice a year and kept in a refrigerator at about 10° C.

The purpose of this paper is to record the taxonomic work on these various Fusarium cultures. No especial endeavor was made to connect any of these species with an ascigeral stage, and in all our work no culture showed any form of growth suggesting such a stage.

SOURCE OF MATERIAL

The tubers from which the isolations were made came from many different localities in the State. In fact, practically all sections except the extreme northwestern are represented. The exact source of each culture will be found in Table I. Of the 97 original isolations only 70 were used.

There is little doubt that dryrot and Fusarium wilt are of large economic importance to all potato growers in the State. In 1917, this Station reported in the plant disease survey a loss of 4 per cent in the potato crop due to wilt and a loss of 3 per cent due to dryrot. The year 1917 is considered a normal one. These figures therefore represent the average yearly loss due to potato diseases caused by species of Fusarium. This loss is fairly evenly distributed over the State.

LITERATURE REVIEWED

A great deal of work has been done and a number of papers have been written on Fusarium troubles of potatoes, but the viewpoint in nearly all of this work is economic. However, in reporting on the economic phase many of the authors include some taxonomic data, and for this reason the following reviews are given. Occasional comment on individual papers has been made, but this has not been done consistently throughout. For convenience the various papers are taken up in chronological order.

Smith and Swingle (*19*) [2] found that there was always present in the darkened vascular bundles of tubers affected with wilt, which had not at that time been separated from dryrot, a fungus which on culturing they found to be a species of Fusarium. In order to determine the characters of this fungus they grew it on about 40 different media and under various temperature conditions. The bulletin gives in detail the results of these

[1] Accepted for publication July 24, 1922.

[2] Reference is made by number (italic) to "Literature cited," p. 363–364.

studies. To this organism the authors applied the name *Fusarium oxysporum* Schlechtendal because they felt it "not at all certain" that the various names given to species of Fusarium growing on potatoes really stood for distinct forms. They therefore considered them as synonyms and used the earliest available name. This paper was the pioneer for Fusarium work in this country, and in addition to the careful work done, mycologists and pathologists are greatly indebted to its authors for the impetus given to the study of potato Fusaria, both in this country and abroad.

In 1910 Appel and Wollenweber (*2*) published a basis for a monograph of the genus Fusarium Link. This paper was the first to give a comparatively exhaustive treatment of the species of Fusarium. It is divided into two parts. In the first part the following subjects are discussed in detail: Methods, including media, inocula, nutrients, light, temperature, color standards, variation in forms appearing in the cultures, mycelium, etc., lack of distinction between microconidia, and macroconidia and characters which constitute a "normal" culture or "normal" spore. These authors give a description of the genus Fusarium, discuss its relationships, and list its synonomy.

The second part relates entirely to taxonomy. Thirteen known species are described with the greatest care and detail, each description being the record of a research problem in itself. This paper is, without doubt, the most fundamental in the literature on Fusarium. It is not here reviewed in proportion to its worth, because our experimental work was not directly influenced by it. The paper was published in Germany 10 years ago and did not include many of the common American species. Therefore, it was not well fitted for our identification work. The paper contains a very good bibliography of the Fusarium problem.

In 1912 Jamieson and Wollenweber (*9*) described the symptoms of a disease causing dryrot of potato tubers, first noted on tubers sent from Spokane, Wash., in February, 1910. To the causal organism of this disease Wollenweber gave the name *Fusarium trichotheciodes*, placing it in the Discolor group. Inoculation experiments proved the pathogenicity of this organism in producing the characteristic dryrot of tubers.

In 1912 Wilcox, Link, and Pool (*20*) published a research bulletin in which they described a rot which is practically identical with that described by Jamieson and Wollenweber (*9*), but due to the fact that the two papers were published so close together and that the investigational work was being carried on simultaneously and independently, these authors gave to the casual organism the name *Fusarium tuberivorum* Wilc. and Link, which we know now to be a synonym of *F. trichothecioides* Wollenw. Wilcox, Link, and Pool's paper is of particular value to our work because of its emphasis on taxonomy.

This paper gives the history and distribution of the dryrot of tubers and the economic importance and symptoms of the particular dryrot under discussion. The authors give a résumé of the genus Fusarium and allied genera from 1809 to date, concluding with Appel and Wollenweber's description of it.

Details are given concerning the technic for studying the fungus, such as temperature, light, single-spore isolation, and color standard; and a discussion is made of macroscopic characters, mycelium, influence of temperature, influence of humidity, color characteristics, conidiophores, chlamydospores, spore measurements, and spore septations. Considerable space is given to the last-mentioned topic. The importance of

various conditions which might influence the number of septations is discussed, and the results of actual investigations are recorded in detail.

In summarizing the taxonomic portion of the paper the authors endeavor to find a logical place for their species, and after several suggestions and comparisons they leave it unplaced, but give a detailed description of the organism. Some pathological studies are discussed with emphasis on the mode of infection, resistance, and susceptibility of varieties and methods of control.

Wollenweber (*21*), studying the genus Fusarium taxonomically, came to a few definite conclusions: The stroma as a taxonomic character in species determination is unreliable. A pure culture method which gives the normal stages is necessary, and the culture media recommended was steamed stems of trees, shrubs, or herbaceous plants for conidia and chlamydospore production and rice, potato tubers, and other starchy media for secondary characters, such as color, extra large sclerotia, and stromata. He is not sure that Fusarium is an obligiate conidial stage of an ascomycete. He puts much emphasis on the importance of the proof of pathogenicity of the organisms, and in his discussions of each species he indicates the kind of parasite.

Wollenweber was the first to assemble into sections species of Fusarium having related characters. He considered a uniform shape of conidia the most important of the characters on which the division could be based. The sections which he described in this paper have been used in all taxonomic work with this genus since that time. Other sections, of course, have been added, but the original sections have for the most part never needed to be amended.

The 20 species of Fusarium described, of which only 3 are new, are grouped under the sections, practically all of which include wound parasites capable of destroying parenchymatous tissue, except the first, which includes vascular parasites only. Wollenweber's sections in their order are: Elegans, Martiella, Discolor, Gibbosum, Roseum, and Ventricosum.

Later Wollenweber (*22*), in morphological and pathological study of the divisions of fungi having cylindrical and crescent-shaped conidia, states definitely that fungi with cylindrical septate conidia fall outside the genus Fusarium, and belong, when the perfect form is known, to the genera Nectria, Hypomyces, and Mycosphaerella, and, when the perfect form is not known, to Cylindrocarpon when chlamydospores are absent and to Ramularia when they are present.

Lewis (*11*), working in Maine, carried on comparative studies with some 40 different cultures of Fusarium isolated from various hosts, including 7 isolated from potatoes. He noted how the growth of the cultures was effected by different media, different quantities of acid and alkali, and different temperatures. He tested for gas in fermentation tubes, but obtained negative results only. Tests of pathogenicity were carried on with all of the cultures, and considerable cross inoculation work was done. He made no attempt to identify the species, because the published descriptions were so incomplete as to make critical comparisons with them impossible. However, Wollenweber was in the United States at that time, and Lewis sent his cultures to Wollenweber, who identified most of them. His results were added as an appendix to the bulletin.

Four of these species—*Fusarium poae, F. helianthi, F. conglutinans*, and *F. pirium*—do not later appear in the literature as occurring on potato. Sherbakoff (*18*) says of the first two that they are closely

related to *F. sporotrichioides* n. sp. and belong to the section Sporotrichiella; the third species belongs to section Elegans and is closely related to *F. orthoceras;* and the fourth may belong to section Arthrosoriella. Sherbakoff does not recognize any of these species but disposes of them all in saying:

> No technical description, except results of inoculations for potatoes, always negative, and certain characters of color and of colony growth, is given, and thus a proper identification is rendered impracticable.

Harter and Field (*7*) from the results of their work on the stemrot of the sweet potato concluded, as Appel and Wollenweber (*2*), that the type of inoculum—mycelium or spores—has a marked influence on the culture. They also proved the pathogenicity of *Fusarium hyperoxysporum* Wr. and *F. batatis* Wr. for the sweet potato and obtained negative results in their inoculation experiments with *F. oxysporum* Schlecht., *F. orthoceras* Ap. and Wr., *F. caudatum* Wr., and *F. radicicola* Wr. on the same host.

Wollenweber (*23*) discussing the species of Fusarium occurring on sweet potatoes, points out the necessity of agreeing on the criteria by which a "normal" culture may be known, to avoid wide discrepancies in describing what is in reality the same species. He describes 11 species of Fusarium, two species of Hypomyces and one of Gibberella occurring on sweet potatoes. This included all the species of Fusarium then known to occur on sweet potatoes. Under each species is given a "diagnosis" or description of the type culture, habitat, and a general discussion of its history and relationships. In the case of new species, of which there are 6, the relationships are taken up with a great deal of care.

A descriptive key to all these species is included based upon the characteristics of pure cultures grown in daylight. Regarding the key the author makes the following comment:

> This key might have been based entirely upon the morphological characters and curvature of the conidia but since the color reactions offer a simpler, though less trustworthy means of identification, they have been employed. This key, therefore, should be regarded only as an aid in identification, not as a guide to the morphology, which has been discussed in the diagnosis and illustrated in detail in the illustrations.

Sherbakoff (*18*) realizing the chaotic condition of the genus Fusarium, especially those species occurring on potato, conducted a research problem to discover on what basis the American species as well as those discussed by Wollenweber could best be separated.

In general, Sherbakoff verified the principles and the results of Appel and Wollenweber's (*2*) work in Germany. He believed, however, disagreeing with Appel and Wollenweber, that there should be a distinction drawn between microconidia and macroconidia, and that the presence or absence of the microconidia may be used in distinguishing species. He also disagrees with Appel and Wollenweber in believing that species can be distinguished—

> when grown on almost any medium, including artificial media, provided that the medium is not extremely poor or rich in food materials, and also provided that the moisture supply in the medium is well regulated.

Sherbakoff found no coremia nor typical pionnotal form of fructification as did Appel and Wollenweber.

The author outlined the scope of the work, discussed the source of material and methods of isolation, culture media, effect of light and temperature, variability in the species of Fusarium, relative taxonomic

importance of different characters, defined certain forms of fructification, reviewed the genus Fusarium, and explained the difficulties of identifying species of Fusarium with previously described species because of the scarcity of taxonomic detail in the descriptions. The greater part of the memoir is taken up by the description of sections, genera, species, and varieties. He listed 20 previously described species, including parasitic and nonparasitic forms—all that were known to occur on potatoes—and 41 new species and varieties. These 61 forms he distributed under Wollenweber's eight sections and three additional ones that he himself originated and defined. Three species of Ramularia, a genus closely related to Fusarium and also occurring on potatoes, are included. There are drawings of practically all species and varieties, showing conidia, conidiophores, and occasionally mycelium. Spore measurements are given for spores grown on various culture media.

Based on the ideas of Appel and Wollenweber, Sherbakoff (*18*) worked out a dichotomous key for the species of Fusarium described, which, while the best yet published, is far from perfect. Imperfections in keys are rather inevitable until methods used in the identification of the species are better standardized. Until standard methods are adopted, the boundaries of species can not be closely enough defined to prevent investigators from introducing numerous varieties, separating one from another on minor characters that are not stable under all conditions.

In the key, Sherbakoff uses septations and shape of conidia most often as differential characters. Presence or absence of microconidia, chlamydospores, sclerotia, sporodochia, type of fructification, and color and type of conidiophores are also used. Difficulty with the key arises most often in the case of varieties. Individual difficulties of this sort will be noted later. Confusion often arises from the misuse of the terms macroconidia and microconidia, but this may be due to typographical errors.

C. W. Carpenter (*4*), in a paper on tuber-rots caused by species of Fusarium, includes a section on taxonomy which gives the description of eight species. Among these one is new, *F. eumartii*, which falls into the section Marteilla.

Link (*13*) shows some very interesting results from physiological studies on *Fusarium oxysporum* Schlect and *F. trichothecioides* Wr. Comparisons of the two species are given to show temperature relations, growth, habit, and food requirements and pathogenicity to tubers and growing plants.

Pratt (*15*), in a paper on control of powdery dryrot caused by *Fusarium trichothecioides* Wr., concludes that this species is of the highest economic importance of all of the Fusarium species in the irrigated portion of the West, and in another paper (*14*) notes that *F. radicicola* Wr. is rather common in desert soils.

Hawkins (*8*), in studying the effect of certain species of Fusarium on the composition of the potato tuber, found that *Fusarium oxysporum* and *F. radicicola* secrete sucrass, maltase, xylase, and diastase. The last-mentioned enzym is apparently incapable of acting on the ungelatinized potato starch. The purpose of the study was to find out what constituents of the potato are most easily destroyed by the fungus and what compounds can not be utilized by it either in respiration or in building its own tissues. Their results are not conclusive as to whether kind or quantity of secretion is in the least specific, as only three organisms were used in their experiments, but this article introduces an interesting phase of the Fusarium problem.

Pratt (*16*), in studying the relation between soil fungi and diseases of the Irish potato in southern Idaho, isolated among many other fungi 14 species of Fusarium. Five of the strains isolated "apparently differed from all species heretofore described," and Pratt, therefore, named them, giving in this publication the original descriptions, which include habitat, cultural characteristics, spore shape, septations, and size. Septations and size are given of spores grown on various media for different ages.

The new species described are: Section Gibbosum, *Fusarium lanceolatum;* Section Elegans, *F. elegantum* and *F. Idahoanum;* Section Discolor, *F. aridum* and *F. nigrum.*

The other nine species were identified, but in only one case, that where Pratt's culture showed some differences from the "authentic culture" which he used for comparison, are there any taxonomic notes.

Bisby (*3*) in his studies on Fusarium diseases in Minnesota notes that *Fusarium oxysporum* and *F. discolor* var. *sulphureum* are of large economic importance in Minnesota. His results with certain temperatures and media in studying these diseases are of interest to use, but otherwise the bulletin is strictly economic in its outlook.

Edson and Shapovalov (*5*) made a careful study of the relations of growth of certain species of Fusarium to temperature. The species they used in the studies were: *Fusarium discolor,* var. sulphureum (Schlect) Ap. and Wollenw., *F. eumartii* Carp., *F. oxysporum* Schlect, *F. radicicola* Wollenw., *F. trichothecioides* Wollenw.

Two species of Verticillium were also used. For each of these species they made nine plate cultures and incubated them at nine different temperatures from 1° C. up to 40° C. at 5° intervals, taking readings of the size of the colony at the end of each 24 hours. The results, aside from aiding in control determinations, proved to the authors that temperature tests in certain cases may serve as a useful supplementary method for the identification of fungi exhibiting contrasting thermal relations.

THE GENUS FUSARIUM LINK.

The genus Fusarium is classified according to Engler and Prantl Naturliche Pflanzenfamilien (*6*) as belonging to the section Mucediacae Pharagmosporae of the family Tuberculariaceae of the order Hyphomycetes of that heterogenous class known as the Fungi Imperfecti. It is, consequently, a form genus, and already the ascigerous stage of a number of its species has been found. A few of these are *Nectria solani* (Ren. and Bert.), which has been reported as the ascigerous stage of *Fusarium solani; Nectria graminicola* B and N., as the ascigerous stage of *F. nivale;* and *Gibberella saubinetii* (Durieu and Mont.) Sacc. to which species *F. tulmorum, F. avenaceum, F. hordei,* and *F. heterosporum* have all been referred. It seems very probable that more and more species of this genus will be connected with genera of the Ascomycetes, though as Wollenweber states (*21*)—

> We are still far from having conclusive proof of the widely recognized theory that *Fusarium* is the obligate conidial stage of Ascomycetes.

The genus Fusarium was described in 1809 by Link (*12*), together with the allied genera Fusidium, Fusisporium, and Atractium. Later Link dropped one or the other or combined them in various ways. Schlectendahl, Corda, Fries, and Saccardo worked on this group of organisms and

classified them in various ways, but they all recognized the imperfections of the classification. In their monograph Appel and Wollenweber (*2*) have established the boundaries of the genus Fusarium, using Atractium Link, Fusidium Link, Fusisporium Link, Selenosporium Corda, Fusoma Corda, and Pionnotes Fries, either in toto or in part, as synonyms.

The synonomy of the genus Fusarium given by Appel and Wollenweber (*2*) is quoted below, and a translation is given of their description and notes.

Synonomy:
Atractium Link pr. p. *in* Mag. Ges. Nat. Freunde III, S. 10 (1809).
Fusidium Link pr. p. *in* Mag. Ges. Nat. Freunde VII, S. 31 (1816).
Fusidium Link pr. p. *in* Spec. Plant 11. S. 96 (1825).
Fusisporium Link *in* Spec. Plant 1, S. 30 (1824).
Selenosporium Corda Icon. I. S. 7 (1837).
Fusoma Corda Icon. I. S. 7 (1837).
Pionnotes Fries Sum. Veg. Scand., S. 481 (1849) Sacc. Syll. IV, S. 725.

Conidia more or less polar, mostly dorsiventral, seldom distinctly round (radiär), more or less curved; when ripe usually septated; more or less colored when in masses; borne one after another in the same spot, but not connected in chains on the end of simple or branched septate conidiophores which appear spread out between the hyphae or joined as they are in coremia, or grouped together in sporodochia. Conidia spread out in a powdery form between the hyphae or tubercular-like on a limited gelatinous sporodochia, a slimy layer or occasionally as pionnotes without definite boundaries.

Chlamydosphores, oval or pear shaped, single or in bunches, in chains or bunched up, remaining joined for some time, terminal or intercalary, not more than one borne in the same place. The chlamydophore is not very different from the conidiophore, and it has no distinctive color. It never gathers in gelatinous layers.

Hyphae septate, variously branched epi- and endo-phytic, occurring sparingly or in great quantity, either isolated or together, curly or thick, partly like coremia, or especially like a stroma to plectychymatic form with definite shape or without definite shape, more often similar to an even growth all over, limited or spread out, often closed up together on the inside, occasionally building up brightly colored mycelium.

Note that it is undecided whether species that do not have septate conidia should be kept separate from the genus or be placed in a subgenus *Fusamen* according to Saccardo (*17*); but there is no question about those which have a tendency toward septation as *F. orthoceras*. It is also undecided in what order of importance the characters should be taken. The choice is between septations, dorsiventrality, polarity and the curve of the long axis of the conidia. It is very questionable whether *Fusarium* should be placed under Leptosporium as in Saccardo, and nothing but the study of the different forms can decide the boundaries of the genus. Concerning the color of the conidia masses it can be said that black does not appear normally, neither does black mycelium. Light orange and ochre colors predominate in the conidia. The mycelium also has yellow, red and blue. The term sclerotium as used in *Fusarium* is disputable. Researches have not shown that the term sclerotia was justifiable for the plectychymatic structures found.

In 1913 Wollenweber (*22*) excluded from the genus all species having septocylindrical conidia. In bringing this genus to date, therefore, this fact should be incorporated. Sherbakoff (*18*) describes the genus concisely as follows:

Hyphomycetes, with from hyaline to bright, but never plain gray nor black, conidia and mycelium; conidia sickle-shaped, septate (usually 3 or more septate), apically pointed, mostly pedicillate, not appendiculate, noncatenulate; conidia scattered over substratum, in pseudopionnotes or in sporodochia, the latter without or with from flat to wart-like plectenchymic substratum, and always without any differentiated enclosing or surrounding structures; conidiophores from simple to irregularly verticillate.

ISOLATION OF CULTURES

The original cultures were all obtained from infected tubers by the following method:

The infected tubers were thoroughly washed, dipped into a 1 to 1,000 solution of mercuric chlorid, and cut open. In order not to contaminate the infected parts of the tuber, the healthy portion was cut almost to the edge of the discolored portion and the tuber then broken open, care being taken that nothing should touch the advancing margin of the fungus. A piece of discolored tissue was taken from the advancing margin and put into a tube of melted agar, which was then poured into a Petri dish and incubated at room temperature. Six to 10 plates were made from a single tuber. The plates were watched carefully to be sure that a pure culture was the result of the isolation. When this was assured a transfer was made to agar slants, and these were kept as stocks. It was surprising how very few contaminations appeared in the plates. Each culture was numbered, and all notes, including source, were kept under this number.

SINGLE-SPORE ISOLATIONS

In 1916 when detail work with these cultures was anticipated it seemed best to take every precaution to be assured of pure cultures, for a mixed culture of two or more species of Fusarium could easily pass unnoticed. Therefore, a single-spore isolation was made from each culture in the following manner:

A sterile platinum needle was used to transfer spores from the stock culture to tube 1, which contained 5 cc. of distilled sterile water. From this tube a series of five dilutions was made into tubes, each containing 5 cc. of sterile distilled water. Three 3-mm. loops of material were taken from tube 1 and put into tube 2; three 3-mm. loops of material were taken from tube 2 and put into tube 3; and three 3-mm. loops of material were taken from tube 3 and put into tube 4. Tubes of standard beef agar were melted and poured into Petri dishes and allowed to cool. From each dilution tube ½ cc. of material was made to flow over the surface of the plated agar. Any excess material was drained off. The plates were allowed to incubate at room temperature for about 16 hours, when they were searched with a microscope for germinating spores. All microscopic examination was made through the bottom of the inverted Petri dish.

Fusarium spores are for the most part hyaline and to locate them on a plate of clear agar is very difficult. The scheme was devised of sprinkling a few sterile spores of *Tilletia foetans* of wheat over the surface of the agar with a tiny sterile spatula. These spores were easy to locate and gave the plane of focus in which the Fusarium spores could be found. The use of the sterile smut spores proves to be a great timesaver.

Usually the search for spores began with the more heavily sown plates, down through the more dilute ones until a spore was found which was sufficiently isolated so that it alone could be removed. The position of this spore was marked by a ring of India ink on the glass and it was then cut out by means of a stiff platinum cylinder illustrated in Phytopathology (*10*) and placed on the upper end of an agar slant. It was carefully watched by means of the microscope to be sure that all growth came from the spore and not from a piece of mycelium from the edge of the piece of agar, as often happened when the germinating spores were not sufficiently isolated. When it was assured that all growth came

from the spore it was considered pure and the culture was kept as a stock. If there was any question as to the source of the growth the culture was discarded. Sometimes a number of attempts had to be made before the stock culture was obtained.

COMPARATIVE CULTURAL STUDIES

Since the purpose of our work was the identification of the species of Fusarium that we had found occurring on potatoes in Montana, we began by comparing the cultural characteristics of each isolation product, hoping to be able to group together those which belonged to one species so that we might eliminate unnecessary duplication with cultures of the same species. This hope was realized only in one group. After a very few trials with different media and at different ages, one group, containing about 20 per cent of all the cultures, separated itself out very constantly. Its growth was so characteristic that we then believed and have since proved that it was *Fusarium trichothecioides* Wr. There were other groups, some seven of them, each containing from 2 to 10 cultures; but the identity of the members of the groups was not sufficiently striking to warrant leaving any of them out of further studies. However, these groupings aided materially when actual identification work began. Seventeen series of cultures have been studied. A series consisted of all the transfers made at the same time and incubated under similar conditions for the purpose of comparative studies. At least four sets of individual notes were taken on each series, so as to bring in the influence of age on the characters.

The characters emphasized in these notes were color, amount, and nature of the growth of mycelium, absence or presence and color of pseudopionnotes or sporodochia. All color determinations were based on Ridgway's "Color Standards." [3]

In practically all the series the cultures were inoculated in triplicate, so that when variations arose between cultures inoculated from the same stock culture a decision as to which was the more normal could be made.

As a preliminary to note taking, those cultures which had sufficient similar cultural characters to suggest identity were put into groups. Each group was designated by a letter, and these were placed in a table in parallel vertical lines, a column for each note taking, so that gross group comparisons could easily be made. Notes on each group were made in detail at the end of the table. If a culture showed only a slight variance from a group it was put into the group, but with special additional notes, and was designated in the table by the group letter with a subnumber. In so far as possible the same letters were used throughout for the same group. After a few sets of notes were taken one or two cultures which seemed most typical of a group were chosen as type cultures, and these numbers were given the same letter each time and served as the nucleus for the group represented by that letter.

Some few cultures, the number varying with the medium on which they were grown, did not develop any distinctive microscopic characters; others developed distinctive characters on certain media; and occasionally the same culture when on one medium was placed in a certain group, while on another medium it would be placed in a different group, or, as

[3] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

often happened, would seem to fit none of the groups. These refractory cultures caused considerable trouble, and some of them have not yet been identified.

MICROSCOPIC STUDIES

USE OF PHOTOMICROGRAPHS

The microscopic studies that are most extensively used in classifying Fusaria are the size, shape, and septation of the spores. In view of the fact that different kinds of spores are produced and that in each kind there is much individual variation, the problem becomes in some cases rather complex.

In a goodly portion of the microscopic studies photomicrographs were used. The spores were mounted in water containing a small quantity of dilute Myer's flagella stain. The cover glass was blotted with a coarse filtered paper, pressed down, and sealed with paraffin to prevent evaporation and consequent movement of the spores. The pictures were taken soon after the slides were made, for the mounts gradually dried out. If they were put into a moist chamber they could sometimes be kept intact for 24 hours or more.

The Leitz horizontal photomicrographic apparatus 1A No. 398, with Leitz microscope stand, having an apochromatic condenser, was used for this work. All pictures were taken magnified 500 diameters.

The photographic method proved very convenient, for by means of it actual reproductions of spore material were made when the spores were at their best and the data were studied when convenient. This permitted a massing of data by means of which close comparative studies of microscopic characters could be made, which without photographs could not have been done.

Drawings, of course, might have been used instead, but there is no doubt that the photographic method is far superior to that of drawing. In the first place, actual reproductions of selected fields of spores are made instead of a few spores selected in accord with the personal preference of the worker and idealized and perfected in the process of drawing. Drawings are often misleading in their fine definitions and detail. Secondly, if the worker is careful to photograph an average field, which in most cases is easy to do, a great deal of additional data are recorded on the picture, which the one who was drawing would have to add in notes that take too much time when the critical stage of a large group of cultures is demanding his attention. Such data, for instance, are the percentage of spores with a certain number of septa, the percentage of macroconidia and microconidia, limits of size of different types of spores, etc.

Two complete sets of pictures were taken, except for cultures that would not fruit. One was of the fungi from cultures about 47 days old, grown on oat agar, and the other of fungi grown on lima bean agar when the cultures were about 10 days old.

The spores of some cultures would not take the stain; others, particularly those having mostly microconidia, even though perfectly sealed exhibited Brownian movement, and still other cultures produced so few spores that a field suitable to photograph was impossible to find. Many attempts, some of which were successful, were made to grow these refractory cultures on a medium that would produce a greater abundance of spores, and whenever successful pictures were taken. Notable

advance was made with some of them when grown on sterilized tomato stems and leaves.

In order to see what effect age and medium had on spore characters, three species, each typical of a group, were chosen and about 10 photographs were taken of them, grown on different media and at different ages. Our conclusions are indicated on page 354.

MICROSCOPIC NOTES

The only key published which includes the greater number of American species of Fusarium is Sherbakoff's (*18*), and it was therefore used as a basis for this work. In determining the various sections this key was very useful, but owing to the fine distinctions made between species and especially between varieties much difficulty was experienced in identifying an unknown culture.

In order to secure the necessary data for the identification work microscopical study was made of all cultures grown on hard oak agar and on lima bean agar, in addition to the data secured from our microphotographs of the cultures grown upon the same media, but under somewhat different conditions. The cultures grown on hard oat agar were about 15 weeks old and had been kept in the refrigerator at 10° C. The cultures grown on lima bean agar were about 4 weeks old and had been kept in a dark incubator at about 21° C.

As with the series of cultures used for the microphotographs, we had difficulty here also with certain ones not fruiting; and additional notes were made for certain cultures at about 4 weeks of age when grown on potato glucose agar and at various ages, depending upon the organism, when grown on tomato stems, potato plug, and lima bean agar.

In these examinations particular care was taken to include some mycelium in order to determine whether or not chlamydospores were present, for in the photographic work it was natural to select a field filled with spores rather than one filled with mycelium, and the presence or absence of chlamydospores, which character Sherbakoff used considerably, was often overlooked in the earlier studies.

MEDIA USED

A limited number of media was used in our work, for the concensus of opinion of those investigators who have used a large variety, notably Smith and Swingle (*19*), Apple and Wollenweber (*2*), and Sherbakoff (*18*), seems to be that little is gained from so doing. Sherbakoff believed that all important characters were brought out on hard oat agar, certain vegetable stems, tuber plugs, and potato agar containing about 5 per cent glucose. We followed the suggestion of Sherbakoff but used a few additional media. The formulas of the media follow:

OAT AGAR.—One hundred gm. rolled oats were put in 1,000 cc. of water and cooked for an hour in an Arnold steamer which varied in temperature from 50° to 75° C.

The product was strained through cheesecloth and the volume of liquid was brought up to 1,000 cc. Thirty gm. of agar were added, and the mixture was put in the autoclave and the pressure allowed to rise gradually to 15 pounds, where it remained for 15 minutes. The material was then tubed, and the tubes were plugged and autoclaved.

LIMA BEAN AGAR.—This was made in the same way as the oat agar except that the decoction was made by heating 100 gm. of broken pieces of lima beans in 1,000 cc. of water.

POTATO GLUCOSE AGAR.—This medium was made in the same way as the oat agar except that the decoction was made from 100 gm. of sliced potato tubers, and just before the mixture was tubed 50 gm. of glucose (T. J. Baker's c. p.) was added.

RICE.—About 3 gm. of rice were put into each tube, 10 cc. of tap water were added, and the tubes were plugged and autoclaved.

POTATO PLUG.—Cylinders cut from potato tubers were slanted and placed in tubes with enough water added to about cover the cylinder. The tubes were plugged and autoclaved.

SWEET CLOVER STEMS.—Stems of sweet clover (*Melilotus alba*) were cut into convenient pieces. If the stems were large enough only one was placed in a tube. Most of them, however, were small and two to four pieces were used. The stems were dry, so water enough to cover them was added. The tubes were plugged and autoclaved.

TOMATO STEMS.—Stems and leaves of young tomato plants (*Lycopersicum esculentum*) were cut into convenient pieces, put into tubes with distilled water added to within about ½ inch of the top of the stems. The tubes were plugged and autoclaved.

Five series of cultures were grown on oat agar, five on potato glucose agar, three on lima bean agar, two on potato tuber plug and one each on the other media mentioned above.

TEMPERATURE AND LIGHT CONDITIONS USED

By far the greater number of the series of cultures were grown in the dark in an incubator regulated between 20° and 22° C. This temperature, according to data of various investigations, seemed to be nearest the optimum for the greater number of species.

In all cases where the "dark" incubator is mentioned it refers to one the temperature of which was regulated by burning a 20-watt carbon light automatically controlled by a thermostat in the lower portion of the incubator. The cultures were kept in cans on wire screen shelves, and the light given off from this bulb should possibly be considered as influencing the results, though it does not seem probable that it did as the temperature of the room was such that the bulb was lighted but a small portion of the time.

One series of cultures grown on potato glucose agar was kept in diffused light and incubated at room temperature which varies from about 18° to 25° C.

Another series grown on potato glucose agar was inoculated in quadruplicate, and two tubes of each culture were grown in the "dark" incubator at 20° C. and two similar tubes grown in an incubator with glass doors, designated as the "light" incubator, which was in the greenhouse so placed that the cultures were in strong diffused light during the day. The tubes were kept in glass beakers and only a few in each beaker. The temperature of the "light" incubator was kept at 21.5° C.

Still another comparison between cultures grown in light and darkness was made with lima bean agar. In this case two tubes of each culture were kept in the dark incubator at 20° C. and two each were kept in diffused light in a room at a temperature which varied between 18° and 25°.

GENERAL DISCUSSION OF METHODS

EFFECT OF MEDIA

No distinct effort was made to determine the comparative values of different media, for in order to determine such values a large number of trials should be made on each medium under observation. However, the results which we gained from a few trials on a few media may be of some value.

EFFECT ON CULTURAL CHARACTERS.—The growth made on sweet clover stems lacked color. In fact certain cultures (those ordinarily grouped under K, a Discolor group, and under D, an Elegans group) which constantly produced color on all other media did not exhibit any on sweet clover. The growth was scanty in proportion to that on other media. This may have been due to the fact that the stems used were old ones which had been very dry. However, Sherbakoff (*18*) notes that the presence of the epidermis on stems seems to lessen the development of aerial mycelium and to favor production of fewer but better developed sporodochia. We also found that sweet clover stems seemed to favor the production of sporodochia and pseudopionnotes. A few cultures which had not at any time formed sporodochia did so on this medium. The sweet clover stems were sometimes covered with a thick, tough layer of plectenchymatic-like tissue which seemed never to bear spores.

The little experience we had with tomato stems suggested that they, too, favored the production of spores. However, our work with this medium was very limited, as we used it only on refractory cultures.

Lima bean agar proved to be another medium that did not stimulate color production. For instance on Group F (a Martiella group) notes taken 12 days after inoculation read, "a slight appearance of greenish blue growth," while cultures of the same group grown on oat at 11 days showed "various combinations of blues, greens, and purples." The D group (an Elegans group) which was mulberry purple on oat showed white or gray on lima bean.

That series of cultures (p. 350) growing on lima bean which was divided and part grown in the diffused light and part in the dark incubator showed practically no difference in color production. Only two cultures produced color in the dark that did not produce any in the light. The amount of mycelium on the lima bean cultures varied somewhat with the groups.

The mycelial growth on potato plug was abundant, and color appeared in varying degrees. That is, more color was produced on potato plugs than on lima bean or on the stems of sweet clover, but less than on the oat or potato glucose agars. Thick, tough layers of plectenchymatic tissue formed over the tuber plugs, as it did over the stems of sweet clover, but sporodochial growth was rare.

The greatest variety of colors was produced on rice, and finer group distinctions were brought out on this medium than on any other used in the cultural studies, but the colors were very mixed and they seemed not to stay true to group. Very little reliance was put on these results at the time the notes were taken. It is interesting to look back and see how nearly true to group the color determinations were. However, the mixture of colors produced was so difficult to describe that except for grouping purposes it is doubtful if rice as a cultural medium would have any specific value.

Again and again in the literature is noted the fact that for color production agar with glucose is the medium to use. Our results showed that the cultures grown on potato glucose agar did develop color, but not much more so than did those on oat agar. The color on the glucose agar in some cases was deeper than on the oat. For instance, notes taken at practically the same age on both media give for the color of group A (a Discolor group) on potato glucose "vinaceous cinnamon to orange cinnamon" and on oat it is "salmon buff to salmon color." For group C (a Discolor group) it reads "Bordeaux" on the glucose and "spinel pink" on the oat. However, the deepness of color is not constant throughout for special notes on culture No. 16 give "seashell pink" as the color on glucose and "cinnamon" as the color on oat. Our results would indicate that the two media are about equal in color production value and the amount of growth is practically the same, being abundant on both.

EFFECT ON MICROSCOPIC CHARACTERS.—No striking difference microscopically was noted between spores grown on lima bean, potato glucose, or oat agars (Pl. 1, A and B; 2, C). We found that the series of oat cultures kept in the refrigerator was in the best condition for spore study of all with which we worked, but it seems probable that this was due to the temperature rather than the culture medium, as the other oat series was about equal to the lima bean.

The few refractory cultures that were grown on tomato stems led us to believe that that medium might prove to be very good. At least it would be worth while to try it out further.

Appel and Wollenweber (*2*) concluded from their work that agar media were by no means so sure of producing normal conidia as the tubers, and the stems were found to be the most satisfactory of all. Wilcox, Link, and Pool (*20*) state that cultures grown on gelatin and agar media are not normal and can not be used in the determination of characters. Our results do not support these conclusions, but they confirm a statement made by Sherbakoff (*18*):

> An agar, especially such a one as oat hard agar, often gives all the forms of fructification for these fungi, with "normal" spores and more or less typical and brilliant color production.

It might be well to add here a footnote given by Sherbakoff (*18*) in explanation of the variance between his results and those of Appel and Wollenweber (*2*):

> This observation is apparently in some contradiction to the observations of Appel and Wollenweber (1910: 12–13), but indeed it is not so; because, judging by the "artificial" media actually used by them, their observations of unfitness of such media for study of "normal" growth of the Fusaria was based on "soft" agars too rich in sugar. The writer also found that such agars produced abnormal growth.

We found that oat agar more than any other medium used combined the qualities necessary to produce good cultural characters such as growth and color and normal spores.

EFFECT OF LIGHT

So little comparative work was done upon the effect of light on growth and color that our results are of rather limited value. The effect of light on Fusarium has never been thoroughly studied. Our conclusions and those of a few other investigators are included below.

Using lima bean agar for a medium, we found practically no difference in the amount of growth or the amount of color produced in cultures

grown in the dark incubator and those grown in diffused light, but the lima bean is not a good color producer under any condition.

With potato glucose agar there was some difference, though it was not striking. These comparisons were drawn, however, from cultures grown in the light incubator and others grown in the dark incubator (p. 350). Our notes taken when the cultures were a week old show that in the dark group A produced rufous pseudopionnotes; in the light they were ferruginous. A more intense purple color showed in group D when grown in the light than when grown in the dark. The most striking comparison, in fact practically the only striking one, was in the case of culture No. 69 which produced carmine mycelium in the light and white mycelium when grown in the dark.

Notes taken on a complete series of week-old cultures grown on potato glucose in diffused light showed practically the same results as those taken on the cultures grown in the dark incubator.

Smith and Swingle (*19*) found that often cultures which produced—

a beautiful, rich salmon colored mycelium when grown in sunlight produced white mycelium when grown in a dark closet.

A difference in color was not noted on all media tried. Except for a difference in color these men concluded that light had no material effect on the growth.

Appel and Wollenweber (*2*) noted that conidia masses were much richer in color when grown in the light than in the dark. They also noted that when cultures were grown in the dark, poorly developed conidia with uneven septations and form appeared. Although direct sunlight was not exactly injurious the diffused daylight was most favorable in every way for the product of morphological characters.

EFFECT OF TEMPERATURE

We did no work with the effect of temperature on the fungi, but a summary of the conclusions of other workers may be of value.

In working with *Fusarium oxysporum*, Smith and Swingle (*19*) found that the fungus grows well on boiled potatoes at a temperature of from 15° to a little above 30° C. Below 15° the growth became slower and slower until 5° was reached, when practically no growth took place. Above 37½° no growth took place.

Link (*13*) did some detailed work on temperature relations of *Fusarium oxysporum* and *F. trichothecioides*. The optimum temperatures for the two are different. However, at temperatures between 15° and 20° C. a good growth was made by both.

Lewis (*11*) in his work with 24 cultures of Fusaria found that 20° to 25° C. seemed to be the best range of temperature for most of the cultures.

Appel and Wollenweber (*2*) in their summary of conditions which will guarantee a "normal growth" say that room temperature should be used, that is, "between 12° and 25° C. neither higher nor lower."

Edson and Shapovalov (*5*), working on temperature relations of six of the more common species of Fusarium, found that growth took place in varying amounts between 2° and 38° C. The minimum for growth was shown by *F. discolor* var. *sulphureum*, the maximum by *F. radioicola*. The maximum growth for all cultures took place between 25° and 30°, though growth was abundant between 15° and 30°.

A temperature of between 19° and 22° C. was used for practically all the work reported in this paper. Incidentally it was found that cultures kept in the refrigerator at 10° showed an unusually good spore condition. It was very likely due to the fact that so low a temperature inhibited the growth and was about the optimum temperature for preservation of spores after their formation. Cultures kept in the refrigerator were always kept at room temperature for about a week after they were inoculated.

During the later spring months the light incubator in the greenhouse warmed up in the middle of the day to 30° or 35° C. We found that cultures kept in this incubator during that time deteriorated quickly.

The conclusion drawn from our own experience and that of others was that cultures can be grown as well at room temperature as at a fixed temperature, provided the temperature does not go lower than 12° or higher than 25° C. In case it is desirable to keep spores in a normal condition for a longer period of time than is possible at room temperature this can be done by keeping them in a refrigerator at 10° or less.

EFFECT OF AGE

Careful study of the series of cultures grown for the purpose of noting the effect of age on spore formation was in some measure disappointing. Isolated examples could be found that would illustrate practically any theory one might wish to propound. Too many factors enter in, such as moisture, nutrients, temperature, etc., for one to be able to make definite conclusions as to the effect of age. It seems that if all conditions are right to produce a "hoch" [4] stage of a normal culture, age does not enter in more than that a very young culture or a very old one can not produce a "Hochkulture." Only relative age then, would seem to be of importance.

CONCLUSIONS AND SUGGESTIONS ON METHODS

The effects of media, temperature, light, and age, though not always very great on cultural and spore characters, are sufficient to make it advisable in describing characters to describe the culture media and conditions so freely as to make the repetition of the culture upon the same medium and under approximately the same conditions easily possible, to note under what conditions these results were observed, and to keep them within certain limits.

Our experience would suggest that cultures grown on a hard oat agar, in diffuse light, and at room temperature will give the best satisfaction.

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[4] Appel and Wollenweber (2) in their attempt to find distinctive terms by which to designate the degree of development and the age of the cultures created six terms which are briefly defined as follows:

ANKULTURE: A little mycelium from the original substance of some Fusaria is inoculated on tubers or stems. A pure, rich mycelial culture results in which there are either no conidia or only a very few, and these are apt to be irregular in shape and septation and are not suitable for morphological research.

NORMKULTURE: A culture which produces conidia readily and in which the spores are regular in form and septation.

ABKULTURE: A culture in which deterioration has set in, and the spores which have not disintegrated are small and usually have fewer septations than do those in the Normculture. The Normkulture is subdivided into three stages:

The JUNGKULTURE, usually less than 8 days old, is one in which the spores have not reached a constant form of development, and spores of varying sizes and septations are found.

In the HOCHKULTURE the spores are truly normal, that is, comparatively even in size, shape, and septations.

In the ALTKULTURE the spores, due to lack of moisture or food, shrink a little in size; or if new spores are formed they are undersized, yet not deteriorated enough in form to belong to the Abkulture.

Plate 1, C and D, illustrate the "alt" and "hoch" stage of the Normkulture as seen in our work.

The cultures may be kept in a normal condition for 12 weeks or more by keeping them at a temperature of 10° C. or less.

Were the standardization work suggested on page 356 to be attempted, we would suggest as the media to be tested out a hard oat agar, potato stems, potato plugs, and possibly potato glucose agar. It would be advisable to try several regulated temperatures, together with a room temperature, the limits of which should be given, probably 12° to 25° C. Tests should be made to determine that time nearest which all the species reach the normal stage of their growth, and if possible some method should be devised to standardize moisture and humidity conditions.

PITFALLS IN IDENTIFICATION WORK WITH SPECIES OF FUSARIUM

The greatest obstacle in the way of accurate determination of species of Fusarium is the lack of a good monograph of the genus, and this lack is due in part to nonstandardization of the methods used in identification work, especially as regards kinds of media, environmental conditions, and the relative value ascribed to various characteristics of the fungus when grown in pure culture under laboratory conditions. The species and varieties intergrade and the differential characters used in the keys are not sufficiently distinct to permit any but an experienced investigator to use the key. To become an authority one must work long enough and with large enough numbers of species so that he can create within himself a conception of the species. In other words, he judges to what species the fungus in question belongs rather than actually identifying it.

Pathologists in various parts of the world often in connection with some pathological studies isolate a species of Fusarium. In their eagerness to name the organism which is causing economic loss they describe it so incomprehensively that future workers are not able to identify their cultures with it and therefore more new names appear.

Appel and Wollenweber (*2*) made a good beginning toward a monograph, but it was merely a beginning. Sherbakoff (*18*) has helped the situation somewhat with his "Fusaria of Potatoes," but his key is deserving of the criticism given above. He has split species up into so many varieties that to identify a specimen beyond its section becomes a tedious task of scientific guessing.

In the near future the botanical world, especially the mycological world, must determine and actively promote some policy with regard to trinomial nomenclature. If all the flowering plants were split into varieties on as many minor characters as are the fungi, binomial nomenclature would before long be a thing of the past. The American Code of Botanical Nomenclature (*1*) states in regard to categories of classification that the terms "subspecies" and "subgenus," etc., may be used when additional categories are necessary for the convenience in presentation of relationships, but "the term variety is relegated to horticultural usage."

The mycologist's difficulty arises largely from lack of perspective. The person who has worked on a single group for some time sees very real, fine distinctions which would not be at all significant to other mycologists. These distinctions may not be of enough importance to justify his making a new species, but they are too real to him to be overlooked, and he therefore originates a variety. Would it not be better to keep in mind that a species must have more or less flexible boundaries due to evolution which has taken and is now taking place,

and in describing new species or in identifying new cultures allow for a certain amount of variance? The code quoted just above defines species as "connected or coherent groups of individuals."

There is little doubt that in some cases physiological strains of species must be recognized by some system to be agreed upon, possibly by making new species, but until formally adopted by a representative body varietal names, especially those based on morphological characters, should be avoided.

SUGGESTION FOR A STANDARD METHOD FOR FUSARIUM STUDY

An important step in taxonomic work on Fusarium would be to standardize the methods for growing Fusarium species in somewhat the same way that certain bacteriological methods are standardized.

In order to do this it would first be necessary to carry on a comprehensive preliminary study. Interested workers in different localities would grow a large number of species of Fusarium, preferably subcultures from common stocks. The conditions of media composition, light, temperature, and humidity should be as nearly uniform as possible, selecting those suggested by the results of previous workers.

The method of note taking in the work should be sufficiently uniform to facilitate comparative studies of the results. From these comparative studies it could be concluded what conditions proved most satisfactory in growing species of Fusarium.

Selecting the most promising method thus obtained as a provisional standard, cultural work should again be carried on by a very large number of workers and with a very large number of species, and notes taken in a uniform manner. A comparison of the various notes on single species would determine whether or not the method used could be adopted as "a permanent standard."

By careful study of the various notes taken on all species of Fusarium used, the most stable characters could be determined and a really workable key made.

Such a procedure would involve a large expenditure of time and money and much care on the part of the workers. To find enough interested workers with the time to devote to such a study might in itself be a difficult task. However, until a key based on comprehensive data of this kind is made we see little hope for accuracy in identification. (See also page 354.)

RESULTS OF IDENTIFICATION WORK

The cultural and microscopic data, acquired as described above, were carefully studied and with the aid of Sherbakoff's (*18*) key many of the cultures under investigation were identified. Their descriptions and identifications follow according to groups. Practically all the cultures were found to be included under Wollenweber's three sections: Elegans, Discolor, and Martiella. Notable exceptions to this are No. 20, 69, and 75. For various reasons some few of the cultures are only provisionally identified, and two are not identified at all. The summary given in Table I will show which these are and give the reason for indecision. (See page 362.)

Since Sherbakoff's key was used, the descriptions of species given by him were taken as the standard in most cases. If questions arose about

individual characters, comparisons were made with descriptions by other investigators whenever such descriptions were available.

SECTION ELEGANS

One group (Group D) fell within this section. It included No. 21, 24, 25, 27, 28, 29, 31, 45, 46, 58, and 59. This was a difficult group to identify because of the scarcity of macrospores and the variations in color. Microscopically the group (with the exception of No. 59) falls into the species *Fusarium oxysporum*, but no tube culture produced sclerotia, which according to Smith and Swingle (*19*) were green in color and always found in cultures grown on potato plug, and according to Sherbakoff (*18*) were:

Bluish black in color, constantly present on potato tuber plug and sometimes on different agars.

In plate cultures grown on potato agar with 5 per cent of glucose, a few of the numbers (21, 28, and 46) produced small, dark purple spots, which on examination proved to be masses of nonsporing mycelium; but after four weeks of growth these small masses of mycelium seemed to be too loose to be called sclerotia. The fact that they did not form consistently throughout the group also suggests that they are not the sclerotia noted by the authors mentioned above.

The color of our cultures, also, does not quite agree with former descriptions of the species. Sherbakoff gives "macroconidia in mass usually of pinkish buff color" but neglects to state on what medium this is true.

On potato-glucose agar plates kept in the light most of our cultures (No. 24, 25, 27, 29, 45, 58, and 59) showed salmon coloring varying from light buff to ochraceous salmon, but shades of purple are typically found. Combinations with pinks and sometimes with greens occur but a greater or less amount of purple was characteristic of the group under all conditions. The only media used in common with Smith and Swingle were boiled rice and potato tuber plugs. Smith and Swingle found the color on the former when grown in the dark "mixed pink and lilac shading into white." Our notes show a production of purple (true) to resolane purple. On potato tuber plug these authors noted the growth when made in the darkness was "pure white changing to creamy white." We noted a slight development of a pinkish and purple pigment when grown in the dark.

However, these discrepancies in color do not seem sufficient to throw these cultures out of *Fusarium oxysporum*, but the lack of sclerotia seems important We would, therefore, identify these cultures as *F. oxysporum* var. *asclerotium*,[5] a variety described by Sherbakoff which differs from *F. oxysporum*—

by the absence of sclerotia, and definite plectenchymic sporodochia, in color of the mycelium and somewhat longer and narrower macroconidia.

Sherbakoff neglects to state in what way the color differs.

Macrospores were very scarce in all the cultures of this group, and in No. 24 none at all were found. In the other numbers they varied

[5] It might seem inconsistent after the discussion on binomial nomenclature on page 355 to make use of varietal names in our classification. We recognize the disadvantages of trinomial nomenclature but feel justified in following it to avoid still further confusion of the names used in this genus. We feel, however, that some official recommendations and action should be taken upon the important question by societies qualified to represent mycology.

considerably in size. This variation in size seemed to bear no consistent relationship to numbers, age, media, or temperature. We are therefore including in the limits given the various sizes of the spores measured on the different cultures.

Fusarium oxysporum Schlect. var. *asclerotium* Sherb. (Description taken from No 21, 24, 25, 27, 28, 29, 31, 45, 46, and 58.

Macroconidia typically dorsiventral, doesiventrality sometimes slight, usually distinct, and if so, ventrally curved. More or less uniform in diameter, with more or less abruptly attenuated apex; base pedicillate.

Microconidia very abundant. Oftentimes no macroconidia present, especially in cultures that have been kept for some time in stock. Chlamydospores common, mycelial intercalary and terminal, conidial intercalary.

Mycelium abundant, fine and long, from white to slight purple tint to haematoxylin violet to mulberry purple on hard oat agar grown in the dark; white to slight development of pinkish pigment and sometimes purple color on potato plug grown in dark; white to slight purple tint on potato-glucose agar grown in darkness and white to cameo pink to petunia violet when grown in the light.

Substratum colorless to purple to dull purplish black on potato-glucose agar.

The conidial measurements are as follows:

1-septate, few, 22.5 by 4.5 microns.

2-septate, rare, none measured.

3-septate, 50 to 100 per cent, 34 by 4 (22 to 52 by 3 to 5 microns).

4-septate, 0 to 40 per cent, 48 by 4.5 (35 to 60 by 4 to 5 microns).

5-septate, 0 to 20 per cent, 48 by 4.5 (35 to 60 by 4 to 5 microns).

Fusarium sclerotioides Sherb. var. *brevius* Sherb. (Description from No. 59.) See Plate 2, A.

Macroconidia typically dorsiventral; dorsiventrality distinct, ventrally curved, dorsally elliptic, typically broader toward apex, indistinctly pedicillate; gradually attenuated, pointed apex.

No sclerotia, no plectenchymic sporodochia.

Microconidia abundant, oval, 0–and 1–septate.

Sometimes far in excess of macroconidia.

Intercalary mycelial chlamydospores common. Mycelium well developed, white to mulberry purple on hard oat agar; white to cameo pink and petunia violet when grown in the light on potato glucose agar; slight development of pinkish pigment on potato plug. Substratum on potato glucose agar livid pink to dark maroon purple.

The conidia measurements are as follows:

1-septate rare, 22 by 4 microns.

2-septate rare, no measurements made.

3-septate 50 to 80 per cent, 36 by 5 (25 to 40 by 4.5 to 5.5) microns.

SECTION DISCOLOR

Three distinct groups, namely A, B, K, and one group C, which varied considerably within itself, contained species included in Wollenweber's section Discolor. The description of these cultures with their identification follows.

Fusarium trichothecioides Wr.; (Description taken from No. 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 14, 33, 38, 61, 63, 64, 76, 79, 81, 83, 91, and 97.)

Conidia not differentiated into macroconidia and microconidia, but there are two types of spores, the comma and the discolor types. The discolor type, that is conidia shaped like those of *Fusarium discolor*, is very rare. Comma type of spore slightly dorsiventral to straight, diameter more or less uniform, apex and base not differentiated rounded. (Pl. 2, B.)

Spores in powdery masses, at first on aerial mycelium, which soon covers the surface completely.

Terminal and intercalary chlamydospores occasionally noted. Mycelium abundant at first and white, soon becoming covered with powdery spore masses, which vary from pale flesh, salmon buff, chamois to buckthorn brown on potato-glucose agar; pale flesh to pale pink to pale pinkish buff on potato plug; white to pale flesh to safrano pink to light ochre to salmon buff on oat agar.

Substratum colorless to somewhat darkened on potato-glucose agar. This species is easily identified by its very characteristic powdery growth.

The conidial measurements of the comma type of spore are as follows:
0-septate, 11 by 4 (6 to 19 by 3.5 to 4.5) microns.
1-septate, 15 by 4 (12 to 24 by 3.5 to 5) microns.
2-septate, 20 by 4.5 (18 to 26 by 4 to 5) microns.
3-septate, 26 by 5 (19 to 34 by 4.5 to 6) microns.
4-septate, 32 by 5 microns.

The percentages of 0-, 1-, 2-, and 3- septate conidia vary in different cultures. In most cases 1-septate conidia predominate.

Fusarium subpallidum var. *roseum* Sherb. (Description taken from No. 15, 17,[6] 22, and 40.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, more or less uniform diameter, apex not long, rounded; base pedicillate.

Spores in small salmon-colored sporodochia, occasionally merging into pseudopionnotes. Microconidia absent. Chalmydospores sometimes found, both conidial and mycelial intercalary. Mycelium somewhat varied in color, white above to salmon orange below with some shades of purple on potato plug; flesh pink to light coral pink and rose pink with occasional slight tint of purple or yellow on potato agar with 5 per cent glucose.

Substratum, colorless to rose pink on potato agar with glucose.

The conidial measurements are as follows:
1-septate, 0 to 20 per cent, 18 by 4 microns.
2-septate, rare (no measurements made).
3-septate, 50 to 90 per cent, 25 by 4.5 (16 to 30 by 3.5 to 6) microns.
4-septate, 0 to 15 per cent, 34 by 5 microns.
5-septate, 0 to 12 per cent, 34 by 5 microns.

It is doubtful whether there is enough difference between the species *Fusarium subpallidum*, *F. subpallidum* var. *roseum*, *F. clavatum*, and *F. discolor* to warrant more than one species.

Fusarium clavatum Sherb. (Description taken from No. 23, 41, 42, and 43.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, slightly broader toward the apex, apex rather abruptly attenuated, base distinctly pedicillate.

Spores in small sporodochia, later merging into pseudopionnotes from pale flesh to salmon colored on oat and lima bean; light coral red to coral red on potato agar with 5 per cent glucose.

Microconidia absent.

Intercalary conidial chlamydospores sometimes present, mycelial intercalary chlamydospores occasionally found or scattered.

These cultures showed unusually close identity culturally and microscopically throughout.

The conidial measurements are as follows:
1-septate, rare.
2-septate, 2 to 6 per cent.
3-septate, 75 to 90 per cent, 27 by 4.5 (16 to 40 by 4 to 6) microns. Average limits 20 to 30 by 4 to 5 microns.
4-septate, 15 to 30 per cent, 30 by 5 microns.
5-septate, 5 to 15 per cent, 30 by 5 microns.

Practically speaking, the spore measurements and the percentages of the variously septated spores varied no more between the different media (potato glucose, oat, and lima bean agars) than between different cultures grown on the same medium.

No. 16 seems to vary between *F. clavatum* and *F. subpallidum* var. *roseum* and *F. discolor* in color characters, but the spores agree with *F. discolor* in shape and size, and sometimes in average number septations. We, therefore, are identifying it as that species.

[6] Practically no normal spores were found in any of the cultures of No. 17 on any of the media used. The culture seems attenuated. However, from the few spores and from earlier cultural notes we identified it with this species.

Fusarium discolor Ap. and Wr. var. *sulphurum* (Schlect) Ap. and Wr. (Description taken from No. 77 and 85.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, more or less uniform in diameter. Apex not long, typically slightly broader toward the apex, more or less abruptly attenuated, base distinctly pedicillate.

Spores in pseudopionnotes, flesh ochre to salmon color on all media used.

Microconidia absent.

Conidial chlamydospores often found; mycelial chlamydospores never noted, due, perhaps, to scarcity of mycelium.

Mycelium white at first, but soon becoming entirely covered by pseudopionnotes.

Substratum colorless to slight salmon coloring.[7]

(For photograph of No. 77, see Pl. 1, C and D.)

The conidial measurements are as follows:

2-septate, rare.

3-septate, 15 per cent, 24 by 4 (22 to 32 by 4 to 4.5) microns.

4-septate, 15 per cent, 35 by 5 microns.

5-septate, 70 per cent, 40 by 5 (35 to 45 by 4 to 5) microns.

6-septate, rare, 42 by 6 microns.

The percentages of the different septate spores vary in different cultures. For instance, 5-septate spores were 97 per cent on 7-day culture on lima bean and only 40 per cent on oat about 15 weeks old. The size also varies. The cause of difference in size would seem to depend on temperature and moisture conditions quite as much as on the medium used.

No. 84 was much the same as No. 77 and 85 but showed the following variations in spore measurements:

1-septate, rare.

2-septate, 4 per cent.

3-septate, 40 per cent, 26 by 4.5 microns.

4-septate, 30 per cent, 32 by 4.5 microns.

5-septate, 25 per cent, 36 by 5 microns.

6-septate, rare, 38 by 5 microns.

Color of growth lighter on potato glucose, orange tinge with bacterial-like growth below.

No. 52 was much the same as No. 77 and 85 but showed the following variations in spore measurements:

3-septate, 12 per cent, 25 to 40 by 4.5 microns.

4-septate, 8 per cent, 38 by 4.6 microns.

5-septate, 80 per cent, 42 by 5 (35 to 52 by 5) microns.

6-septate, rare.

Spores seem to be slightly less curved than in No. 77, though they grade into each other.

Color of growth slightly different, apricot orange rather than flesh ocre on all media used. On lima bean mycelium was medium in growth, contrasted with its scarcity in No. 77.

The darkening of the medium mentioned in note on *Fusarium discolor* var. *sulphureum* was never noticed in cultures of this number.

F. culmorum (W. Smith) Sacc. (Description taken from No. 84, which was the only isolation made of this species.) See Plate 2, D.

Macroconidia dorsiventral, ventrally straight or very slightly curved, slight constriction at the apical end and the pedicillate base, quite uniform diameter throughout, typically 5-septate 36 by 6 microns. 3- and 4-septate conidia are not uncommon. Conidia have thick membranes and very pronounced septa. Orange-colored sporodochia found.

Conidial chlamy dospores abundant on lima bean agar, age 175 days.

On potato glucose agar mycelium abundant, bright pink above, carmine to ox-blood red below.

Substratum ox-blood red.

SECTION MARTIELLA

The members of the one group (F) that fell within this section were not identical in their cultural characters but were sufficiently similar to suggest a group. Microscopically it is quite easy to recognize the group

[7] On potato glucose agar, both in the light and in the dark, the medium sometimes darkened to a brown black and the growth became more or less powdery, from Sanford's brown to a nigger brown in color, the pseudopionnotes disappearing. Spores mounted from such cultures appeared more or less disintegrated.

Martiella, for the blunt spores are very characteristic. The size of the spores and number of septations vary considerably, and to identify the species and varieties offers many difficulties.

Sherbakoff (*18*), in this group also, has made varieties that could have been avoided had he made his species a little more comprehensive, and from our experience the characters of some of his species are not sufficiently stable. Such characters are comparative width and length of spores, "somewhat narrower macroconidia," "color of conidia and substratum usually paler," "frequent occurrence of bluish plectenchyma," etc.

After considerable comparative study we have identified No. 30, 35, 36, 47, 49, 51, 53, 71, 74, 80, 89, 90, 94, and 95 as *Fusarium solani.* Slight differences occur in these cultures, which, judging from a single photograph or a single set of notes, might suggest a variety of *F. solani* or of *F. Martii* or even a new species, but study of all of the data shows that these cultures do not have sufficiently stable characters to identify them as varieties or species. The cultures vary from one another and from the descriptions of *F. solani* only in minor details. Some of these variations are shown in the two photographs of *F. solani.* (Pl. 3, A and B.)

Fusarium solani (Mart. p. par.) Ap. et. Wr. (Description taken from No. 95 as a type.)

Macroconidia typically somewhat broader in upper half, rounded to slightly constricted apex, slightly if at all pedicillate. Normally 3-septate 28.75 by 4.5 microns (limits 27 to 38.5 by 4 to 5 microns), sometimes 2- and 4-septate, rarely 5-septate.

Pseudopionnotes and sporodochia occur commonly on most media.

Microconidia may or may not be present. When present usually abundant, round or oval in shape. Chlamydospores in mycelium terminal and intercalary, common in old cultures.

Aerial mycelium weak to well developed, typically white, neutral gray, sometimes with a purple tint.

Substratum on potato glucose agar usually from deep purplish vinaceous to dull violet black. Color on oat agar a mixture of blue, green, and purple.

Fusarium coeruleum (Lib.) Sacc. (Description taken from No. 55, which was the only isolation made of this species.)

Macroconidia dorsiventral, slightly ventrally curved. Basal end distinctly pedicillate. Apex rounded, more or less abruptly attenuated. Uniform diameter throughout.

Three-septate spores dominant, quite variable, 31 to 42 microns by 5 to 6 microns.

Aerial mycelium feltlike in age, appressed, white to bluish white and olive buff to dusky slate, violet on potato glucose agar.

Plectenchymatic tissue and substratum on potato glucose agar violet to indigo blue and bluish black.

Chlamydospores very abundant in old cultures, terminal and intercalary and in long chains and masses.

OTHER SECTIONS AND UNIDENTIFIED ISOLATIONS

The few cultures that fell outside of the three sections just discussed were identified by means of Sherbakoff's key and descriptions (*18*), but since we did not have known cultures for comparison, no descriptions of them are included here. The identification of each as we determined it is as follows:

Section Gibbosum: No. 20, *Fusarium gibbosum.*

Section Roseum: No. 13, *F. subulatum* var. *brevius.*

Section Arthrosporiella: No. 69, *F. arthrosporioides;* No. 72, *F. anguioides*? (Chlamydospores were sometimes found.)

Section Ferruginosum: No. 75, *F. bullatum* (may be variety *roseum*).

TABLE I.—*Summary of identifications made, together with source of diseased tubers, character of disease and date of isolation*

Isolation No.	Source of tubers.	Date of isolation.	Suspected disease.	Determination.
1	Roundup	Feb. 20, 1914	Dryrot	*F. trichothecioides.*
3	do	do	do	Do.
5	do	do	do	Do.
6	do	do	do	Do.
7	Stevensville	do	do	Do.
8	do	do	do	Do.
9	do	do	do	Do.
10	do	do	do	Do.
11	Lavina	Mar. 15, 1914	do	Do.
12	do	do	do	Do.
13	Miles City	Apr. 1, 1914	do	*F. subulatum* var. *brevius.*
14	do	do	do	*F. trichothecioides.*
15	Moccasin	Jan. 15, 1915	do	*F. subpallidum* var. *roseum.*
16	do	do	do	*F. discolor.*
17	do	do	do	*F. subpallidum* var. *roseum.*
20	do	do	do	*F. gibbosum.*
21	do	do	do	*F. oxysporum* var. *sclerotium.*[1]
22	do	do	do	*F. subpallidum* var. *roseum.*
23	do	do	do	*F. clavatum.*
24	Rockvale	do	Wilt	*F. oxysporum* var. *sclerotium.*[1]
25	do	Jan. 16, 1915	do	Do.[1]
27	do	do	do	Do.[1]
28	do	do	do	Do.[1]
29	do	do	do	Do.[1]
30	do	do	do	*F. solani.*
31	do	do	do	*F. oxysporum* var. *sclerotium.*[1]
33	Great Falls	Jan. 15, 1915	Dryrot	*F. trichothecioides.*
35	Rockvale	Jan. 21, 1915	Wilt	*F. solani.*
36	do	do	do	Do.
38	Fallon	Jan. 22, 1915	do	*F. trichothecioides.*
40	Moccasin	Jan. 26, 1915	do	*F. subpallidum* var. *roseum.*
41	do	do	do	*F. clavatum.*
42	do	do	do	Do.
43	do	do	do	Do.
45	Corvallis	Feb. 3, 1915	do	*F. oxysporum* var. *sclerotium.*[1]
46	do	do	do	Do.[1]
47	do	do	do	*F. cF. solani.*
48	do	do	do	*ulmorum.*
49	do	do	do	*F. solani.*
51	Bozeman	June 5, 1915	Dryrot	Do.
52	Wibaux	do	do	*F. discolor* var. *sulphureum.*
53	Bozeman	June 7, 1915	do	*F. solani.*
55	do	June 22, 1915	do	*F. coeruleum.*
58	Lewistown [2]	Aug. 23, 1915	Wilt	*F. oxysporum* var. *sclerotium.*[1]
59	do	do	do	*F. sclerotioides* var. *brevius.*[1]
61	Great Falls	Sept. 7, 1915	do	*F. trichothecioides.*
63	Havre	Dec. 10, 1915	Dryrot	Do.
64	Roundup	Mar. 21, 1916	do	Do.
69	Billings	Apr. 20, 1916	do	*F. arthrosporioides.*
71	Darby	May 4, 1916	do	*F. solani.*

[1] See notes on Elegans group p. 357.

[2] There is some confusion in the record of the source of this culture. It is recorded in one place as Big Arm and in another as Lewistown. Circumstantial evidence points to the latter as correct.

TABLE I.—*Summary of identifications made, together with source of diseased tubers, character of disease and date of isolation*—Continued

Isolation No.	Source of tubers.	Date of isolation.	Suspected disease.	Determination.
72	Bozeman	May 1, 1916	Dryrot	*F. anguioides.*
74	Havre	June 27, 1916	do	*F. solani.*
75	Forsyth	Feb. 1, 1917	do	*F. bullatum.*
76	do	May 22, 1917	do	*F. trichothecioides.*
77	do	do	do	*F. discolor* var. *sulphureum.*
79	Stone Shack	do	do	*F. trichothecioides.*
80	Bozeman	do	do	*F. solani.*
81	Glendive	do	do	*F. trichothecioides.*
83	Billings	do	do	Do.
84	do	do	do	*F. discolor* var. *sulphureum.*
85	do	do	do	Do.
89	Glasgow	June 12, 1917	do	*F. solani.*
90	Boulder	do	do	Do.
91	Hardin	do	do	*F. trichothecioides.*
94	Joliet	June 14, 1917	do	*F. solani.*
95	Bozeman	June 16, 1917	do	Do.
97	Circle	Mar. 23, 1918	do	*F. trichothecioides.*

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PLATE 1

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B.—*Fusarium clavatum*, No. 41, grown on oat agar. Age 47 days.
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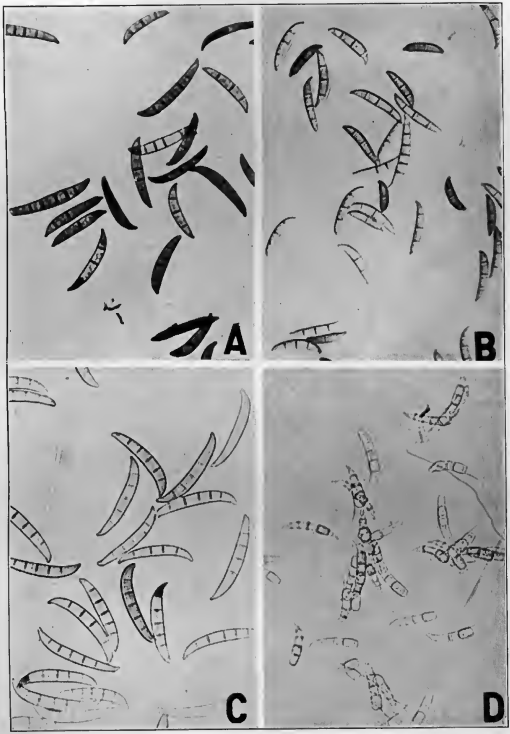
D.—*Fusarium discolor* var. *sulphureum*, No. 77, grown on lima bean agar. Age 91 days. (Altkulture.)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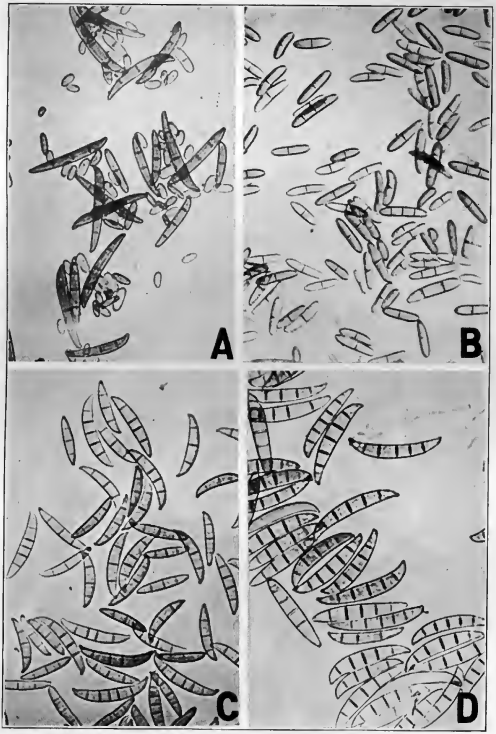
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A.—*Fusarium sclerotioides* var. *brevius*, No. 59, grown on lima bean agar. Age 9 days.
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A.—*Fusarium solani*, No. 95, grown on tomato leaves and stems. Age 37 days.
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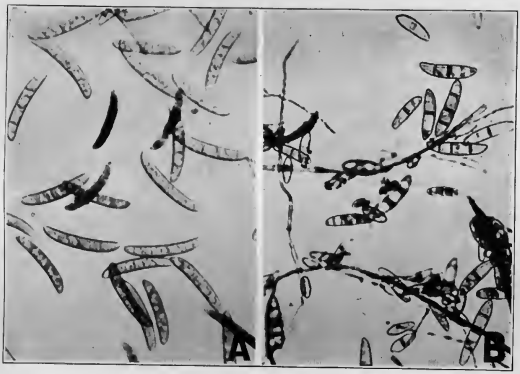
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DETERMINATION OF FATTY ACIDS IN BUTTER FAT: II [1]

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INTRODUCTION

Since the publication of an earlier report (7) [2] on the subject, work has been continued under rather adverse conditions due to numerous changes in staff and other unavoidable interruptions. The study of technical methods for fat analysis was undertaken solely for the purpose of evolving some scheme for determining the constituents of butter fat, particularly the different fatty acids, whereby the influence of various physiological factors might be more accurately measured. Sufficient progress having been made in the methods to warrant their application, several experiments were planned with the view of obtaining information relative to the effect of breed, period of lactation, and of different oils and fats in the ration. The aim of each experiment was to determine some distinct phase of the problem, supplemental to the others, and finally to summarize all available data, as indicated by the following synopsis:

I. Composition of butter fat:
   1. From the milk of mixed herd, grade Holsteins and grade Jerseys, fed normal rations.
   2. From the milk of single animals, grade Holsteins and grade Jerseys, comparatively fresh in lactation, fed normal rations.
   3. From the milk of single animals, grade Holsteins and grade Jerseys, fresh, intermediate, and late in lactation, fed normal rations.
   4. From mixed milk of grade Holsteins fed a normal ration with and without the addition of various oils and fats.

II. Summary of data from Massachusetts and elsewhere, together with such general deductions as seem warranted.

It is obvious at the outset that the number of trials will be too small and too limited in scope to furnish even a tithe of the information necessary to a full understanding of the problem, but the authors hope that the investigation may at least throw some light on a difficult subject. All data, both descriptive and analytical, not deemed absolutely essential have been omitted to economize space.

APPLICATION OF THE METHOD

The cows used in the several experiments herein reported were grade Holsteins and grade Jerseys of the experiment station herd. They were housed in comfortable, well-lighted, and well-ventilated stables

[1] Accepted for publication July 11, 1922. Contribution from the Department of Chemistry, Massachusetts Agricultural Experiment Station. Submitted with the approval of the Director of the Station.

[2] Reference is made by number (italic) to "Literature cited," pp. 397-398.

and turned out into the yard for several hours every day for exercise, weather permitting. They were fed and milked twice daily, had access to water at all times, and in general received excellent care.

The cream was separated by gravity (Cooley system) and churned as sweet as possible to preserve the several milkings required. The resulting butter fat was melted, filtered, and retained for analysis in glass-stoppered bottles in a partially darkened room.

I. COMPOSITION OF BUTTER FAT

1. FROM THE MILK OF MIXED HERD, GRADE HOLSTEINS AND GRADE JERSEYS, FED NORMAL RATIONS

The analysis given in Tables I and II was published in the earlier article (7), but the percentage of oleic acid has been recalculated from the iodin number of the fat instead of from that of the insoluble acids, and the results are offered as probably typical of butter fat produced by grade animals under the care and feeding practiced at the experiment station. The figures may at least serve as a tentative basis for comparison in the other experiments.

TABLE I.—*Analysis of butter fat*

Saponification number (s)	(mgm.)	231.453
Acid number (a)	(mgm.)	2.183
Ether number (e)	(mgm.)	229.270
Iodin number		27.999
Equivalent in oleic acid	(per cent)	31.145
Total fatty acids (T) (1.00−0.00022594 e)	(per cent)	94.819
Neutralization number (n) s/T	(mgm.)	244.100
Free fatty acid (A) a/n	(per cent)	.894
Soluble fatty acids (S) ($T-I$)	(per cent)	7.319
Neutralization number	(mgm.)	509.619
Insoluble fatty acids (I) by alcoholic potash	(per cent)	87.500
Neutralization number	(mgm.)	221.890
Stearic acid (by crystallization)	(per cent)	13.010
Glycerol (0.00054703 e)	(per cent)	12.542

TABLE II.—*Fatty acids in butter fat*

Fatty acids.	Per cent.
Soluble acids:	
Butyric acid (by difference)	3.153
Caproic acid	1.360
Caprylic acid	.975
Capric acid	1.831
Total	7.319
Insoluble acids:	
Lauric acid	6.895
Myristic acid	22.618
Palmitic acid (by difference)	15.458
Stearic acid	11.384
Oleic acid	31.145
Total	87.500
Total fatty acids	94.819

As decomposition vitiates certain determinations in the analysis of oils and fats, the results fail to create a perfect entity, but with experienced workers such errors may gradually be reduced to a minimum.

2. FROM THE MILK OF SINGLE ANIMALS, GRADE HOLSTEINS AND GRADE JERSEYS, COMPARATIVELY FRESH IN LACTATION, FED NORMAL RATIONS

The cows in this test were fair types of their respective breeds and comparable in age. Cecile II had freshened more recently than the others. The Holsteins were a high-fat strain of moderate milk yield. The milk produced was the daily average for the period in which cream was saved for churning. The milk analyzed was a five-days' composite, taken about the same time as the cream samples. The feeds, both grain and roughage, were average products of their kind of which analyses were not considered necessary. The results of this test are given in Table III.

TABLE III.—*Records of cows and milk analysis*

	Colantha II.	Samantha II.	Cecile II.	Peggy.
Breed	Grade Holstein	Grade Holstein	Grade Jersey	Grade Jersey.
Date of birth	Oct. 14, 1914	Aug. 18, 1909	Dec. 18, 1912	.... 1910.
Last calf dropped	July 3, 1917	Aug. 16, 1917	Oct. 19, 1917	Aug. 9, 1917.
Condition on calving	Good flesh	Good flesh	Good flesh	Good flesh.
Date served	Oct. 22, 1917	Oct. 26, 1917	Nov. 24, 1917	Nov. 8, 1917.
Weight of animal	1,000 pounds	1,192 pounds	700 pounds (?)	780 pounds.
Daily ration:				
Hay	18 pounds	24 pounds	7 pounds	17 pounds.
Alfalfa			4.5 pounds	
Corn stover			16	
Gluten feed	4 pounds	4.5 pounds	3.8 pounds	3 pounds.
Wheat bran			2.5 pounds	
Ground oats	2 pounds	2.5 pounds		1 pound.
Corn bran	4 pounds	4 pounds		4 pounds.
Milk produced (daily average).[1]	Oct. 22 to 25, 1917, 23.7 pounds.	Oct. 26 to 29, 1917, 29.7 pounds.	Nov. 5 to 8, 1917, 24.3 pounds.	Oct. 18 to 21, 1917, 17.6 pounds
Milk analysis:				
Solids (gravimetric)	13.38 per cent	12.95 per cent	14.43 per cent	15.82 per cent.
Fat (Babcock)	4.40 per cent	4.55 per cent	5.40 per cent	6.60 per cent.
Proteids (N × 6.25)	3.37 per cent	3.22 per cent	3.40 per cent	3.98 per cent.
Lactose (by difference)	4.87 per cent	4.47 per cent	4.92 per cent	4.45 per cent.
Ash	.74 per cent	.71 per cent	.71 per cent	.79 per cent.

[1] For the days cream was saved for churning.

ANALYSIS OF BUTTER FAT

The methods employed for the ordinary analysis of butter fat have been described previously (*8*). The use of glycerol potash for the determination of insoluble acids and the preparation of stock has been superseded, however, by alcoholic potash as less drastic in its action on the unsaturated acids; but even the latter under careful manipulation tends to induce intramolecular changes resulting in a loss of iodin absorption and to some extent an increase in alkali-consuming power due to instability of the resulting molecule, particularly with linolic, linolenic, and other highly unsaturated acids, as shown by Fittig, Varrentrapp, and Schrauth, summarized in another article (*10, p. 362*). Recent experiments, although incomplete, indicate that under control conditions such decomposition can be largely prevented with normal butter fat. The determination of stearic acid by crystallization from alcohol is unquestionably simple in theory but rather difficult in practice except in a cold, dry atmosphere, since condensed moisture is a vitiating factor. The analysis of butter fat is given in Table IV.

TABLE IV.—*Analysis of butter fat*

	Colantha II.	Samantha II.	Cecile II.	Peggy.
Saponification number (s).....mgm..	229.466	230.324	230.907	230.968
Acid number (a).....mgm..	.927	2.386	1.626	3.657
Ether number (e).....mgm..	228.539	227.938	229.281	227.311
Iodin number.....	30.622	29.605	28.159	22.720
Equivalent in oleic acid.....per cent..	34.063	32.931	31.323	25.273
Total fatty acids (T) (1.00−0.00022594 e).....per cent..	94.836	94.850	94.820	94.864
Neutralization number (n) s/T .....mgm..	241.961	242.830	243.521	243.473
Free fatty acids (A) a/n.....per cent..	.383	.983	.668	1.502
Soluble fatty acids (S) ($T-I$) .....per cent..	6.470	7.023	7.226	6.510
Neutralization number.....mgm..	503.184	513.356	517.409	495.637
Insoluble fatty acids (I) by alcoholic potash.....per cent..	88.366	87.827	87.594	88.354
Neutralization number.....mgm..	222.835	221.197	220.927	224.893
Stearic acid by crystallization .....per cent..	13.709	16.997	20.321	13.398
Glycerol (0.00054703 e).....per cent..	12.502	12.469	12.542	12.435

The percentages of total fatty acids were 94.843 in the Holsteins and 94.842 in the Jerseys and their neutralization numbers 242.396 and 243.497 mgm., respectively. The percentages of free fatty acids were 0.683 and 1.085, indicating a greater tendency to hydrolyze in the Jerseys. The percentages of soluble fatty acids were 6.747 and 6.868, and their neutralization numbers 508.270 and 506.523. The percentages of insoluble fatty acids were 88.097 and 87.974, and their neutralization numbers 222.016 and 222.910. The nature of these differences is apparent from the data, but the extent can not be actually apportioned with five components involved. The two breeds showed a remarkably close agreement in the several groups of fatty acids and of glycerol, but the proportion of high molecular weight acids in the insolubles was slightly more pronounced in the Holsteins.

The composition of neither breed conformed particularly to that of the herd sample (Table I), although the difference in actual percentage of soluble and of insoluble acids was more appreciable than the difference in proportion of constituent acids in each group, as indicated by the neutralization numbers.

ESTERIFICATION PROCESS

The method (7) of esterification, purification, and fractionation of the ethyl esters remains substantially as published. Attention might be called, however, to some minor modifications that have since been adopted. Alcohol for esterification is prepared by distilling approximately 2 liters in a water bath over 600 to 700 gm. of granulated caustic lime (95 per cent CaO) and 30 to 40 gm. of yellow ceresin wax and redistilling over fresh lime and ceresin until free from water, as indicated by the absence of lime from solution. On the final distillation the first and last portions are rejected for additional treatment and the main portion is preserved. This process has proved the most reliable so far tested. Three cc. of concentrated sulphuric acid have been substituted as a catalyzer in place of dry hydrochloric acid at a material saving in time and convenience and considerable gain in efficiency.

A second extraction of the alcoholic residue and combined washings with ether is no longer considered necessary. A "high" side tube distillation flask is employed in both the preliminary distillation of the esters and the fractionation. The number of fractions has been increased to 7. Fraction 1, ranging to about 100° C.[3], is the most volatile portion derived from the esters, largely ether but containing considerable ethyl butyrate. It is made to a volume of 500 cc. with ether and the alkali-consuming power of an aliquot is determined; from this the grams of ethyl butyrate can be calculated. Fractions 2 to 6 are now largely determined by weight, a small Troemner scale with prepared counterpoise being used, since the temperature uncorrected and influenced by the speed of distillation has not proved entirely a safe guide. The content of ethyl oleate is no longer considered a prominent factor. By using an adapter on the end of the condenser little difficulty is encountered in changing flasks. The recovery in fractions 2 to 7 has been greatly increased and now averages 185 gm. from 300 gm. of butter fat, with a maximum of 216 gm. thus far.

TABLE V.—*Weight and analysis of fractions (ethyl esters)*

COLANTHA II

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	° C.	*Gm.*	*Mgm.*		*Per cent.*
1	±70 to 90	.....	[a] 1,453.197	.....	.....
2	90 to 165	5.6093	396.205	3.776	4.618
3	165 to 217	3.9590	337.924	7.241	8.854
4	217 to 257	6.4436	267.689	11.872	14.518
5	257 to 286	13.7516	238.602	14.216	17.384
6	286 to 317	57.4804	218.942	16.275	19.902
7	317 to 332	97.4166	200.959	22.324	27.299
Total	.....	184.6605	.....	.....	.....

SAMANTHA II

1	±70 to 90	.....	[a] 1,655.186	.....	.....
2	90 to 166	5.5964	385.988	5.055	6.182
3	166 to 218	3.9766	335.668	8.944	10.937
4	218 to 258	5.4594	270.757	12.834	15.694
5	258 to 286	14.2372	238.847	14.605	17.860
6	286 to 317	53.7426	217.020	16.714	20.439
7	317 to 327	79.3705	200.880	19.797	24.209
Total	.....	162.3827	.....	.....	.....

CECILE II

1	±70 to 100	.....	[a] 2,084.412	.....	.....
2	100 to 170	5.6765	405.373	2.048	2.504
3	170 to 220	4.0712	357.188	3.725	4.555
4	220 to 261	4.6266	287.805	7.613	9.309
5	261 to 286	8.5821	249.297	10.207	12.481
6	286 to 318	55.7372	218.974	14.914	18.238
7	318 to 326	71.7496	204.804	19.707	24.098
Total	.....	150.4432	.....	.....	.....

[a] Total alkali-consuming power of the fraction.

[3] With a minimum of 100° C. the complete elimination of ether and alcohol is more definitely assured; otherwise these might vitiate fraction 2.

TABLE V.—*Weight and analysis of fractions*—Continued

PEGGY

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1	±70 to 90	.....	[a] 1,239.987	.....	.....
2	90 to 167	4.5351	412.882	2.279	2.787
3	167 to 220	4.3029	343.102	5.383	6.583
4	220 to 260	5.7014	273.896	9.093	11.119
5	260 to 286	14.9828	240.286	11.265	13.775
6	286 to 317	54.2113	218.762	12.135	14.839
7	317 to 328.5	92.9064	203.218	14.813	18.114
Total	.....	176.6399	.....	.....	.....

[a] Total alkali-consuming power of the fraction.

TABLE VI.—*Fatty acids in butter fat*

Fatty acids.	Colantha II.	Samantha II.	Cecile II.	Peggy.
Soluble acids:	Per cent.	Per cent.	Per cent.	Per cent.
Butyric acid (by difference)	2.260	2.928	3.007	2.726
Caproic acid	1.588	1.688	1.794	1.290
Caprylic acid	.648	.744	.881	.779
Capric acid	1.974	1.663	1.544	1.715
Total	6.470	7.023	7.226	6.510
Insoluble acids:				
Lauric acid	7.618	6.317	5.616	6.598
Myristic acid	19.768	17.455	20.534	21.782
Palmitic acid (by difference)	14.803	16.196	12.321	22.863
Stearic acid	12.114	14.928	17.800	11.838
Oleic acid	34.063	32.931	31.323	25.273
Total	88.366	87.827	87.594	88.354
Total fatty acids	94.836	94.850	94.820	94.864

The percentage of butyric acid was 2.594 in the Holsteins and 2.867 in the Jerseys; of caproic acid, 1.638 and 1.542; of caprylic acid, 0.696 and 0.830; and of capric acid, 1.819 and 1.630. The differences were compensating, the high percentages alternating, but were not sufficiently indicative to warrant deductions. The percentage of lauric acid was 6.968 in the Holsteins and 6.107 in the Jerseys; of myristic acid, 18.612 and 21.158; of palmitic acid, 15.500 and 17.592; of stearic acid, 13.521 and 14.819; and of oleic acid, 33.497 and 28.298. The outstanding feature was the low oleic acid content of Peggy's fat, which necessarily affected the results. Despite this fact, however, a lower percentage of myristic and stearic acids and a higher percentage of oleic acid appeared to be characteristic of the Holsteins as contrasted with the Jerseys.

As compared with the herd sample (Table II) neither breed showed any appreciable differences to which attention has not already been called. That the percentage of caproic acid in the herd sample was apparently abnormal, being noticeably lower than is indicated by a subsequent average, is taken into consideration.

3. FROM THE MILK OF SINGLE ANIMALS, GRADE HOLSTEINS AND GRADE JERSEYS, FRESH, INTERMEDIATE, AND LATE IN LACTATION, FED NORMAL RATIONS

The cows were grades, the Jerseys on the average were appreciably older, but all had freshened within a few weeks. Cecile II was the same cow used the year previous. The Holsteins were a 4 per cent strain of fair milk yield. Fancy III, although a high-grade Jersey, did not produce as rich milk as many of that breed.

In the sampling of milk and cream there was an interval of four months [4] between the so-called fresh and intermediate periods of lactation and an additional three months [4] to the late period, during which time the milk yield decreased nearly 32 per cent in the Holsteins and 28 per cent in the Jerseys, and in general the percentages of solids and fat increased but not as consistently as might have been expected.

The same grain mixture and similar hay were fed throughout the different lactation periods, but the amounts varied as stated. Analysis of the hay and various grains indicates their quality (Table X).

TABLE VII.—*Records and analysis of milk from cows fresh in lactation*

	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Breed	Grade Holstein	Grade Holstein	Grade Jersey	Grade Jersey.
Date of birth	Nov. 16, 1913	Aug. 25, 1914	Dec. 18, 1912	Aug. 11, 1908.
Last calf dropped	Aug. 20, 1918	Oct. 6, 1918	Aug. 26, 1918	Oct. 12, 1918.
Condition on calving	Good flesh	Good flesh	Thin	Thin.
Date served	Dec. 9, 1918	Nov. 15, 1918	Jan. 9, 1919	Mar. 26, 1919.
Weight of animal	1,195 pounds	1,113 pounds	713 pounds	863 pounds.
Daily ration:				
Hay	26 pounds	24 pounds	18 pounds	22 pounds.
Grain mixture	12 pounds	12 pounds	8 pounds	9 pounds.
Cottonseed meal, 20 per cent.				
Gluten feed, 20 per cent.				
Wheat bran, 30 per cent.				
Corn feed meal, 30 per cent.				
Milk produced (daily average).[1]	Sept. 23 to 26, 1918, 33.5 pounds.	Oct. 31 to Nov. 3, 1918, 41.4 pounds.	Sept. 23 to 27, 1918, 23.7 pounds.	Oct. 31 to Nov. 3, 1918, 33.5 pounds.
Milk analysis:				
Solids (gravimetric)	12.47 per cent	12.80 per cent	14.43 per cent	12.50 per cent.
Fat (Babcock)	3.93 per cent	4.38 per cent	5.20 per cent	4.18 per cent.
Proteids (N×6.25)	3.12 per cent	3.02 per cent	3.66 per cent	3.06 per cent.
Lactose (by difference)	4.69 per cent	4.69 per cent	4.85 per cent	4.51 per cent.
Ash	.73 per cent	.71 per cent	.72 per cent	.75 per cent.

[1] For the days cream was saved for churning.

TABLE VIII.—*Records and analysis of milk from cows intermediate in lactation*

	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Weight of animal	1,280 pounds	1,163 pounds	753 pounds	878 pounds.
Daily ration:				
Hay	24 pounds	22 pounds	18 pounds	20 pounds.
Grain mixture	13 pounds	13 pounds	8 pounds	10 pounds.
Milk produced (daily average).[1]	Jan. 26 to 29, 1919, 27.9 pounds.	Jan. 30 to Feb. 2, 1919, 33.6 pounds.	Jan. 26 to 29, 1919, 16.7 pounds.	Jan. 30 to Feb. 2, 1919, 29.8 pounds.
Milk analysis:				
Solids (gravimetric)	12.84 per cent	12.66 per cent	15.52 per cent	13.00 per cent.
Fat (Babcock)	4.28 per cent	4.25 per cent	6.13 per cent	4.80 per cent.
Proteids (N×6.25)	3.32 per cent	3.07 per cent	4.10 per cent	3.10 per cent.
Lactose (by difference)	4.52 per cent	4.68 per cent	4.58 per cent	4.40 per cent.
Ash	.72 per cent	.66 per cent	.71 per cent	.70 per cent.

[1] For the days cream was saved for churning.

[4] Approximate length of period

TABLE IX.—*Records and analysis of milk from cows late in lactation*

	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Weight of animal	1,336 pounds	1,196 pounds	730 pounds	899 pounds.
Daily ration:				
Hay	22 pounds	22 pounds	16 pounds	20 pounds.
Grain mixture	13 pounds	13 pounds	8 pounds	10 pounds.
Milk produced (daily average).[1]	Apr. 23 to 30, 1919, 23.5 pounds.	Apr. 19 to 25, 1919, 27.7 pounds.	Apr. 19 to 23, 1919, 16.6 pounds.	Apr. 21 to 27, 1919, 25.1 pounds.
Milk analysis:				
Solids (gravimetric)	13.45 per cent	13.12 per cent	15.07 per cent	13.00 per cent.
Fat (Babcock)	4.65 per cent	4.40 per cent	5.80 per cent	4.80 per cent.
Proteids (N×6.25)	3.52 per cent	3.18 per cent	3.93 per cent	3.27 per cent.
Lactose (by difference)	4.54 per cent	4.85 per cent	4.64 per cent	4.19 per cent.
Ash	.74 per cent	.69 per cent	.70 per cent	.74 per cent.

[1] For the days cream was saved for churning.

TABLE X.—*Analysis of feeds*

	Hay.	Cottonseed meal.	Gluten feed.	Wheat bran.	Corn feed meal.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	12.35	7.50	9.32	11.90	16.70
Dry matter:					
Ash	6.03	6.06	4.08	6.93	1.55
Protein (N×6.25)	8.09	38.51	26.77	16.44	10.31
Fiber	30.16	14.18	8.49	11.84	2.38
Extract matter	53.07	34.10	56.81	59.31	84.27
Fat	2.65	7.15	3.85	5.48	1.49

TABLE XI.—*Analysis of butter fat from cows fresh in lactation*

	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Saponification number (s) (mgm.)	232.561	233.016	232.456	235.333
Acid number (a) (mgm.)	2.850	1.663	1.997	2.803
Ether number (e) (mgm.)	229.711	231.353	230.459	232.530
Iodin number	30.135	28.285	26.900	25.477
Equivalent in oleic acid (per cent)	33.521	31.463	29.922	28.340
Total fatty acids (T) (1.00—0.00022594 e) (per cent)	94.810	94.773	94.793	94.746
Neutralization number (n) (s/T) (mgm.)	245.292	245.867	245.225	248.383
Free fatty acids (A) a/n (per cent)	1.162	.676	.814	1.128
Soluble fatty acids (S) ($T-I$) (per cent)	7.668	8.008	7.382	8.601
Neutralization number (mgm.)	513.641	505.132	509.428	510.662
Insoluble fatty acids (I) by alcoholic potash (per cent)	87.142	86.765	87.411	86.145
Neutralization number (mgm.)	221.678	221.939	222.913	222.196
Stearic acid by crystallization (per cent)	17.399	18.669	23.304	20.991
Glycerol (0.00054703 e) (per cent)	12.566	12.656	12.607	12.720

The percentages of total fatty acids were 94.792 in the Holsteins and 94.770 in the Jerseys and their neutralization numbers 245.580 and 246.804 mgm., respectively, indicating a slightly smaller proportion of high molecular weight acids in the Jerseys due to Fancy III's fat. The

percentages of free fatty acids were 0.919 and 0.971. The percentages of soluble fatty acids were 7.838 and 7.992, and their neutralization numbers 509.387 and 510.045, respectively. The percentages of insoluble fatty acids were 86.954 and 86.778, and their neutralization numbers 221.809 and 222.555. The percentage was exceptionally low in Fancy III's fat. The percentages of glycerol were 12.611 and 12.664.

The two breeds contained similar quantities of the several groups of fatty acids and of glycerol, and their acid mixtures, both soluble and insoluble, were of like character, although the neutralization number of the insoluble was slightly higher in the Jerseys.

Both breeds exceeded the herd sample (Table I) materially in soluble fatty acids.

TABLE XII.—*Analysis of butter fat from cows intermediate in lactation*

	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Saponification number (s).....(mgm.)..	229.741	229.190	231.781	234.107
Acid number (a).....(mgm.)..	1.646	2.029	2.728	1.732
Ether number (e).....(mgm.)..	228.095	227.161	229.053	232.375
Iodin number.....	31.501	31.189	24.382	26.750
Equivalent in oleic acid....(per cent)..	35.040	34.693	27.122	29.756
Total fatty acids (T) (1.00—0.00022594 e).....(per cent)..	94.846	94.868	94.825	94.750
Neutralization number (n) (s/T).....(mgm.)..	242.225	241.588	244.430	247.079
Free fatty acids (A) a/n (per cent).....	.680	.840	1.116	.701
Soluble fatty acids (S) ($T-I$) (per cent).	6.744	6.703	6.773	7.542
Neutralization number.....(mgm.)..	535.083	539.370	527.122	538.345
Insoluble fatty acids (I) by alcoholic potash.....(per cent)..	88.102	88.165	88.052	87.208
Neutralization number.....(mgm.)..	219.808	218.949	222.687	221.889
Stearic acid by crystallization.....(per cent)..	16.923	17.732	20.493	18.287
Glycerol (0.00054703 e)....(per cent)...	12.477	12.426	12.530	12.712

The percentages of total fatty acids were 94.857 in the Holsteins and 94.788 in the Jerseys, and their neutralization numbers 241.907 and 245.755 mgm., respectively. The percentages of free fatty acids were 0.760 and 0.908. The percentages of soluble fatty acids were 6.724 and 7.158, and their neutralization numbers 537.227 and 532.734, which were considerably higher than usual. The percentages of insoluble fatty acids were 88.134 in the Holsteins and 87.630 in the Jerseys, and their neutralization numbers 219.379 and 222.288. The percentages of glycerol were 12.452 and 12.621, a noticeable difference.

The Holsteins exceeded the Jerseys in total and insoluble fatty acids and contained a considerably higher proportion of high molecular weight acids in the insolubles. The Jerseys suffered a smaller loss of soluble acids and of glycerol.

The Holsteins exceeded the herd sample (Table I) in insoluble acids with a corresponding loss of soluble acids and a material loss in neutralization number of the insolubles. The Jerseys averaged substantially the same as the herd sample.

TABLE XIII.—*Analysis of butter fat from cows late in lactation*

	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Saponification number (*s*).....(mgm.)..	224.626	225.709	230.834	231.259
Acid number (*a*).....(mgm.)..	2.440	2.701	3.161	2.432
Ether number (*e*).....(mgm.)..	222.186	223.008	227.673	228.827
Iodin number.....	36.241	33.649	26.952	29.472
Equivalent in oleic acid....(per cent)..	40.313	37.430	29.980	32.783
Total fatty acids (*T*) (1.00−0.00022594 *e*) .....(per cent)..	94.980	94.961	94.856	94.830
Neutralization number (*n*) (*s*/*T*) .....(mgm.)..	236.498	237.686	243.352	243.867
Free fatty acid (*A*) *a*/*n*.....(per cent)..	1.032	1.136	1.299	.997
Soluble fatty acids (*S*) (*T*−*I*) .....(per cent)..	6.509	6.577	7.232	7.526
Neutralization number.....(mgm.)..	519.511	519.340	516.081	527.106
Insoluble fatty acids (*I*) by alcoholic potash.....(per cent)..	88.471	88.384	87.624	87.304
Neutralization number.....(mgm.)..	215.676	216.727	220.842	219.450
Stearic acid by crystalization .....(per cent)..	21.628	20.399	21.033	21.512
Glycerol (0.00054703 *e*).....(per cent)..	12.154	12.199	12.454	12.518

The percentages of total fatty acids were 94.971 in the Holsteins and 94.843 in the Jerseys, and their neutralization numbers 237.092 and 243.610 mgm., respectively. The percentages of free fatty acids were 1.084 and 1.148. The percentages of soluble fatty acids were 6.543 and 7.379, and their neutralization numbers 519.426 and 521.594, which were appreciably high. The percentages of insoluble fatty acids were 88.428 and 87.464, and their neutralization numbers 216.202 and 220.146. The percentages of glycerol were 12.177 and 12.486, which was decidedly low in the Holsteins.

The Holsteins further exceeded the Jerseys in total and insoluble fatty acids, with a still greater difference in neutralization number of the insolubles. The Jerseys maintained a high soluble acid content and only a moderate loss of glycerol.

The Holsteins exceeded the herd sample (Table I) in total and insoluble fatty acids by a larger amount than in the previous period and with a greater loss in neutralization number of the insolubles and of glycerol. The Jerseys again averaged practically the same as the herd sample except for an appreciable loss in neutralization number of the insolubles.

TABLE XIV.—*Weight and analysis of fractions of milk from cows fresh in lactation (ethyl esters)*

COLANTHA

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	°*C.*	*Gm.*	*Mgm.*		*Per cent.*
1.....	±70 to 85	.....	[a] 1,472.835	.....	.....
2.....	85 to 145	5.6846	398.375	2.098	2.565
3.....	145 to 190	5.1990	361.366	4.514	5.520
4.....	190 to 230	3.8515	283.442	10.292	12.586
5.....	230 to 278	13.9373	242.332	14.268	17.448
6.....	278 to 310	57.5422	214.019	19.080	23.331
7.....	310 to 328.5	128.4525	197.332	26.687	32.634
Total.....	.....	214.6671	.....	.....	.....

[a] Total alkali-consuming power of the fraction.

TABLE XIV.—*Weight and analysis of fractions of milk from cows fresh in lactation (ethyl esters)*—Continued

SAMANTHA IV (260.1 GM. OF FAT)

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1	±70 to 80	.....	[a] 1,329.760	.....	.....
2	80 to 141	4.2115	391.789	3.229	3.948
3	141 to 186	4.9620	377.169	4.997	6.111
4	186 to 224	3.6875	290.948	8.754	10.705
5	224 to 271	10.8334	242.858	12.122	14.823
6	271 to 306	45.2200	216.148	15.072	18.430
7	306 to ?	87.4847	199.026	20.981	25.656
Total	.....	156.3991	.....	.....	.....

CECILE II (261.96 GM. OF FAT)

1	±70 to 80	.....	[a] 1,085.690	.....	.....
2	80 to 144	4.6898	397.378	2.622	3.206
3	144 to 191	5.0742	345.943	4.517	5.523
4	191 to 220	3.7697	279.378	7.469	9.133
5	220 to 259	11.5881	242.983	10.301	12.596
6	259 to 310	47.8509	214.861	14.929	18.256
7	310 to 325	96.3112	197.850	21.972	26.868
Total	.....	169.2839	.....	.....	.....

FANCY III

1	±70 to 82	.....	[a] 1,822.107	.....	.....
2	82 to 125	5.9548	414.095	2.389	2.921
3	125 to 155	5.6521	376.234	3.962	4.845
4	155 to 192	4.1875	295.894	7.721	9.441
5	192 to 254	13.4642	244.204	11.283	13.797
6	254 to 298	55.5961	215.916	14.377	17.581
7	298 to 314	104.4243	199.966	19.299	23.599
Total	.....	189.2790	.....	.....	.....

[a] Total alkali-consuming power of the fraction.

TABLE XV.—*Fatty acids in butter fat from cows fresh in lactation*

Fatty acids.	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Soluble acids:	Per cent.	Per cent.	Per cent.	Per cent.
Butyric acid (by difference)	3.179	.....	2.998	4.230
Caproic acid	2.210	.....	1.857	2.400
Caprylic acid	.778	.....	1.030	.714
Capric acid	1.501	.....	1.497	1.257
Total	7.668	8.008	7.382	8.601
Insoluble acids:				
Lauric acid	4.949	4.831	4.533	4.804
Myristic acid	20.129	18.445	19.097	20.746
Palmitic acid (by difference)	13.381	15.828	13.419	14.172
Stearic acid	15.162	16.198	20.370	18.083
Oleic acid	33.521	31.463	29.992	28.340
Total	87.142	86.765	87.411	86.145
Total fatty acids	94.810	94.773	94.793	94.746

The percentage of butyric acid was 3.179 in the Holsteins (1 sample) and 3.614 in the Jerseys; of caproic acid, 2.210 and 2.129; of caprylic acid, 0.778 and 0.872; and of capric acid, 1.501 and 1.377. The differences were not pronounced, but there was a tendency toward a higher content of butyric and caprylic acids in the Jerseys.

The percentage of lauric acid was 4.890 in the Holsteins and 4.669 in the Jerseys; of myristic acid, 19.287 and 19.922; of palmitic acid, 14.605 and 13.796; of stearic acid, 15.680 and 19.227; and of oleic acid, 32.492 and 29.166. The percentage of myristic and stearic acids was lower and that of oleic acid higher in the Holsteins than in the Jerseys.

The soluble acids of the Holsteins were of the same general character as those of the herd sample (Table II), differing somewhat in total but more particularly in the amount of each acid in the mixture, although such differences were largely compensating. This was equally true of the insolubles. In the Jerseys these differences were even more marked than in the Holsteins.

TABLE XVI.—*Weight and analysis of fractions of milk from cows intermediate in lactation (ethyl esters)*

COLANTHA

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1	±70 to 80	.....	[a] 1,397.089	.....	.....
2	80 to 148	5.5029	392.083	3.112	3.806
3	148 to 210	5.7301	350.465	6.245	7.637
4	210 to 248	4.1855	273.351	11.659	14.257
5	248 to 283	13.0994	238.216	15.290	18.697
6	283 to 318	54.5059	214.841	18.830	23.026
7	318 to 332	110.1808	198.452	25.566	31.263
Total	.....	193.2046	.....	.....	.....

SAMANTHA IV

1	±70 to 80	.....	[a] 1,383.062	.....	.....
2	80 to 139	5.4437	397.596	2.984	3.649
3	139 to 195	5.6695	351.131	6.143	7.512
4	195 to 235	4.3474	269.935	11.561	14.137
5	235 to 275	13.3353	236.493	14.739	18.023
6	275 to 315	54.8424	213.021	18.383	22.480
7	315 to 329	108.1717	198.017	25.176	30.786
Total	.....	191.8100	.....	.....	.....

CECILE II

1	±70 to 80	.....	[a] 1,377.451	.....	.....
2	80 to 153	5.4392	390.056	2.688	3.287
3	153 to 218	5.8774	340.175	4.798	5.867
4	218 to 257	4.4112	273.495	8.220	10.052
5	257 to 287	13.3860	240.218	10.728	13.119
6	287 to 317	54.8585	216.355	13.844	16.929
7	317 to 336	131.1700	198.795	20.386	24.929
Total	.....	215.1423	.....	.....	.....

[a] Total alkali-consuming power of the fraction.

TABLE XVI.—*Weight and analysis of fractions of milk from cows intermediate in lactation (ethyl esters)*—Continued

FANCY III

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1	±70 to 80		[a] 1,500.889		
2	80 to 132.5	5.3816	401.690	1.797	2.197
3	132.5 to 180	5.6924	364.612	3.901	4.770
4	180 to 216	4.0433	291.181	8.069	9.867
5	216 to 264	12.9128	245.121	12.093	14.788
6	264 to 308	54.4026	216.675	15.754	19.265
7	308 to 327	109.9412	200.142	21.291	26.035
Total		192.3739			

[a] Total alkali-consuming power of the fraction.

TABLE XVII.—*Fatty acids in butter fat from cows intermediate in lactation*

Fatty acids.	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Soluble acids:	Per cent.	Per cent.	Per cent.	Per cent.
Butyric acid (by difference)	2.525	2.777	2.492	3.028
Caproic acid	2.118	2.029	1.862	2.253
Caprylic acid	.763	.660	1.041	.881
Capric acid	1.338	1.237	1.378	1.380
Total	6.744	6.703	6.773	7.542
Insoluble acids:				
Lauric acid	5.409	4.534	5.403	5.272
Myristic acid	19.188	19.624	21.287	20.763
Palmitic acid (by difference)	13.555	13.681	16.196	15.469
Stearic acid	14.910	15.633	18.044	15.948
Oleic acid	35.040	34.693	27.122	29.756
Total	88.102	88.165	88.052	87.208
Total fatty acids	94.846	94.868	94.825	94.750

The percentage of butyric acid was 2.651 in the Holsteins and 2.760 in the Jerseys; of caproic acid, 2.074 and 2.058; of caprylic acid, 0.712 and 0.961; and of capric acid, 1.288 and 1.379. In the main the results confirmed those of the previous period as to breed differences.

The percentage of lauric acid was 4.972 in the Holsteins and 5.338 in the Jerseys; of myristic acid, 19.406 and 21.025; of palmitic acid, 13.618 and 15.833; of stearic acid, 15.272 and 16.996; and of oleic acid, 34.867 and 28.439. The breed differences as to myristic and oleic acids were more decisive than in the previous period.

In the Holsteins all the soluble acids decreased in the second period, butyric most of all, and nearly all the insolubles increased, particularly oleic acid, but the agreement with the herd sample (Table II) was not materially improved. In the Jerseys the butyric and caproic acids decreased, and several of the insolubles increased, but stearic and oleic acids decreased, as a whole substantially conforming to the herd sample.

TABLE XVIII.—*Weight and analysis of fractions of milk from cows late in lactation (ethyl esters)*

COLANTHA

Fraction No.	Range of fraction.	Weight of fraction.	Saponification No.	Iodin No.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1	±70 to 90	.....	[a]1, 366. 230	.....	.....
2	90 to 146	4. 6359	400. 958	6. 415	7. 845
3	146 to 211	4. 8609	355. 525	10. 366	12. 676
4	211 to 243	4. 1081	282. 435	14. 466	17. 689
5	243 to 283	14. 2259	244. 859	15. 928	19. 477
6	283 to 310.5	52. 3540	220. 233	18. 799	22. 988
7	310.5 to 331.5	67. 4810	202. 637	23. 845	29. 158
Total	.....	147. 6658	.....	.....	.....

SAMANTHA IV

1	±70 to 90	.....	[a]1, 371. 841	.....	.....
2	90 to 140	4. 6752	404. 831	4. 620	5. 650
3	140 to 207	4. 8510	357. 845	8. 077	9. 877
4	207 to 244	3. 9896	285. 900	12. 249	14. 979
5	244 to 280	14. 3177	243. 531	14. 524	17. 761
6	280 to 311	52. 8224	218. 164	17. 529	21. 435
7	311 to 326	71. 0477	202. 061	22. 429	27. 427
Total	.....	151. 7036	.....	.....	.....

CECILE II

1	±70 to 90	.....	[a]1, 357. 814	.....	.....
2	90 to 147	5. 0608	406. 357	5. 070	6. 200
3	147 to 191	5. 3511	351. 782	7. 401	9. 050
4	191 to 231	4. 9009	282. 018	10. 028	12. 263
5	231 to 277	14. 3388	245. 102	11. 208	13. 706
6	277 to 304	52. 3918	220. 609	13. 141	16. 069
7	304 to 321	86. 4479	202. 460	18. 036	22. 055
Total	.....	168. 4913	.....	.....	.....

FANCY III

1	±70 to 80	.....	[a]1, 402. 700	.....	.....
2	80 to 143	4. 7724	390. 725	4. 607	5. 634
3	143 to 199	5. 3625	369. 784	6. 796	8. 311
4	199 to 235	4. 1495	289. 912	10. 379	12. 692
5	235 to 279	14. 2308	244. 922	12. 682	15. 508
6	279 to 310	53. 1995	218. 487	15. 436	18. 876
7	310 to 327	89. 7879	200. 926	20. 772	25. 401
Total	.....	171. 5026	.....	.....	.....

[a] Total alkali-consuming power of the fraction.

TABLE XIX.—*Fatty acids in butter fat from cows late in lactation*

Fatty acids.	Colantha.	Samantha IV.	Cecile II.	Fancy III.
Soluble acids:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Butyric acid (by difference)	2.241	2.494	2.864	3.059
Caproic acid	1.765	1.784	1.726	2.301
Caprylic acid	.653	.733	.857	.616
Capric acid	1.850	1.566	1.785	1.550
Total	6.509	6.577	7.232	7.526
Insoluble acids:				
Lauric acid	7.687	6.592	7.087	6.333
Myristic acid	15.554	17.059	18.985	18.484
Palmitic acid (by difference)	5.782	9.274	13.142	10.923
Stearic acid	19.135	18.029	18.430	18.781
Oleic acid	40.313	37.430	29.980	32.783
Total	88.471	88.384	87.624	87.304
Total fatty acids	94.980	94.961	94.856	94.830

The percentage of butyric acid was 2.368 in the Holsteins and 2.962 in the Jerseys; of caproic acid, 1.775 and 2.014; of caprylic acid, 0.693 and 0.737; and of capric acid, 1.708 and 1.668. The breed differences are concealed by the higher content of soluble acids in the Jerseys.

The percentage of lauric acid was 7.140 in the Holsteins and 6.710 in the Jerseys; of myristic acid, 16.307 and 18.735; of palmitic acid, 7.528 and 12.033; of stearic acid, 18.582 and 18.606; and of oleic acid, 38.872 and 31.382. The breed differences were substantially of the same character as in the intermediate period.

The Holsteins differed materially from the herd sample (Table II), particularly in the insoluble acid mixture. In a general way the Jerseys agreed with the herd sample except in stearic acid content.

EFFECT OF LACTATION

In the Holsteins the percentage of total fatty acids increased 0.065 in four months and 0.179 in seven months, and their neutralization number decreased 3.673 and 8.488 mgm.; the soluble fatty acids decreased 1.114 and 1.295 per cent; the insoluble fatty acids increased 1.180 and 1.474, and their neutralization number decreased 2.430 and 5.607 mgm.; and the glycerol decreased 0.159 and 0.434 per cent. The change in soluble fatty acids consisted of a decreasing loss from butyric acid to caprylic with a slight increase in capric acid. There was an appreciable gain in lauric acid, loss in myristic acid, and marked gain in oleic acid.

In the Jerseys the percentage of total fatty acids increased 0.018 in four months and 0.073 in seven months, and their neutralization number decreased 1.049 and 3.194 mgm.; the soluble fatty acids decreased 0.834 and 0.613 per cent; the insoluble fatty acids increased 0.852 and 0.682 per cent, and their neutralization number decreased 0.267 and 2.409 mgm.; and the glycerol decreased 0.043 and 0.178 per cent. The change in soluble fatty acids was more gradual than in the Holsteins; butyric, caproic, and caprylic acids decreased slightly; capric acid increased; lauric acid increased noticeably; myristic acid decreased; and oleic acid increased, but less than in the Holsteins.

4. FROM MIXED MILK OF GRADE HOLSTEINS FED A NORMAL RATION, WITH AND WITHOUT THE ADDITION OF VARIOUS OILS AND FATS

Four grade Holsteins of similar age, comparatively fresh in lactation, were divided into two groups, known as herds A and B. All of the cows, except Samantha III, had been used in previous experiments. The same hay and grain mixture was fed throughout the experiment to both herds, but Colantha was reduced 2 pounds of grain in the last period. Herd B received, in addition, various oils and fats in four periods. The hay and various grains in the mixture were of fair average quality, as shown by analysis (Table XXI). The coconut fat was a refined produet said to be used in the manufacture of confectionery, and the peanut and corn oils were refined for salad purposes. The soybean oil was the ordinary commercial product. They were all analyzed (Table XXII) by the common methods but were not esterified.

The milk produced was the daily average for the three days on which cream was saved for churning. The milk analyzed was a five-days' composite taken at substantially the same time as the cream sample.

TABLE XX.—*Records of cows and milk analysis*

	Herd A.		Herd B.	
	Colantha.	**Samantha IV.**	**Colantha II.**	**Samantha III.**
Breed	Grade Holstein	Grade Holstein	Grade Holstein	Grade Holstein
Date of birth	Nov. 16, 1913	Aug. 25, 1914	Oct. 14, 1914	Aug. 25, 1913
Last calf dropped	Sept. 19, 1919	Aug. 20, 1919	Aug. 8, 1919	Sept. 12, 1919.
Condition on calving	Good flesh	Good flesh	Good flesh	Good flesh.
Date served	Dec. 5, 1919; Feb. 4, Mar. 14, 1920.	Dec. 4, 1919	Nov. 3, 1919	Dec. 8, 1919.
PERIOD I				
Sample No.	A1	A1	B1	B1.
Daily ration:				
Hay	24 pounds	24 pounds	22 pounds	24 pounds.
Grain mixture	10 pounds	10 pounds	10 pounds	10 pounds.
Gluten feed, 40 per cent.				
Wheat bran, 20 per cent.				
Barley meal, 40 per cent.				
Weight of animal	1,348 pounds	1,160 pounds	1,093 pounds	1,210 pounds.
Milk produced (daily average).[1]	Nov. 18 to 20, 1919, 29.6 pounds.	Nov. 18 to 20, 1919, 34.7 pounds.	Nov. 18 to 20, 1919, 31.4 pounds	Nov. 18 to 20, 1919, 29.2 pounds.
Milk analysis:				
Solids (gravimetric)	12.66 per cent	12.84 per cent	13.29 per cent	13.25 per cent.
Fat (Babcock)	4.23 per cent	4.50 per cent	4.60 per cent	4.58 per cent
Proteids (N×6.25)	3.27 per cent	3.05 per cent	3.29 per cent	3.48 per cent
Lactose (by difference)	4.39 per cent	4.60 per cent	4.69 per cent	4.44 per cent.
Ash	.77 per cent	.69 per cent	.71 per cent	.75 per cent.
Sample No.	A2	A2	B2	B2.
Weight of animal	1,308 pounds	1,125 pounds	1,083 pounds	1,183 pounds.
Milk produced (daily average).[1]	Nov. 27 to 29, 1919, 24.6 pounds.	Nov. 27 to 29, 1919, 31.6 pounds.	Nov. 27 to 29, 1919, 31.0 pounds.	Nov. 27 to 29, 1919, 25.2 pounds.
Milk analysis:				
Solids (gravimetric)	12.65 per cent	12.91 per cent	13.10 per cent	13.35 per cent.
Fat (Babcock)	4.43 per cent	4.50 per cent	4.38 per cent	4.48 per cent
Proteids (N×6.25)	3.43 per cent	3.09 per cent	3.35 per cent	3.58 per cent.
Lactose (by difference)	4.00 per cent	4.63 per cent	4.64 per cent	4.54 per cent.
Ash	0.79 per cent	0.69 per cent	0.73 per cent	0.75 per cent.

[1] For the days cream was saved for churning.

TABLE XX.—*Records of cows and milk analysis*—Continued

PERIOD 2

	Herd A.		Herd B.	
	Colantha.	Samantha IV.	Colantha II.	Samantha III.
Sample No.	A_3	A_3	B_3	B_3.
Daily ration:				
Hay	24 pounds	24 pounds	22 pounds	24 pounds.
Grain mixture	10 pounds	10 pounds	10 pounds	10 pounds.
Coconut fat			.75 pounds	.75 pounds.
Weight of animal	1,298 pounds	1,183 pounds	1,108 pounds	1,213 pounds.
Milk produced (daily average.)[1]	Dec. 11 to 13, 1919, 18.2 pounds.	Dec. 11 to 13, 1919, 31.5 pounds.	Dec. 11 to 13, 1919, 28.5 pounds.	Dec. 11 to 13, 1919, 25.6 pounds.
Milk analysis:				
Solids (gravimetric)	12.66 per cent	13.04 per cent	13.81 per cent	14.12 per cent.
Fat (Babcock)	4.10 per cent	4.60 per cent	5.20 per cent	5.35 per cent.
Proteids (N ×6.25)	3.47 per cent	3.09 per cent	3.29 per cent	3.46 per cent.
Lactose (by difference)	4.29 per cent	4.65 per cent	4.59 per cent	4.54 per cent.
Ash	.80 per cent	.70 per cent	.73 per cent	.77 per cent.
Sample No.	A_4	A_4	B_4	B_4.
Weight of animal	1,333 pounds	1,203 pounds	1,123 pounds	1,255 pounds.
Milk produced (daily average).[1]	Dec. 25 to 27, 1919, 23.7 pounds.	Dec. 25 to 27, 1919, 31.6 pounds.	Dec. 25 to 27, 1919, 24.3 pounds.	Dec. 25 to 27, 1919, 24.6 pounds.
Milk analysis:				
Solids (gravimetric)	12.96 per cent	12.87 per cent	13.31 per cent	14.20 per cent.
Fat (Babcock)	4.38 per cent	4.40 per cent	5.20 per cent	5.60 per cent.
Proteids (N ×6.25)	3.44 per cent	3.09 per cent	3.05 per cent	3.44 per cent.
Lactose (by difference)	4.35 per cent	4.68 per cent	4.35 per cent	4.40 per cent.
Ash	.79 per cent	.70 per cent	.71 per cent	.76 per cent.

PERIOD 3

Sample No.	A_5	A_5	B_5	B_5.
Daily ration:				
Hay	24 pounds	24 pounds	22 pounds	24 pounds.
Grain mixture	10 pounds	10 pounds	10 pounds	10 pounds.
Peanut oil			.75 pounds	.75 pounds.
Weight of animal	1,315 pounds	1,178 pounds	1,080 pounds	1,230 pounds.
Milk produced (daily average).[1]	Jan. 8 to 10, 1920, 21.8 pounds.	Jan. 8 to 10, 1920, 30.1 pounds.	Jan. 8 to 10, 1920, 33.9 pounds.	Jan. 8 to 10, 1920, 27.7 pounds.
Milk analysis:				
Solids (gravimetric)	12.69 per cent	13.06 per cent	12.92 per cent	13.39 per cent.
Fat (Babcock)	4.15 per cent	4.60 per cent	4.20 per cent	4.78 per cent.
Proteids (N ×6.25)	3.47 per cent	3.12 per cent	3.22 per cent	3.29 per cent.
Lactose (by difference)	4.29 per cent	4.64 per cent	4.77 per cent	4.57 per cent.
Ash	.78 per cent	.70 per cent	.73 per cent	.75 per cent.
Sample No.	A_6	A_6	B_6	B_6.
Weight of animal	1,338 pounds	1,200 pounds	1,080 pounds	1,223 pounds.
Milk produced (daily average).[1]	Jan. 22 to 24, 1920, 20.8 pounds.	Jan. 22 to 24, 1920, 31.8 pounds.	Jan. 22 to 24, 1920, 33.1 pounds.	Jan. 22 to 24, 1920, 29.9 pounds.
Milk analysis:				
Solid (gravimetric)	13.09 per cent	13.13 per cent	13.17 per cent	13.14 per cent.
Fat (Babcock)	4.45 per cent	4.65 per cent	4.35 per cent	4.40 per cent.
Proteids (N ×6.25)	3.51 per cent	3.17 per cent	3.31 per cent	3.34 per cent.
Lactose (by difference)	4.37 per cent	4.62 per cent	4.80 per cent	4.67 per cent.
Ash	.76 per cent	.69 per cent	.71 per cent	.73 per cent.

PERIOD 4

Sample No.	A_7	A_7	B_7	B_7.
Daily ration:				
Hay	24 pounds	24 pounds	22 pounds	24 pounds.
Grain mixture	10 pounds	10 pounds	10 pounds	10 pounds.
Corn oil			.75 pound	.75 pound.
Weight of animal	1,348 pounds	1,182 pounds	1,085 pounds	1,233 pounds.
Milk produced (daily average).[1]	Feb. 5 to 7, 1920, 22 pounds.	Feb. 5 to 7, 1920, 29.6 pounds.	Feb. 5 to 7, 1920, 35.4 pounds.	Feb. 5 to 7, 1920, 31 pounds.
Milk analysis:				
Solids (gravimetric)	13.27 per cent	13.11 per cent	12.58 per cent	12.92 per cent.
Fat (Babcock)	4.45 per cent	4.65 per cent	4 per cent	4.33 per cent.
Proteids (N×6.25)	3.58 per cent	3.16 per cent	3.14 per cent	3.26 per cent.
Lactose (by difference)	4.47 per cent	4.61 per cent	4.74 per cent	4.61 per cent.
Ash	.77 per cent	.69 per cent	.70 per cent	.72 per cent.
Sample No.	A_8	A_8	B_8	B_8.
Weight of animal	1,343 pounds	1,168 pounds	1,085 pounds	1,218 pounds.

[1] For the days cream was saved for churning.

TABLE XX.—*Records of cows and milk analysis*—Continued

PERIOD 4—Continued

	Herd A.		Herd B.	
	Colantha.	Samantha IV.	Colantha II.	Samantha III.
Milk produced (daily average).[1]	Feb. 19 to 21, 1920, 20.6 pounds.	Feb. 19 to 21, 1920, 31.3 pounds.	Feb. 19 to 21, 1920, 33.7 pounds.	Feb. 19 to 21, 1920. 29 pounds.
Milk analysis:				
Solids (gravimetric)	13.06 per cent	12.96 per cent	12.47 per cent	12.63 per cent.
Fat (Babcock)	4.40 per cent	4.50 per cent	4.00 per cent	4.20 per cent.
Proteids (N×6.25)	3.45 per cent	3.14 per cent	3.09 per cent	3.08 per cent.
Lactose (by difference)	4.45 per cent	4.61 per cent	4.66 per cent	4.61 per cent.
Ash	.76 per cent	.71 per cent	.72 per cent	.74 per cent.

PERIOD 5

Sample No.	A_9	A_9	B_9	B_9.
Daily ration:				
Hay	24 pounds	24 pounds	22 pounds	24 pounds.
Grain mixture	8 pounds	10 pounds	10 pounds	10 pounds.
Soybean oil			.75 pounds	.75 pounds.
Weight of animal	1,353 pounds	1,193 pounds	1,098 pounds	1,220 pounds.
Milk produced (daily average).[1]	Mar. 4 to 6, 1920, 21.6 pounds.	Mar. 4 to 6, 1920, 29.8 pounds.	Mar. 4 to 6, 1920, 31.8 pounds.	Mar. 6 to 8, 1920, 29.1 pounds.
Milk analysis:				
Solids (gravimetric)	12.75 per cent	13.16 per cent	12.99 per cent	12.83 per cent.
Fat (Babcock)	4.25 per cent	4.60 per cent	4.20 per cent	4 per cent.
Proteids (N×6.25)	3.37 per cent	3.17 per cent	3.22 per cent	3.18 per cent
Lactose (by difference)	4.38 per cent	4.69 per cent	4.84 per cent	4.91 per cent.
Ash	.75 per cent	.70 per cent	.73 per cent	.74 per cent.
Sample No.	A_{10}	A_{10}	B_{10}	B_{10}.
Weight of animal	1,345 pounds	1,170 pounds	1,088 pounds	1,245 pounds.
Milk produced (daily average).[1]	Mar. 18 to 20, 1920, 22.3 pounds.	Mar. 18 to 20, 1920, 30.1 pounds.	Mar. 18 to 20, 1920, 27.3 pounds.	Mar. 18 to 20, 1920, 27.5 pounds.
Milk analysis:				
Solids (gravimetric)	12.95 per cent	13.30 per cent	13.79 per cent	12.91 per cent.
Fat (Babcock)	4.30 per cent	4.70 per cent	4.40 per cent	4.20 per cent.
Proteids (N×6.25)	3.37 per cent	3.15 per cent	3.61 per cent	3.19 per cent.
Lactose (by difference)	4.55 per cent	4.77 per cent	5.02 per cent	4.80 per cent.
Ash	.73 per cent	.68 per cent	.76 per cent	.72 per cent.

[1] For the days cream was saved for churning.

TABLE XXI.—*Analysis of feeds*

	Hay.	Gluten feed.	Wheat bran.	Barley meal.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	10.59	10.20	12.19	12.77
Dry matter:				
Ash	6.03	4.52	7.31	2.45
Protein (N× 6.25)	8.73	27.94	16.75	12.32
Fiber	32.38	6.32	11.29	4.86
Extract matter	50.55	58.43	59.74	77.91
Fat	2.31	2.79	4.91	2.46

TABLE XXII.—*Analysis of oils and fats fed*

	Coconut fat.	Peanut oil.	Corn oil.	Soybean oil.
Saponification number (s).....(mgm.)..	256.600	189.835	190.761	192.337
Acid number (a).....(mgm.)..	.092	.532	.347	2.594
Ether number (e).....(mgm.)..	256.508	189.303	190.414	189.743
Iodin number.....	9.447	94.751	123.319	131.180
Equivalent in oleic acid....(per cent)..	10.508	.....	.....	.....
Total fatty acids (T) (1.00−0.00022594 e) .....(per cent)..	94.204	95.723	95.698	95.713
Neutralization number(n)s/T(mgm.)..	272.388	198.317	199.336	200.952
Free fatty acids (A) a/n....(per cent)..	.034	.268	.174	1.291
Soluble fatty acids (S) ($T-I$) (per cent)..	9.372	None.	None.	None.
Neutralization number.....(mgm.)..	341.539	.....	.....	.....
Insoluble fatty acids (I) by alcoholic potash.....(per cent)..	84.832	96.005	96.026	95.953
Neutralization number.....(mgm.)..	264.748	[a] 211.577	[a] 219.463	[a] 219.425
Stearic acid by crystallization .....(per cent)..	2.755	None.	.523	.334
Glycerol (0.00054703 e).....(per cent)..	14.032	10.355	10.416	10.380
Refractive index (Abbe) 25° C.....	1.4550	1.4689	1.4724	1.4737
Refractive index (Abbe) 40° C.....	1.4491	1.4632	1.4671	1.4677
Colorimeter (Lovibond) ½-inch cell....	[b] .57	[b] 1.05	[b] 2.70	[b] 16.10
	[c] .03	[c] .45	[c] .30	[c] 3.90
Viscosity (Redwood) 70° F.....	8.691	11.554	10.203	9.730

[a] Exceeds the neutralization number of the total fatty acids due to decomposition difficult to control in such unsaturated products.
[b] Yellow.
[c] Orange.

The composition of the milk (Table XX) produced by the control herd A on hay and grain was fairly constant during the several periods of the experiment (four months), as was also the yield except in the case of Colantha in the second period when there was a noticeable decrease due to a sore teat. The average analysis of the milk of herd A for all periods and that of herd B for each period, together with the yields, are presented in Table XXIII.

TABLE XXIII.—*Summarized milk data*

	Herd A.	Herd B.				
	Periods 1 to 5.	Period 1, preliminary.	Period 2, coconut fat.	Period 3, peanut oil.	Period 4, corn oil.	Period 5, soybean oil.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Solids.....	12.96	13.25	13.86	13.16	12.65	13.13
Fat.....	4.44	4.51	5.34	4.43	4.14	4.20
Proteids.....	3.28	3.43	3.31	3.29	3.14	3.30
Lactose.....	4.51	4.57	4.47	4.71	4.65	4.89
Ash.....	.73	.74	.74	.73	.72	.74
Average production for the period.....	*Pounds.* 27.3	*Pounds.* 29.7	*Pounds.* 25.2	*Pounds.* 31.5	*Pounds.* 32.4	*Pounds.* 29.7

There was a substantial agreement in composition of the milk of herd A and that of herd B in the preliminary period, and the yield was similar. It is logical to assume that the composition of the milk of herd B in subsequent periods would have remained fully as constant as that of herd A provided the oils and fats fed were without influence. As compared with the preliminary period, coconut fat increased the solids 0.61 per cent and the fat 0.83 per cent, but at a loss of 4.5 pounds of milk a day for the period. On peanut oil the milk practically reverted to its original composition, with a gain in yield of 1.8 pounds daily over the preliminary period.

By a like comparison, corn oil decreased the solids 0.60 per cent and the fat 0.37 per cent but with an increased yield of 2.7 pounds of milk, and on soybean oil the solids nearly equaled those of the preliminary period, with a loss of 0.31 per cent of fat and approximately the same yield.

ANALYSIS OF BUTTER FAT FROM HERD A

Colantha and Samantha IV were considered as herd A. The cream from these two cows was mixed and churned, and samples of butter fat were analyzed; but as the maximum range did not exceed a reasonable variation for such a physiological product over a period of four months, three samples, A_2, A_6, and A_{10} (averaging practically the same as the 10 samples) were deemed sufficient to fully represent the herd and for subsequent esterification.

The percentages of total fatty acids in A_2, A_6, and A_{10} were 94.771, 94.806, and 94.802 (average 94.793) with no appreciable variation, and their neutralization numbers were 246.212, 244.913, and 243.878 mgm. (average 245.001), with a loss of 2.334 mgm., largely due in advancing lactation to a slight increase of insoluble acids at the expense of the soluble. The percentages of free fatty acids were 0.772, 0.936, and 0.458 (average 0.722), indicating a moderate amount of hydrolysis largely due to manipulation. The percentages of soluble fatty acids were 7.591, 7.302, and 7.258 (average 7.384), and their neutralization numbers 496.614, 491.413, and 498.044 mgm. (average 495.357).

The percentages of insoluble fatty acids were 87.180, 87.504, and 87.544 (average 87.409), and their neutralization numbers 224.409, 224.343, and 222.806 mgm. (average 223.853), indicating a slight increase in the proportion of high molecular weight acids with advancing lactation. The percentages of glycerol were 12.660, 12.576, and 12.586 (average 12.607), indicating no marked change.

As compared with the herd sample (Table I) the average of A_2, A_6, and A_{10} showed a close agreement in percentage of total, soluble, and insoluble fatty acids and glycerol; but the neutralization number of the total fatty acids was higher by 0.901 mgm., that of the soluble acids was lower by 14.262 mgm., and that of the insoluble acids was higher by 1.963 mgm., indicating some difference in proportion of constituent acids.

TABLE XXIV.—*Analysis of butter fat*

	Period 1.		Period 2.		Period 3.		Period 4.		Period 5.	
Sample No.	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9	A_{10}
Saponification number (s) (mgm.)	233.179	233.338	230.973	232.471	232.986	232.192	231.213	231.818	231.171	231.201
Acid number (a) (mgm.)	1.661	1.900	1.787	1.923	1.972	2.292	1.474	1.851	1.228	1.118
Ether number (e) (mgm.)	231.518	231.438	229.186	230.548	231.014	229.900	229.739	229.967	229.943	230.083
Iodin number	27.312	28.017	28.831	27.361	27.631	27.714	27.893	28.253	28.294	28.649
Equivalent in oleic acid (per cent)	30.381	31.165	32.070	30.435	30.736	30.828	31.027	31.428	31.473	31.868
Total fatty acids (T) (1.00−0.00022594 e) (per cent)	94.769	94.771	94.822	94.791	94.780	94.806	94.809	94.804	94.805	94.802
Neutralization number (n) s/T (mgm.)	246.050	246.212	243.586	245.246	245.818	244.913	243.872	244.523	243.838	243.878
Free fatty acids (A) a/n (per cent)	.675	.772	.734	.784	.802	.936	.604	.757	.504	.458
Soluble fatty acids (S) ($T-I$) (per cent)	7.987	7.591	7.586	7.216	7.367	7.302	7.579	7.596	7.288	7.258
Neutralization number (mgm.)	462.927	496.614	460.664	505.626	506.570	491.413	481.330	488.112	488.378	498.044
Insoluble fatty acids (I) by alcoholic potash (per cent)	86.782	87.180	87.376	87.391	87.413	87.504	87.230	87.208	87.517	87.544
Neutralization number (mgm.)	226.089	224.409	224.349	224.262	223.842	224.343	223.241	223.306	223.474	222.806
Stearic acid by crystallization (per cent)	9.967	10.345	8.188	5.544	9.303	9.950	11.141	11.346	12.150	12.256
Glycerol (0.00054703 e) (per cent)	12.665	12.660	12.537	12.612	12.637	12.576	12.567	12.580	12.579	12.586
Refractive index (Abbe) 40° C.	1.4536	1.4536	1.4536	1.4536	1.4536	1.4536	1.4536	1.4536	1.4535	1.4534
Colorimeter (Lovibond) ½-inch cell	[a] 3.20	[a] 3.70	[a] 3.50	[a] 3.00	[a] 2.20	[a] 2.80	[a] 2.00	[a] 2.10	[a] 2.30	[a] 2.20
	[b] 1.80	[b] 1.70	[b] 1.50	[b] 1.30	[b] 1.00	[b] 1.00	[b] 1.20	[b] 1.10	[b] 1.20	[b] 1.20

[a] Yellow. [b] Orange.

TABLE XXV.—*Weight and analysis of fractions (ethyl esters)*

A_2

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1			[a] 1,585.051		
2	90 to 144.5	4.7939	401.384	2.593	3.171
3	144.5 to 199	5.4274	347.415	6.021	7.362
4	199 to 238	5.1062	272.158	11.306	13.825
5	238 to 278.5	15.7266	238.796	14.195	17.358
6	278.5 to 310	54.5945	215.353	16.903	20.669
7	310 to 325	109.4460	199.769	22.105	27.031
Total		195.0946			

A_6

1	±70 to 90		[a] 1,419.532		
2	90 to 148	4.7288	398.816	2.991	3.658
3	148 to 199.5	5.1199	356.153	5.810	7.105
4	199.5 to 238.5	4.8099	281.241	10.578	12.935
5	238.5 to 281	15.7429	240.099	13.946	17.054
6	281 to 312.5	53.7111	215.163	16.509	20.187
7	312.5 to 328	125.3339	198.528	22.400	27.391
Total		209.4465			

A_{10}

1	±70 to 90		[a] 1,447.586		
2	90 to 149.5	4.6372	396.237	2.414	2.951
3	149.5 to 214	5.2042	363.557	5.083	6.216
4	214 to 258.5	4.9114	274.142	11.359	13.891
5	258.5 to 294.5	15.6960	235.719	14.710	17.988
6	294.5 to 318.5	54.0467	212.990	17.636	21.566
7	318.5 to 337	128.5638	197.798	24.760	30.277
Total		213.0593			

[a] Total alkali-consuming power of the fraction.

TABLE XXVI.—*Fatty acids in butter fat*

Fatty acids.	A_2.	A_6.	A_{10}.
Soluble acids:	Per cent.	Per cent.	Per cent.
Butyric acid (by difference)	3.407	3.120	3.480
Caproic acid	1.731	1.872	2.064
Caprylic acid	.810	.820	.527
Capric acid	1.643	1.490	1.187
Total	7.591	7.302	7.258
Insoluble acids:			
Lauric acid	5.806	5.415	4.913
Myristic acid	20.682	20.297	20.931
Palmitic acid (by difference)	20.508	22.257	19.103
Stearic acid	9.019	8.707	10.729
Oleic acid	31.165	30.828	31.868
Total	87.180	87.504	87.544
Total fatty acids	94.771	94.806	94.802

The percentages of butyric acid in A_2, A_6, and A_{10}, from cows receiving grain and hay, were 3.407, 3.120, and 3.480 (average 3.336); of caproic acid 1.731, 1.872, and 2.064 (average 1.889); of caprylic acid 0.810, 0.820, and 0.527 (average 0.719); and of capric acid 1.643, 1.490, and 1.187 (average 1.440). The butyric and caproic acids fully maintained their percentage during the four months of the experiment, but the caprylic and capric acids decreased.

As compared with the herd sample (Table II) the butyric acid averaged higher by 0.183 per cent, the caproic acid higher by 0.529 per cent, the caprylic acid lower by 0.256 per cent, and the capric acid lower by 0.391 per cent.

The percentages of lauric acid were 5.806, 5.415, and 4.913 (average 5.378); of the myristic acid 20.682, 20.297, and 20.931 (average 20.637); of the palmitic acid 20.508, 22.257, and 19.103 (average 20.623); of the stearic acid 9.019, 8.707, and 10.729 (average 9.485); and of the oleic acid 31.165, 30.828, and 31.868 (average 31.287). The lauric acid decreased with advancing lactation; otherwise there was no consistent change noted.

As compared with the herd sample (Table II) the lauric acid was low by 1.517 per cent, the myristic acid low by 1.981 per cent, the palmitic acid high by 5.165 per cent, the stearic acid low by 1.899 per cent, and the oleic acid high by 0.142 per cent, a noticeable difference in proportion of constituent acids.

ANALYSIS OF BUTTER FAT FROM HERD B

Colantha II and Samantha III were considered as herd B. The cream was mixed and churned and 10 samples of butter fat, representing five feeding periods, were analyzed. The first sample in each period was considered indicative, but the second, taken after the same feeding had been continued two weeks longer, was regarded as a better criterion of the effect of the oils and fats fed and consequently for esterification.

Fat samples B_1 and B_2 were of similar character, differing somewhat in content of soluble fatty acids. As compared with A_2, B_2 contained practically the same amount of total, free, soluble, and insoluble fatty acids and of glycerol; but the neutralization number of the insoluble acids was slightly higher, 1.174 mgm. It again appears reasonable to assume that, if the feeding of oils and fats was without influence, the composition of the butter fat of herd B should continue fully as uniform as that from herd A, thus permitting a direct comparison of the preliminary B_2 and subsequent feeding periods with possibly a slight allowance for advancing lactation.

The soluble fatty acids in B_4, from cows receiving coconut fat, decreased 1.243 per cent, as compared with B_2; the insoluble fatty acids increased 1.249 per cent and their neutralization number 3.194 mgm., a decided change both in quantity and character of the insoluble acids.

The total fatty acids in B_6, from cows receiving peanut oil, increased 0.226 per cent, and their neutralization number decreased 11.213 mgm.; the soluble fatty acids decreased 1.206 per cent, and their neutralization number, 20.055; the insoluble fatty acids increased 1.432 per cent, and their neutralization number decreased 6.983; and the glycerol decreased 0.548 per cent, indicating a notable gain in proportion of higher molecular weight acids in both groups.

TABLE XXVII.—*Analysis of butter fat*

	Period 1.		Period 2.		Period 3.		Period 4.		Period 5.	
Sample number	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9	B 10
Saponification number (*s*) (mgm.)	232.634	233.788	234.400	233.234	226.015	223.695	223.837	220.751	222.552	221.622
Acid number (*a*) (mgm.)	1.376	1.866	1.693	1.591	1.498	1.786	2.610	3.915	1.759	1.948
Ether number (*e*) (mgm.)	231.258	231.922	232.707	231.643	224.517	221.909	221.227	216.836	220.793	219.674
Iodin number	27.726	27.663	25.307	26.511	36.248	37.982	39.879	40.423	41.090	41.218
Equivalent in oleic acid (per cent)	30.841	30.771	28.150	29.490	40.321	42.250	44.360	44.965	45.707	45.849
Total fatty acids (*T*) (1.00−0.00022594 *e*) (per cent)	94.775	94.760	94.742	94.766	94.927	94.986	95.002	95.101	95.011	95.037
Neutralization number (*n*) *s*/*T* (mgm.)	245.459	246.716	247.408	246.116	238.093	235.503	235.613	232.123	234.238	233.195
Free fatty acids (*A*) *a*/*n* (per cent)	.561	.756	.684	.646	.629	.758	1.108	1.687	.751	.835
Soluble fatty acids (*S*) (*T*−*I*) (per cent)	7.288	7.756	7.276	6.513	6.961	6.550	6.730	6.625	6.271	6.168
Neutralization number (mgm.)	475.329	483.780	469.214	481.053	475.980	463.725	426.850	418.506	473.991	446.174
Insoluble fatty acids (*I*) by alcoholic potash (per cent)	87.487	87.004	87.466	88.253	87.966	88.436	88.272	88.476	88.740	88.869
Neutralization number *I* (mgm.)	226.310	225.583	228.958	228.777	219.269	218.600	221.033	218.167	217.256	218.414
Stearic acid by crystallization (per cent)	10.351	8.969	10.971	9.754	14.137	14.716	13.486	14.998	14.418	15.027
Glycerol (0.00054703 *e*) (per cent)	12.651	12.687	12.730	12.672	12.282	12.139	12.102	11.862	12.078	12.017
Refractive index (Abbe) 40° C	1.4536	1.4536	1.4526	1.4549	1.4551	1.4555	1.4553	1.4553	1.4556	1.4556
Colorimeter (Lovibond) ½-inch cell	[a] 5.80	[a] 5.70	[a] 3.90	[a] 3.60	[a] 2.25	[a] 2.80	[a] 2.00	[a] 2.10	[a] 2.40	[a] 2.90
	[b] 1.70	[b] 1.80	[b] 1.50	[b] 1.25	[b] 0.95	[b] 1.10	[b] 1.40	[b] 1.20	[b] 1.20	[b] 1.50

[a] Yellow. [b] Orange.

The total fatty acids in B_8, from cows receiving corn oil, increased 0.341 per cent as compared with B_2, and their neutralization number decreased 14.593 mgm.; the free fatty acids increased 0.931 per cent; the soluble acids decreased 1.131 per cent, and their neutralization number, 65.274 mgm.; the insoluble acids increased 1.472 per cent, and their neutralization number decreased 7.416 mgm.; and the glycerol decreased 0.825 per cent, a change similar to but more marked than in the previous period.

The total fatty acids in B_{10}, from cows receiving soybean oil, increased 0.277 per cent, and their neutralization number decreased 13.521 mgm.; the soluble fatty acids decreased 1.588 per cent, and their neutralization number 37.606 mgm.; the insoluble fatty acids increased 1.865 per cent, and their neutralization number decreased 7.169 mgm.; and the glycerol decreased 0.670 per cent, a change slightly different but approximately the same as in the previous case.

TABLE XXVIII.—*Weight and analysis of fractions (ethyl esters)*

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
		B_2			
	°C.	Gm.	Mgm.		Per cent.
1	±70 to 90	.....	[a]1,587.856	.....	.....
2	90 to 153	4.7042	387.750	3.158	3.862
3	153 to 207	5.2264	367.367	5.541	6.776
4	207 to 251	4.8993	288.957	10.324	12.625
5	251 to 290.5	15.8992	245.752	13.679	16.727
6	290.5 to 316.5	53.9656	217.611	16.639	20.347
7	316.5 to 334.5	131.8435	199.906	22.974	28.094
Total	.....	216.5382	.....	.....	.....
		B_4			
1	±70 to 90	.....	[a]1,441.976	.....	.....
2	90 to 138	4.4956	403.891	1.939	2.371
3	138 to 201	5.0411	366.749	4.526	5.534
4	201 to 245	4.8221	275.390	9.817	12.005
5	245 to 274.5	15.5843	239.613	12.513	15.301
6	274.5 to 303.5	53.5750	219.938	14.983	18.322
7	303.5 to 327.5	132.2908	202.036	22.110	27.037
Total	.....	215.8089	.....	.....	.....
		B_6			
1	±70 to 90	.....	[a]1,554.192	.....	.....
2	90 to 138.5	4.6859	405.759	3.130	3.828
3	138.5 to 192	5.1400	362.188	6.403	7.830
4	192 to 238	4.8029	270.068	14.655	17.921
5	238 to 284	15.5928	231.380	19.448	23.782
6	284 to 314	53.7289	209.791	24.451	29.900
7	314 to 328	103.0110	195.994	32.311	39.511
Total	.....	186.9615	.....	.....	.....

[a] Total alkali-consuming power of the fraction.

TABLE XXVIII.—*Weight and analysis of fractions*—Continued

B_8

Fraction No.	Range of fraction.	Weight of fraction.	Saponification number.	Iodin number.	Ethyl oleate.
	°C.	Gm.	Mgm.		Per cent.
1	±70 to 95	.....	[a]1,453.197	.....	.....
2	95 to 148.5	4.4388	395.177	3.451	4.220
3	148.5 to 198	4.4753	359.884	6.806	8.323
4	198 to 246	4.6266	267.181	15.492	18.944
5	246 to 288	14.4314	231.499	20.229	24.737
6	288 to 316.5	53.7554	209.624	25.724	31.456
7	316.5 to 333.5	99.2487	195.484	34.729	42.468
Total	.....	180.9762	.....	.....	.....

B_{10}

1	±70 to 97	.....	[a]1,590.662	.....	.....
2	97 to 142	4.3775	402.010	4.151	5.076
3	142 to 186	4.6831	360.950	7.348	8.985
4	186 to 234	4.5210	271.797	15.174	18.555
5	234 to 273	13.8912	231.688	20.457	25.016
6	273 to 317	52.8181	210.453	25.743	31.479
7	317 to 327.5	96.9084	195.983	34.205	41.827
Total	.....	177.1993	.....	.....	.....

[a] Total alkali-consuming power of the fraction.

TABLE XXIX.—*Fatty acids in butter fat*

Fatty acids,	B_2, preliminary.	B_4, coconut fat.	B_6, peanut oil.	B_8, Corn oil.	B_{10}, soybean oil.
Soluble acids:	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
Butyric acid (by difference)	2.669	2.597	3.124	3.272	2.893
Caproic acid	2.263	1.972	1.921	1.829	1.779
Caprylic acid	.816	.452	.468	.401	.474
Capric acid	2.008	1.492	1.037	1.123	1.022
Total	7.756	6.513	6.550	6.625	6.168
Insoluble acids:					
Lauric acid	6.210	8.042	4.681	4.551	4.839
Myristic acid	22.050	24.988	17.223	16.428	16.128
Palmitic acid (by difference)	20.170	17.125	11.268	[a]9.262	[a]8.699
Stearic acid	7.803	8.608	13.014	13.270	13.354
Oleic acid	30.771	29.490	42.250	44.965	45.849
Total	87.004	88.253	88.436	88.476	88.869
Total fatty acids	94.760	94.766	94.986	95.101	95.037

[a] In these abnormal samples the recovery by distillation exceeded the results reported by 0.698 per cent in B_8 and by 0.684 in B_{10}. It is impossible to ascribe definitely the source of this error at the present writing.

As compared with A_2, B_2 on a normal ration contained less butyric acid by 0.738 per cent, more caproic acid by 0.532 per cent, more caprylic acid by 0.006 per cent, and more capric acid by 0.365 per cent, differences too great to permit the direct use of the former as a basis.

In the case of the insoluble acids, B_2 contained more lauric acid by 0.404 per cent, more myristic acid by 1.368 per cent, less palmitic acid by 0.338 per cent, less stearic acid by 1.216 per cent, and less oleic acid by 0.394 per cent—a much closer agreement than in the case of the soluble acids, considering the amounts involved, but still making it advisable to use only B_2 for direct comparison with the other periods.

As compared with the herd sample (Table II) the differences are even greater. B_2 contained less butyric acid by 0.484 per cent, more caproic acid by 0.903 per cent, less caprylic acid by 0.159 per cent, more capric acid by 0.177 per cent, less lauric acid by 0.685 per cent, less myristic acid by 0.568 per cent, more palmitic acid by 4.712 per cent, less stearic acid by 3.581 per cent, and less oleic acid by 0.374 per cent.

The butyric acid in B_4, from the cows receiving coconut fat, decreased 0.072 per cent as compared with B_2; caproic acid decreased 0.291 per cent; caprylic acid 0.364 per cent; capric acid 0.516 per cent; lauric acid increased 1.832 per cent; myristic acid increased 2.938 per cent; palmitic acid decreased 3.045 per cent; stearic acid increased 0.805 per cent; and oleic acid decreased 1.281 per cent.

As compared with B_2, the butyric acid in B_6, from cows receiving peanut oil, increased 0.455 per cent; the caproic acid decreased 0.342 per cent; the caprylic acid decreased 0.348 per cent; capric acid decreased 0.971 per cent; lauric acid decreased 1.529 per cent; myristic acid decreased 4.827 per cent; palmitic acid decreased 8.902 per cent; stearic acid increased 5.211 per cent; and oleic acid increased 11.479 per cent.

The butyric acid in B_8, from cows receiving corn oil, increased 0.603 per cent as compared with B_2; caproic acid decreased 0.434 per cent; caprylic acid decreased 0.415 per cent; capric acid decreased 0.885 per cent; lauric acid decreased 1.659 per cent; myristic acid decreased 5.622 per cent; palmitic acid decreased 10.908 per cent; stearic acid increased 5.467 per cent; and oleic acid increased 14.194 per cent.

As compared with B_2, the butyric acid in B_{10}, from cows receiving soybean oil increased 0.224 per cent; the caproic acid decreased 0.484 per cent; the caprylic acid decreased 0.342 per cent; the capric acid decreased 0.986 per cent; the lauric acid decreased 1.371 per cent; the myristic acid decreased 5.922 per cent; the palmitic acid decreased 11.471 per cent; the stearic acid increased 5.551 per cent; and the oleic acid increased 15.078 per cent.

In brief, the coconut fat decreased all the soluble acids in increasing amounts from butyric to capric acid, increased the lauric and myristic acids, and decreased the oleic acid.

Peanut oil increased the butyric acid, decreased all the other acids from caproic to palmitic in increasing amounts, and increased stearic and oleic acids materially.

Corn oil increased the butyric acid, decreased all the other acids from caproic to palmitic acid in increasing amounts (except caprylic), and increased the stearic and oleic acids.

Soybean oil increased the butyric acid and decreased all the other acids from caproic to palmitic in increasing amounts (except caprylic), and increased the stearic and oleic acids.

The question naturally arises with butter fats B_6, B_8, and B_{10}, from cows receiving peanut, corn, and soybean oils, as to whether the high iodin numbers might not be due in part to the direct assimilation and transference of linolic and linolenic acids from the oils fed to the butter fat. A number of determinations were made by the lead-salt-ether method with a view of obtaining some definite information on the subject, of which those in Table XXX are fairly typical.

TABLE XXX.—*Lead-salt-ether-soluble fatty acids*

Sample No.	Fatty acids recovered.	Iodin number.	Neutralization number.
	Per cent.		*Mgm.*
B_8	41.86	82.11	217.94
B_{10}	42.55	83.02	218.27

Lead-salt-ether-soluble fatty acids recovered from butter fat are rarely pure liquid acids but are more or less contaminated with small quantities of water-soluble acids extremely difficult to remove by washing and of insoluble saturated acids that pass the filter. The results indicate, however, that with due allowance the liquid acids could not have contained any appreciable quantity of higher unsaturated acids than oleic.

II. SUMMARY OF DATA FROM MASSACHUSETTS AND ELSEWHERE, TOGETHER WITH SUCH GENERAL DEDUCTIONS AS SEEM WARRANTED

TABLE XXXI.—*Analysis of 29 samples of butter fat from Massachusetts cows fed normal rations*

	Average.	Range.
Saponification number (s).....(mgm.)..	231.403	224.626 to 235.333
Acid number (a).....(mgm.)..	2.049	.927 to 3.657
Ether number (e).....(mgm.)..	229.355	222.186 to 232.530
Iodin number.....	28.461	22.720 to 36.241
Equivalent in oleic acid.....(per cent)..	31.315	25.273 to 40.313
Total fatty acids (T) (1.00−0.00022594 e) .....(per cent)..	94.818	94.746 to 94.980
Neutralization number (n) s/T.....(mgm.)..	244.051	236.498 to 248.383
Free fatty acids (A) a/n.....(per cent)..	.840	.383 to 1.502
Soluble fatty acids (s) ($T-I$).....(per cent)..	7.297	6.470 to 8.601
Neutralization number.....(mgm.)..	504.787	460.664 to 539.370
Insoluble fatty acids (I) by alcoholic potash .....(per cent)..	87.519	86.145 to 88.471
Neutralization number.....(mgm.)..	222.362	215.676 to 226.310
Stearic acid by crystallization....(per cent)..	15.011	5.544 to 23.304
Glycerol (0.00054703 e).....(per cent)..	12.546	12.154 to 12.720
Refractive index[1] (Abbe) 40° C.....	1.4536	1.4534 to 1.4536
Colorimeter (Lovibond)[1] ½-inch cell.....	Yellow 3.2 Orange 1.4	Yellow 2.0 to 5.8 Orange 1.0 to 1.8

[1] Twelve samples.

The 29 samples of butter fat of grade Holsteins and grade Jerseys on normal rations, reported above, contained on the average 94.82 per cent of total fatty acids with a neutralization number of 244; 0.84 per cent of free fatty acids; 7.30 per cent of soluble fatty acids with a neutralization number of 504; 87.52 per cent of insoluble fatty acids with a neutralization number of 222.36; and 12.55 per cent of glycerol. The range in percentage and in neutralization number of both the soluble and insoluble fatty acids was rather pronounced.

TABLE XXXII.—*Fatty acids in 21 samples of butter fat from Massachusetts cows fed normal rations*

Fatty acids.	Average.	Range.
Soluble acids:	*Per cent.*	*Per cent.*
Butyric acid [a] (by difference)	2.932	2.241 to 4.230
Caproic acid [a]	1.898	1.290 to 2.400
Caprylic acid [a]	.786	.527 to 1.041
Capric acid [a]	1.570	1.187 to 2.008
Total	7.186	6.470 to 8.601
Insoluble acids:		
Lauric acid	5.849	4.533 to 7.687
Myristic acid	19.784	15.554 to 22.618
Palmitic acid (by difference)	15.167	5.782 to 22.863
Stearic acid	14.907	7.803 to 20.370
Oleic acid	31.895	25.273 to 40.313
Total	87.602	86.145 to 88.471
Total fatty acids	94.788	94.746 to 94.980

[a] Twenty samples.

The 20 samples of butter fat (analyzed for fatty acids) from cows on normal rations contained on the average 2.93 per cent of butyric acid, 1.90 per cent of caproic acid, 0.79 per cent of caprylic acid, and 1.57 per cent of capric acid. The range in individual acids averaged about 85 per cent, but in most cases the differences were fairly compensating as determined by the neutralization number.

The 21 samples averaged 5.85 per cent of lauric acid, 19.78 per cent of myristic acid, 15.17 per cent of palmitic acid, 14.91 per cent of stearic acid, and 31.90 per cent of oleic acid. The range in lauric, myristic, and oleic acids was less proportionally than in the solubles, in stearic acid somewhat greater, and in palmitic acid almost 300 per cent. As the neutralization number of palmitic acid is nearly that of the average insoluble acid mixture the other four acids were fairly compensating in most instances.

TABLE XXXIII.—*Fatty acids in butter fat*

	Bell (*1, p. 48*).[1]	Blyth (*2, p. 272*).	Browne (*3, p. 827*).	Crowther and Hynd (*4, p. 145*).			Duclaux (*5, p. 1024*).
				Dairy butter.	First runnings.	Last runnings.	
Soluble acids:							
Butyric acid	6.13	3.49	5.45	4.45	4.30	4.06	3.38 to 3.65
Caproic acid		2.40	2.09	1.45	1.98	1.48	2.00 to 2.26
Caprylic acid	2.09	.80	.49	.99	1.11	1.37	.....
Capric acid		.....	.32	1.10	1.51	.96	.....
Total	8.22	6.69	8.35	7.99	8.90	7.87	.....
Insoluble acids:							
Lauric acid	.....	.....	2.57	3.55	5.08	6.40	.....
Myristic acid		.....	9.89	20.13	10.38	18.78	.....
Palmitic acid	49.46	47.50	38.61	15.24	17.47	11.78	.....
Stearic acid		.....	1.83	1.08	5.93	3.19	.....
Oleic acid	36.10	40.40	32.50	45.47	46.49	41.31	.....
Di-hydroxy stearic acid	.....	.....	1.00	.68	.30	.16	.....
Total	85.56	87.90	86.40	86.15	85.65	81.62	.....
Total fatty acids	93.78	94.59	94.75	94.14	94.55	89.49	.....

	Fleischmann and Warmbold (*6, p. 302*).				Jensen (*11, p. 277*).	Koefoed (*12, p. 133*).[2]	Leathes (*14, p. 30*).[3]	Molinari (*17, p. 387*).	Molt (*13*).[4]
	1	2	3	4					
Soluble acids:									
Butyric acid	5.00	5.00	4.32	4.32	3.92	1.42	5.25 to 6.12	.....	
Caproic acid	2.00	2.00	2.16	2.16	1.88	1.90	.90 to 2.70	.....	7.40
Caprylic acid	.15	.15	.67	.67	.....	.47	Traces.	.....	
Capric acid	.....	.....	.....	.....	.....	1.90	Traces.	.....	
Total	7.15	7.15	7.15	7.15	.....	5.69	.....	.....	7.40
Insoluble acids:									
Lauric acid	.....	.....	.....	.....	.....	7.58	Traces.	14 to 16	.....
Myristic acid	.....	.....	4.46	10.00	.....	20.86	Traces.	11 or more.	.....
Palmitic acid	52.12	51.10	48.94	42.75	.....	26.54	.....	14 to 18	20.00
Stearic acid	.....	3.35	5.54	2.00	.....	1.90	.....	7 to 11	40.93
Oleic acid	35.63	33.30	28.81	33.00	.....	32.23	.....	25 to 30	26.52
Arachidic acid	.....	.....	.....	.....	.....	.....	Traces.	.....	.....
Higher unsaturated acids	.....	.....	.....	.....	.....	.....	.....	4 to 5.7	.....
Total	87.75	87.75	87.75	87.75	.....	89.11	.....	.....	87.45
Total fatty acids	94.90	94.90	94.90	94.90	.....	94.80	.....	.....	94.85

[1] Reference is made by number (italic) to "Literature cited," pp. 397–398.
[2] Results recalculated on a basis of 94.80 per cent total fatty acids.
[3] Recalculated.
[4] Results recalculated by Browne.

TABLE XXXIII—*Fatty acids in butter fat*—Continued

	Partheil and Ferie (*18, p. 566*).		Richmond (*19, p. 42*).	Siegfeld (*20, p. 292, 294, 295*).				Smedley (*21, p. 457*).	Spallazini (*22, p. 808*).[2]
				1	2	3	4		
Soluble acids:									
Butyric acid			3.43	3.53	3.58	3.27	3.35		4.44
Caproic acid			3.25	2.50	1.26	1.73	1.68		.92
Caprylic acid			.51	1.03	2.87	1.89	2.21		.28
Capric acid			1.77						
Total			8.96	7.06	7.71	6.89	7.24		5.64
Insoluble acids:									
Lauric acid	16.40	14.88	6.94						
Myristic acid	11.09	11.88	19.14	25.35	22.94	30.70	26.00		
Palmitic acid	18.24	14.46	24.48	17.18	20.84	16.89	20.96		
Stearic acid	6.65	10.49	1.72					10–15	89.48
Oleic acid	30.67	32.64	33.60	42.75	40.53	37.98	38.09		
Higher unsaturated acids	5.40	4.15							
Total	88.45	88.50	85.88	85.28	84.31	85.57	85.05		89.48
Total fatty acids			94.84	92.34	92.02	92.46	92.29		95.12

	Violette (*23, p. 346*).								Wright (*24, p. 14*).[1]
	Superior qualities of butter.			Inferior qualities of butter.					
	1	2	3	4	5	6	7	8	
Soluble acids:									
Butyric acid	6.07	5.33	5.50	5.05	4.62	4.80	4.76	4.37	5.25
Caproic acid	3.66	3.23	3.34	3.06	2.80	2.92	2.89	2.65	
Capric acid	2.85	3.00	2.80	3.00	2.90	2.40	3.00	2.95	
Total	12.58	11.56	11.64	11.11	10.32	10.12	10.65	9.97	
Insoluble acids:									
Lauric acid									
Myristic acid									
Palmitic acid	82.28	82.63	82.87	83.20	84.32	84.31	83.83	84.62	
Stearic acid									
Oleic acid									
Total	82.28	82.63	82.87	83.20	84.32	84.31	83.83	84.62	
Total fatty acids	94.86	94.19	94.51	94.31	94.64	94.43	94.48	94.59	

[2] Recalculated.

The results of other workers and compilers showed noticeable variations in total, soluble, and insoluble fatty acids (affected no doubt by the method of analysis, as well as by difference in product), but after the elimination of extremes they agreed as to the general character of butter fat better than might be expected. Although any deductions are open to criticism, the results appeared to indicate a total fatty acid content of approximately 94.41 to 94.90 per cent, soluble acids 6.69 to 8.96 per cent, and insoluble acids 86.15 to 88.50 per cent. These figures do not differ materially from those secured on Massachusetts samples.

The percentage of the different soluble fatty acids by other investigators showed decided variations. The results, while not strictly comparable, recognized butyric acid as the most prominent, with caproic acid second. The data for caprylic and capric acids were quite limited. In a few instances caprylic acid was shown to be the smallest constituent,

but in no case did both caprylic and capric acids agree with the average of the samples tested in Massachusetts.

Of the insolubles, lauric acid was seemingly at least one of the smaller and myristic acid one of the larger ingredients, but only in a few cases were the results at all consistent. The range in palmitic acid was rather wide, but on excluding the higher percentages most of the remainder were between 15 and 25 per cent.

In stearic-acid content there was again considerable divergence. The results may be divided into two groups from 1.08 to 3.35 per cent and from 5.54 to 15 per cent. Of the more recent workers Miss Smedley obtained a higher percentage than the others. The Massachusetts average herein reported on normal rations is somewhat higher than previously secured (*9, p. 109–110*) and may prove higher than subsequent figures from a greater number of cows will justify.

The percentage of oleic acid varied from 25 to 46.49. A number of the foreign butter fats had an oleic-acid content similar to those derived in Massachusetts from feeding corn and soybean oils. This acid appears to respond the most readily to the influences of feed and advancement in lactation.

The small quantity of di-hydroxy stearic acid reported in butter fat may arise from decomposition changes accompanying analytical procedure rather than as a natural occurrence. The weight of evidence does not support the contention that unsaturated acids higher than oleic are present in straight butter fat to any appreciable amount.

GENERAL CONCLUSIONS

(1) On normal rations the percentage of total fatty acids in the butter fat of the Holsteins was substantially the same as that of the Jerseys, but their neutralization number was somewhat lower; the free fatty acids and soluble fatty acids were also lower, while the percentage of insoluble fatty acids was higher and their neutralization number and glycerol lower.

(2) The percentages of butyric and caprylic acids were lower in the Holsteins than in the Jerseys; caproic and capric acids higher; lauric and oleic acids higher; and myristic, palmitic, and stearic acids lower.

(3) With advancing lactation the percentages of total fatty acids increased slightly in both breeds, while their neutralization numbers decreased; the soluble fatty acids decreased, while the insoluble fatty acids increased and their neutralization numbers and the glycerol decreased. As a rule the changes were more pronounced in the Holsteins.

(4) The addition of oils and fats to the normal rations increased the insoluble fatty acids, partly at the expense of the solubles and partly by increasing the total fatty acids, and in consequence depressed the glycerol content.

(5) The neutralization numbers of the soluble, insoluble, and total fatty acids were decreased on feeding the peanut, corn, and soybean oils, and that of the insoluble acids was increased by coconut fat.

(6) Of the soluble acids, butyric fluctuated but maintained its percentage, caproic acid decreased appreciably, and caprylic and capric acids decreased about 50 per cent in extreme cases. Of the insoluble acids, lauric and myristic acids increased when coconut fat was fed but decreased when unsaturated oils were fed. In the latter cases oleic acid increased greatly.

(7) In so far as it is possible to draw a general conclusion from the data thus far secured from all experiments (*15*, *16*) made at this station extending over many years, it seems evident that neither protein nor carbohydrates have any appreciable influence in changing the chemical composition of the butter fat; on the other hand, different oils and fats do modify to an extent the chemical composition of butter fat, the modification depending upon the composition of the oils and fats fed.

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STRIPED SOD WEBWORM, CRAMBUS MUTABILIS CLEMENS[1]

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INTRODUCTION

Throughout a wide area *Crambus mutabilis* is one of the most common species of the genus. It ranks well toward the head of the list in destructiveness, although by itself it never has been directly charged with a destructive outbreak. It has not previously received detailed study, and the available information concerning it is scattered and meager. The present paper includes a summary of previously published facts, together with the results of the writer's studies for several years.

SYSTEMATIC HISTORY

Crambus mutabilis was first described by Clemens (*3, p. 204*)[2] in 1860, but he furnished no information as to the source of his material. Three years later Zeller (*15, p. 44*) redescribed it as *Crambus fuscicostellus*, a name which better characterizes the species than Clemens's adjective. Both names appear in the literature for some years, although Grote (*7, p. 79*) early recognized their probable synonymy. Smith (*13, p. 87*) first placed *fuscicostellus* unconditionally as a synonym of *mutabilis*, in which he is fully borne out by Hampson (*8, p. 928*), who had Zeller's type in the British Museum for comparison.

GEOGRAPHICAL DISTRIBUTION

Crambus mutabilis seems to be a purely North American species, for outside of North America it has been reported only by Hedemann (*9, p. 300*), from St. Thomas Island in the West Indies. It is widespread over the eastern half of the United States. A study of the published records and of all the available museum material shows that the outlying points from which reliable records are available are Brownsville and Amarillo, Tex.; Vineyard, Utah; Sioux City, Iowa; and Cartwright, Manitoba. It has been found in most of the States to the eastward of a line connecting these places, although it does not appear in a considerable collection of the genus made in southern Minnesota, and has not been recorded from Wisconsin, West Virginia, and several of the New England States.

The reported occurrence in Nebraska is evidently based on Bruner's paper (*2, p. 262*), in which, however, he does not say that it has been taken in that State. It has been taken at numerous points in Florida, but, except for a single specimen from northwestern Arkansas, has not yet been reported from the tier of States between and including Oklahoma

[1] Accepted for publication July 11, 1922. This paper is the third in a series of Contributions to a Knowledge of the Crambinæ of North America. I, *Crambus hemiochrellus* Zeller, appeared in Annals of the Entomological Society of America for March, 1918, and II, *Crambus laqueatellus* Clemens, in the June, 1922, issue of the same journal.

[2] Reference is made by number (italic) to "Literature cited," p. 414.

and South Carolina, although it probably occurs throughout that section. There is a specimen in Doctor Barnes's collection from Digby, Nova Scotia. It has also been reported from California in a broad way, by various writers following Felt (*4, p. 97*), but the writer has seen nothing to substantiate this record. There is a single specimen in the National Museum labeled "Arizona," from Riley's collection. The accompanying map (fig. 1) shows the points in the United States whence definite records have been obtained. Where no exact locality is given the State is marked with an interrogation point.

FOOD PLANTS

The striped sod webworm has never been recorded as feeding on any plant outside the grass family. In the field, larvæ have been taken on blue grass, corn, wheat, timothy, and once, at Nashville, Tenn., on small clumps of a wild grass which was not at the time in determinable con-

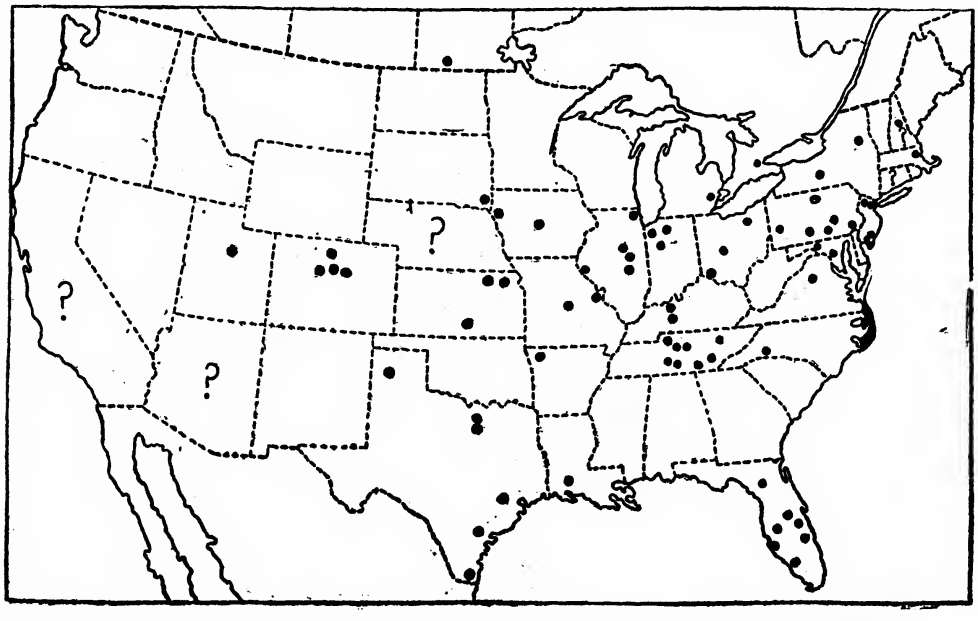

FIG. 1.—Map showing known distribution of *Crambus mutabilis* in North America.

dition. In addition to the foregoing the writer has reared it in cages on crab grass (*Syntherisma sanguinalis*), barley, and rye. Larvæ were also offered oats and orchard grass (*Dactylis glomeratus*) and in the later stages fed freely. The young larvæ, however, seemed to have trouble in subsisting on these grasses, and none were reared on them alone. Undoubtedly the larvæ will thrive on many other grasses.

ECONOMIC HISTORY

Few cases of serious injury are definitely chargeable to *Crambus mutabilis*. This does not mean that it is not an injurious form, but merely that it has not been caught in the act. There is plenty of circumstantial evidence to warrant an indictment aside from the established records. Bruner (*2, p. 262*) first mentions it as a grass insect attacking corn following sod. Riley (*11, p. 36*) says it occasionally attacks corn. Webster (*14, p. 86*) intimates that this species may have had something to do with the destruction wrought in meadows and in

corn and oat fields in northern Ohio in 1895 by his statement that the moths were taken in June following the outbreak, but "in more limited numbers" than those of *C. trisectus*, to which the larger part of the trouble was undoubtedly due. From Forbes (*6, p. 39–42*) there are numerous reports of crambid injuries, especially to corn, but in not a single instance does he attribute the damage to this species alone; always at least one other species is involved.

In the writer's experience larvæ have been found in numerous instances destroying corn plants, and almost invariably either the crop followed sod or the injury was confined to the margin of the field. At Spring Hill, Tenn., one larva was found destroying a wheat plant early in June. A. F. Satterthwait has on three occasions sent the writer larvæ found feeding in the bulbs of timothy plants. In some experimental plantings of corn on sod land near Chapel Hill, Tenn., three of these larvæ were found to every one of *Crambus caliginosellus*, the only other crambid present, and the stand of corn was being very materially injured by them. In 1920, near La Fayette, Ind., this species, associated with *C. trisectus*, caused severe injury in several fields of corn.

Although there is no record of a general outbreak of this species, it is certain that, with favoring conditions, severe but narrowly local outbreaks occur more often than is realized. The moths are often sufficiently numerous to show that a large amount of food has been required to produce them, and as the larvæ seem to be strictly grass feeders the species must be placed well toward the head of the list of injurious forms. It seems especially subject to parasitism and epidemic diseases, and this may explain why it has not increased to such an injurious degree as some of the other species.

SEASONAL HISTORY

In Tennessee, where collections have been constantly made for the last five years, the moths first appear about May 15, the date of their appearance being very uniform year after year. By May 20 they are abundant and continue so for a month in gradually decreasing numbers. During five years' collecting no moths have been taken between June 19 and July 3, and only scattering individuals until about July 10, when the moths of the second generation appear in greater numbers and remain over a longer period than those of the first generation, continuing fairly abundant until August 15. Then after another interval of absence a few appear during the last few days of August and in September, evidently a partial third generation. The latest record for the country is of one moth taken at Knoxville, Tenn., October 26, long after the others were gone.

In Florida, the moths of the first generation appear as early as the latter half of February, but data are lacking for the rest of the year. A fairly complete series of collections made at La Fayette, Ind., shows that in that latitude the periods of abundance fall about a month later than in Tennessee, the first from June 20 to July 10 and the second from August 6 to September 8. Even there the partial third generation appears, as moths were taken October 1 and 2 after having been absent for nearly a month. The La Fayette records accord closely with Forbes's (*6, p. 43*) statement for Illinois—

> two well-marked periods of maximum occurrence, one in July and one in August, with a comparatively sparse showing toward the middle of July.

Both Felt (*4, p. 65*) and Slingerland (*12, p. 210*) report the results of trap lantern collections at Ithaca, N. Y., and, although the number of moths taken is too small to more than indicate the facts, they seem to show that the two periods of greatest abundance lie between June 10 and 20 and July 8 and 20. More than one month is required for the development of a generation, so these Ithaca figures must be taken subject to revision.

REARING RECORDS

Laboratory records fully bear out the foregoing observations. Table I is a compilation of all the rearing records available for individuals under observation from egg to adult. It will be noticed that during the summer the interval between oviposition and the emergence of the adult is from 42 to 70 days, averaging about 55 days. Allowing three days for the preoviposition period brings it to 58 days, slightly less than two months from egg to egg. This works out almost exactly in the table; one generation from May 23 to July 21; the second from July 21 to September 11; and the third, or overwintering generation, from September 11 to the following May. It will be noted also that many of the later larvæ of the second generation, those coming from eggs laid after August 1, emerged the following spring. Thus it is safe to conclude that in Tennessee there are normally three generations per year, some of the second and all of the third overwintering as larvæ. Farther north the third doubtless becomes smaller and may completely disappear, leaving larvæ of only the second generation to pass the winter.

TABLE I.—*Rearing records of Crambus mutabilis under observation in the laboratory or insectary from egg to adult*[1]

Cage No.	Eggs laid.	Moth out.	Number of days.	Sex.	Number of moths.
A	May 23	July 8	46	♂	1
A	23	Aug. 5	74	♂	1
1775	23	July 30	68	♂	1
1775	23	Aug. 2	71	♀	1
B	26	July 7	42	♂	1
C	26	8	43	.....	1
C	26	Aug. 5	71	♂	1
E	27	July 11	45	.....	1
1777	30	31	62	♀	1
15268	June 3	26	53	♂	1
15284	6	26	50	♂	1
H	9	31	52	♂	1
H	9	Aug. 12	64	♂	1
G	12	July 31	49	♂	1
1792	12	Aug. 2	51	♀	1
I	16	12	57	♀	1
J	July 21	Sept. 3	44	.....	2
J	21	4	45	.....	8
J	21	5	46	.....	5
J	21	7	48	6 ♂ 6 ♀	12
J	21	8	49	1 ♂ 1 ♀	2
J	21	9	50	1 ♂ 2 ♀	3
J	21	14	55	♂	1

[1] The records designated by letters were made in 1914; in other cases the first two figures of the cage number indicate the year in which the records were made.

TABLE I.—*Rearing records of Crambus mutabilis under observation in the laboratory or insectary from egg to adult*—Continued

Cage No.	Eggs laid.	Moth out.	Number of days.	Sex.	Number of moths.
J	July 21	Sept. 23	64	♂	1
15406	24	11	49	♂	1
15406	24	13	51	5 ♂ 4 ♀	9
15406	24	14	52	1 ♂ 3 ♀	4
15406	24	17	55	1 ♂ 1 ♀	2
15414	27	18	53	♀	2
16221	29	22	55	♂	1
16221	29	24	57	♂	1
16221	29	29	62	♂	1
16221	29	Oct. 1	64	♀	1
16221	29	3	66	♂	1
16221	29	17	80	♂	1
16221	29	19	82	♀	1
16221	29	Nov. 6	98	♀	1
16221	29	14	102	♀	1
16221	29	Apr. 23	268	♀	1
16221	29	May 21	296	.....	1
15476	Aug. 6	Oct. 19	74	♀	1
16260	11	Apr. 23	255	.....	2
16259	16	May 2	259	.....	1
16259	16	21	278	.....	1
16259	16	27	284	.....	2
R [2]	Sept. 11	Dec. 9	58	♀	1
15607	12	May 26	256	♂	1
15602	17	June 2	258	♀	3
15619	27	1	247	♂	2
15619	27	5	251	♀	1

[2] Reared in laboratory

Table II is a record of all the rearings of larvæ collected in the field, showing in most cases the interval between the activity of the larvæ in the spring and the appearance of the moths of the first generation. The data apply to middle Tennessee unless otherwise stated.

TABLE II.—*Rearing records of Crambus mutabilis larvæ collected in the field*

Cage No.	Larva taken.	Moth out.	Sex.	Source.
182	Apr. 3	May 25	♂	Grass roots; fed blue grass.
15141	20	24	♂	Blue grass.
1655	21	June 27	♀	Grass.
1824	29	3	♀	Corn.
12358	May 9	13	♀	Corn.
Field note	10	.....	.....	Clump grass; not reared.
16104	10	May 29	♂	Grass.
12358	15	June 8	.....	Corn.
15359	July 7	July 27	♀	Blue grass.
15359	7	Aug. 9	♀	Blue grass.
C999G	27	Sept. 10	.....	Timothy, Athens, Ind.
C1037	Aug. 6	5	♂	Timothy, Nortonville, Ky.
15641	Sept. 30	.....	.....	Timothy, La Fayette, Ind.; not reared.
15687	Nov. 9	.....	.....	Blue grass, Nashville, Tenn.; not reared.

A series of 50 larvæ from eggs laid July 23 was reared for individual instar records. Each larva was confined in a half-ounce tin box floored with damp blotting paper and was fed blue grass. A record was made of each molt, and the results are condensed in Table III.

TABLE III.—*Instar records of 50 Crambus mutabilis larvæ from eggs laid July 23, 1915*

Stage.	Maximum.	Minimum.	Average.	Number of individuals included in average.
	Days.	*Days.*	*Days.*	
Egg	7	7	7	.....
Larva:				
Instar I	5	3	3.2	50
II	5	2	3.3	48
III	4	3	3.3	44
IV	5	3	3.3	41
V	7	3	4.1	36
VI	7	3	4.8	29
VII	17	9	14.1	19
Pupa:				
♂	11	7	9.9	8
♀	10	7	8.0	8
Total	56	50	52.6	16

THE MOTH

HABITS

The moths of this species (fig. 2) are essentially grass lovers and are seldom found among weeds or bushes. They are reluctant to leave tall grass for a mown field or closely grazed pasture and when driven out will at once circle back. They alight abruptly, usually on a grass stem, and instantly turn head downward and stand with the head pressed closely to the stem and the body elevated at a considerable angle. When at rest the dark costal margin of the fore-wing contrasts with the paler gray median portion and with the pale gray underbody, giving the moth an almost striped appearance when viewed from the side. This coloring and the characteristic attitude make this species one of the easiest to identify at a distance in the field. The moths are most often found in abundance in the lower and damper portions of the meadow, seemingly attracted by the greater luxuriance of the grass. They also occur in open woodland, especially if the ground is grass-covered. Their flight is erratic and awkward.

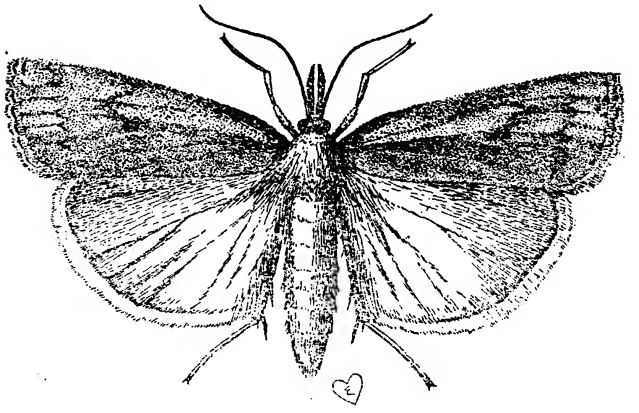

FIG. 2.—*Crambus mutabilis:* Adult female. About three times natural size.

All-night collections at light made throughout the summer in 1915 show that the female moths are attracted during the early part of the evening, between 8 and 9 o'clock and the males not until later, between midnight and 3 a. m. Very few of either sex appeared outside their respective periods.

It was proved by numerous experiments that the moths will feed on dilute honey and will drink water, but that food is not necessary and that they will live longer and the females will produce more eggs on the average when supplied with water alone than when fed honey or when confined in a dry box or vial. Moths in the field have never been observed feeding, or attracted to flowers.

The average length of life of 72 female moths taken at light and in the field and confined, some in dry boxes and some with wet cotton, was 3.86 days. Under the same conditions 115 males averaged 1 day less. One reared female, which had access to water from the time she emerged, lived 10 days and laid 500 eggs, which is probably about normal for a moth in the field. Under optimum conditions 3 males lived 14 days each, but a summary of all records shows that the males are usually shorter-lived than the females.

Practically all eggs obtained from moths taken in the open are fertile, indicating that mating occurs very shortly after the emergence of the females, probably the same night. Occasionally the last few eggs laid by a female prove to be infertile, but in most cases one mating is enough to fertilize the entire supply of eggs, and there is no evidence that the moths normally mate more than once. Female moths isolated and prevented from mating usually deposit some eggs, but these invariably shrivel and fail to hatch.

DESCRIPTION (FIG. 2)

Wing expanse 18 to 24 millimeters, the females averaging larger than the males. General color gray, with a dusky spot near center of forewing, the inner half of costal margin dark brown. Palpi fuscous, the tips of the scales whitish. Head and thorax gray-brown. Male antennæ broadly pectinate (Pl. 2, A), female setaceous. Forewing with costal half slaty gray, sometimes whitish toward the center, anal half with a tinge of luteous. Proximal half of costal margin broadly bronze-brown. A dark brown median line begins near the middle of costal margin, forms a broad angle near end of cell, broadens immediately below it, and continues in an oblique line, gradually narrowing until it reaches the hind margin. In feebly marked specimens this median line is often obsolete except the portion below the end of the cell, which is invariably present as a more or less conspicuous dusky spot. Subterminal line runs nearly straight across the wing, with an acute outward angle at each vein. Terminal line of seven dusky spots at the ends of the veins. Fringes gray, shining. Hind wings gray, a little paler toward base, fringes pale yellow.

GENITALIA.—Male: Body of tegumen (Pl. 2, C) rather long, a little longer than the uncus, rounded above, its limbs long, narrow, turned ventrad, and narrowed at the ends. Uncus nearly straight, rather narrow, with a sharp, nail-like hook at the end, hirsute above. Gnathos long, slender, much exceeding the uncus, tip narrowed and turned slightly ventrad, naked. Aedoeagus (Pl. 2, E) straight, cylindrical, smoothly rounded cephalad, tapering somewhat from the opening to the tip, which flares slightly, terminal opening oblique, with a single long slender heavily chitinized cornutus, about half the length of the organ; anellus reduced to a mere membranous scale on ventral side. Harpes (Pl. 2, D) small, rather weakly chitinized; free costa reduced to a slender sharp spine less than half the length of the free sacculus, outer margin at base hirsute; sacculus with the free portion a flat curved process with rounded tip, hirsute, the hairs on ventral half much shorter than those above, narrowed at base, and with a rounded spined lobe where it joins the base. Vinculum reduced to a small scutate plate lying between the tips of the base of the sacculi. Female: The ventral two-thirds of the genital plate (Pl. 2, F) rounded and somewhat produced, the dorsal lobe smaller, rounded, both lobes hirsute.

THE EGG

DESCRIPTION

Elliptical in outline, bluntly rounded at the ends, one of which is very slightly more obtuse than the other. Length, 0.494 to 0.441 millimeter, average, 0.479 millimeter; width, 0.318 to 0.265 millimeter (average, 0.306 millimeter). The chorion is ornamented with prominent acute longitudinal ridges, usually 17 in number but varying from 16 to 20. These ribs become obsolete at the ends of the egg, where the polar disks are only slightly tuberculate; in the intervals between the ribs are much smaller cross striæ, about 30 in the length of the egg (Pl. 1, C).

The eggs laid by one female are very constant in size and shape, but there is enough variation between those from different moths to make the measurements of little value for specific determination.

The eggs are very pale cream, almost white when laid, but they soon deepen in color, reaching a pale salmon yellow in about three days, after which, until maturity, the color remains unchanged. After the third day the minute dark eye spot is visible within the egg. A few hours before hatching the darkening of the head and thoracic plate gives a purplish tinge to the whole mass of eggs. The egg is cut slightly to one side of the larger end and the larva escapes through a more or less ragged opening, leaving the parchmentlike shell nearly transparent.

NOTES

In summer the eggs hatch in from five to seven days, the variation evidently depending on the temperature. When fully developed the larva leaves the egg regardless of outside conditions and does not remain quiescent as do some others of this group. The eggs are perfectly dry and nonadhesive when laid, and fall down among the grass stems as they are dropped by the moths either in flight or at rest. Their small size and lack of definite location make them hard to discover; and to find one a prolonged search is necessary even when they are known to be abundant.

Dissection of two freshly emerged moths showed, respectively, 560 and 1,120 eggs and egg cells in the ovaries. In the latter case the abdomen also contained a large amount of fat in the form of small bodies about the size of the matured eggs, but whiter and more irregular in shape. The first moth showed a much smaller quantity of this reserve supply, which is probably accounted for by less favorable feeding conditions during the latter part of the larval life. This partial starvation of the larva seems to affect directly and very decidedly the fecundity of the moths.

So far as the writer has been able to determine from many experiments with this and other species of Crambus, the females do not require food other than water for the development of the immature ova in the ovaries at emergence. Spent moths show an entire absence of fat bodies and immature ova, the youngest of these having evidently broken down and been used for the nutrition of the larger eggs. So while there were 1,120 eggs and egg cells in the more fecund of the two moths mentioned above, the actual number of eggs which she would have matured would have been much less than that, perhaps 700, and not over 350 for the other. The largest number of eggs produced by a single moth of this species, of which the writer has record, is 753 eggs, and very few attained 500. The average number produced by moths taken in the field and at light was 170, but many of these had doubtless laid part or all of their supply before capture.

THE LARVA

HABITS

The larva of *mutabilis* is one of the easiest to recognize in the field. The distinct striping of the body and the prominent dark markings of the head distinguish it. When newly hatched the head and cervical plate are shining jet black [3] and the body pale grayish yellow except for the spot of color in the intestinal tract, due to the bit of eggshell eaten in leaving the egg. The pinacula are dusky and unusually conspicuous for the first instar. During the first three instars the head remains black or very deep fuscous. In the fourth and succeeding instars it becomes brownish yellow, marked in a definite pattern with close groups of small, round, dark brown or black spots.

In the writer's experiments many larvæ were reared on blue grass, small sods of which were potted and covered with lantern globes. The newly hatched larvæ begin to feed by cutting small pits between the vascular bundles on the upper surface of the leaf blade, usually toward the base. These pits are soon covered with a few silk fibers, and shortly the larva is concealed by excrement placed systematically in this webbing. During the first three instars, only the green leaf tissue is consumed, leaving the lower epidermis intact; thereafter the entire thickness of the leaf is eaten. As soon as the larva becomes too large to remain sheltered on the leaf blade it descends to the ground and makes a burrow lined with silk and opening usually close beside the stem. In the field there is often a valve-like arrangement at the tip of this tube, so that the larva from within can close it and remain secure from intrusion. Beneath the surface the burrow may run at almost any angle. If the earth is soft it frequently is parallel with and close beside the main stem of the plant; if the ground is hard, it may run off at right angles just beneath the surface. The upper part of the tube is rather substantially constructed and closely lined with silk. Farther down the silk is more sparingly used and often just above the lower end ceases altogether, leaving only the earthen walls. The extreme lower end is again lightly lined with silk. This peculiar construction serves a very useful purpose in protecting the larva. When the plant is disturbed the larva instantly retreats to this silk-lined extremity of its burrow. If the plant and burrow be dug or pulled from the ground the tube breaks at its weakest point. The larva draws the earth-covered silk lining tightly about itself and so closely resembles a mere lump of earth that surprisingly often it escapes further detection. These larvæ become very large and brightly colored and but for the protection of this bit of webbing would be the most easily discoverable of the webworms.

Although in the cornfield the burrow often runs into the ground close by the stem of the plant, very little if any feeding is done beneath the surface. Quite unlike *caliginosellus*, which occupies a half-cylindrical tube lying against the stem and feeds exclusively underground, *mutabilis* always has an exit to the outside and feeds aboveground. The leaves are eaten, beginning at the base, and as they are cut fall away from the plant and lie wilting on the ground. Sometimes so many leaves are cut in this way that to obtain green food the larva is forced to consume

[3] Felt's (*4, p. 65*) surmise as to variations in the color of the head of the newly hatched larva is clearly an error, for the head colors of the first instar of the various species are remarkably constant.

the main stem of the plant, working from the top downward. In such cases the injury closely resembles cutworm work. Only the first-generation larvæ work on corn, and then usually only when it is very small.

In sod the injury is not so characteristic. The larva merely lives in its silk-lined tube, cuts off one blade of grass at a time, and draws it down into the burrow, where it is consumed at leisure. It is doubtful if the larvæ leave their burrows in the daytime, but they will feed during the day, as can be seen by the motion and gradual disappearance of a grass blade inserted into the burrow. All excrement is packed into the lower end of the burrow or sometimes into side pockets forking from the main burrow. When one burrow becomes too small, or is filled with the bright green sawdustlike frass, it is abandoned and another constructed. Occasionally a burrow is found as a silken tube running back under a board or stone lying on the surface of the ground.

Felt (*5, p. 69–74*) and other writers have described and figured definite cylindrical nests made by crambid larvæ, but these have been observed by the present writer in only one instance, and that in connection with this species. In this case numbers of larvæ were confined in lantern-globe cages with small potted sods of blue grass. They had reached about the fifth instar in late September, and at once constructed tubular nests of bits of dry grass blades tightly fastened together and smoothly lined with silk. They were suspended among the leaves of the blue-grass plants, some of them partly in the ground and some entirely above and clear of the ground. (Pl. 1, A.) They contained no excrement and were closed at the bottom, but open at the top. The larvæ seemed to make no effort to close them and wintered successfully in them, although exposed to every change of temperature in an open outdoor insectary. After the construction of these nests the larvæ fed no more until the following spring. It seems probable that such nests are constructed only when the soil is excessively wet, in order that the larva may remain dry during the cold weather. Excessive moisture and dryness are both enemies of larvæ overwintering in cages, and it is difficult to provide exactly proper conditions for them. From these and other observations it may be concluded that in Tennessee the larvæ construct and enter their winter quarters about the last of September. They begin feeding again in April, and the first moths make their appearance about the middle of May.

Two series of 10 larvæ each were run at different times to obtain records of the amount of food eaten. Blue-grass leaves cut into 30-millimeter lengths were used for food. At the close of each instar the uneaten portion was removed and measured. Since the larvæ skeletonize the leaves during the first three instars of their life and the total amount consumed during that time is insignificant, these instars are omitted from the record. The figures represent linear millimeters of blue-grass leaves of an average width of about 3 millimeters. The results of the two series were so similar that they are combined in Table IV.

As indicated by this somewhat incomplete record, the voracity of the larvæ of this species is low compared with that of *Crambus trisectus*, in which the average total consumption amounted to over 2,000 millimeters. This may be another reason why this species has not caused as serious injury as some of the others.

TABLE IV.—*Record of food eaten by larvæ of Crambus mutabilis*

[Linear millimeters of blue-grass leaves, average width about 3 millimeters.]

Instar.	Maximum.	Minimum.	Average.	Number of larvæ.
	Mm.	*Mm.*	*Mm.*	
IV	60	5	33	20
V	135	50	93	20
VI	480	35	183	19
VII	1, 140	90	496	18
VIII	576	68	268	4
Total	.....	.....	1, 073	.....

The normal number of instars for *mutabilis* appears to be seven. Two larvæ pupated from Instar VI, but neither lived until emergence. The males and females develop in very nearly the same time, showing a difference of only 0.2 of a day between the averages for 8 of each sex.

The width of head and average length of the larva in each instar are shown in Table V.

TABLE V.—*Larval measurements of Crambus mutabilis*

Instar.	Number measured.	Head width.			Body length.
		Maximum.	Minimum.	Average.	
		Mm.	*Mm.*	*Mm.*	*Mm.*
I	8	0. 194	0. 194	0. 194	1. 77
II	6	. 301	. 301	. 301	2. 80
III	4	. 459	. 424	. 441	3. 08
IV	5	. 582	. 547	. 570	5. 00
V	5	. 900	. 812	. 847	9. 00
VI	11	1. 306	1. 166	1. 227	13. 00
VII	3	1. 912	1. 586	1. 730	18. 00

DESCRIPTION

INSTAR I.—Head shining black, cervical plate fuscous, prothorax a little darker than abdomen, mesothorax and metathorax concolorous with it. Abdomen pale transparent yellow. Pinacula on thorax and abdomen dusky and conspicuous, unusually so for the first instar in this genus.

INSTAR II.—Head shining black, frons deep fuscous, cervical plate castaneous, a little paler and more reddish than head. Rest of thorax and abdomen pale yellow. Pinacula dusky and conspicuous, for each is surrounded by a small area of brownish overcolor.

INSTAR III.—Head shining black, frons paler, fuscous; cervical plate fuscous, rest of thorax and body pale, tinted by the ingested food, skin finely granular. Pinacula brownish, rugose, more or less conspicuously surrounded by a brownish area. Caudal plate pale with dusky dots.

INSTAR IV.—[No description obtained.]

INSTAR V.—Head dark yellow with clearly defined yellowish brown markings made up of round spots arranged in broken groups but in a definite pattern as follows: One area bordering the vertical suture and extending down on to the face in a branch each side of the frons; another larger triangular area with its base on caudal margin of head, extending forward until its tip joins the forks of the vertical spot; below this a smaller crescent-shaped area midway between the frons and caudal margin of

head; and below this a line running from the ocellar area to the caudal margin of the head. This pattern remains constant throughout the remaining instars. Cervical plate dusky yellow with small dark spots. Body color pale greenish yellow tinged with a faint claret or maroon overcolor. There are four longitudinal rows of whitish spots, one on each side of the narrow middorsal line and a broader one on each latero-dorsal aspect above the spiracles, giving the larva a striped or spotted appearance. Venter pale green, skin finely granular and glistening. Pinacula dark yellowish brown, rugose.

INSTAR VI.—Head dusky yellow with conspicuous groups of round fuscous spots; frons concolorous with head. Cervical plate yellowish brown, with several dark spots. Rest of thorax and abdomen entirely covered with the brown overcolor except for the four longitudinal pale or greenish lines which are more conspicuous in this instar. Pinacula darker than body, nearly concolorous with dark markings on head. Black cicatrices on the pedal segments of the abdomen about the size and shape of the spiracles. Caudal plate dusky yellow with dark markings.

INSTAR VII.—Head as in Instar VI with the markings more sharply defined. Cervical plate dusky yellow with a faint, dark-bordered pale median line, otherwise as in Instar VIII.

INSTAR VIII.—Head dusky or amber yellow, with dark markings composed of close groups of round yellowish brown spots arranged as described under Instar V. Frons dusky yellow, outlined with a very fine dark line and outside a pale V, which at apex continues caudad to the vertical suture. Cervical plate dusky yellow, with narrow, dark-bordered pale median line and some small dark markings. The four rows of irregular whitish spots give the larva a distinctly striped appearance. Pinacula yellowish brown, leathery, shining, rugose. Cicatrices larger than spiracles, black. (Pl. 2, B.)

THE PUPA

DESCRIPTION

The pupa is pale yellow when first formed, soon changed to a golden yellow, the head, thorax, and wing cases darkening as it approaches maturity. Spiracles are present on abdominal segments 3 to 9, inclusive, those on segment 3 almost under the edge of the wing cases, those on segments 5 to 8 elevated, the one on segment 9 pale and merely a scar. Anal process rather narrow (Pl. 2, G), a dorsal rounded ridge of nearly uniform width running to the rather truncate and downwardly bent tip. At the angles of this tip stand the setæ of the dorsal pair, very small, depressed, and inclined cephalad. At each side of the dorsal ridge is a flattened depressed area cut by the deep, wide, curved nasal groove at whose caudal end stands a small tubercle. Ventrad this anal process is more tapering and ends in a rounded elevation from the side of which arises the ventral pair of setæ, somewhat larger than the dorsal pair and diverging. Cephalad of this terminal elevation are four or five shallow, parallel, longitudinal depressions as if made by fingers laid side by side. The tip of the abdomen beneath is flattened but not concave.

THE COCOON

When fully grown the larva makes its cocoon (Pl. 1, B) either by walling off a section of its burrow or constructing a separate chamber near by in the soil, more often the latter. The cocoon is about 15 millimeters long and half as wide, shaped like a peanut meat, rather firm to the touch, lined with soft gray silk and outwardly covered with earth particles so that it is not easily found. It lies close to the surface of the ground and when buried deeper has an extension reaching the surface. In emerging the moth leaves the pupal shell entirely within the cocoon.

SYSTEMATIC RELATIONSHIPS

Crambus mutabilis appears to be the ultimate of one series in this genus. It differs from *hemiochrellus* (*1*, *p.* 57), its nearest relative, in the greater development of the antennæ and the reduction in the male genitalia. The male antennæ are more strongly pectinate than those of *hemiochrellus;* in fact, more so than in any other species of the genus which the

writer has seen. Each of the median segments bears from 14 to 18 sensoria (Pl. 2, A) compared with 8 or 10 in *hemiochrellus*. The male genitalia give the best basis for comparison. The free costa, which in *hemiochrellus* is a stout naked spine as long or longer than the sacculus, is in *mutabilis* a small slender spine less than half the length of the sacculus, which remains practically the same. The tegumen and uncus retain much the same shape, but the cornutus (Pl. 2, E) in the aedoeagus is both shorter and more slender than in *hemiochrellus*. The wing shape and the general uniform coloration remain similar in the two species, and the eggs of the two assume and retain exactly the same color during the incubation period.

NATURAL CONTROL

That this species has not more frequently been recorded as a destructive pest is probably due to two factors—the apparent great susceptibility of the larvæ to disease, and the attacks of parasites.

DISEASES

While making intensive studies of this species at Nashville, Tenn., in rich blue-grass meadows, where the larvæ were known to be abundant, areas were often found in which practically every burrow contained only the flaccid dead body of the maker. The same disease, evidently bacterial, was met with in the laboratory, and it was only by using the strictest care in the sterilization of the tin boxes used as rearing cages that it was possible to bring the larvæ to maturity. The disease first manifests itself in the lack of appetite and sluggishness of the larva. The next day the larva is dead and somewhat softened, but not externally changed. Another day reduces it to a shapeless sack filled with a dark brown semiliquid. Finally the skin also breaks down and the mass gradually dries up, leaving only a dark stain. The writer has not succeeded in getting a determination of the cause of the disease, but that it plays a very large part in keeping this species from becoming a serious pest can not be doubted.

Another disease which is less common kills the larva more gradually. It usually begins at the caudal end and leaves it corky in texture and densely filled with a mass of whitish hyphæ. The fungus causing it has been determined by Dr. A. T. Speare as a species of Isaria.

PARASITES

Because of the fact that the larvæ of this species have a more open method of feeding than some of the others, they appear to be more subject to the attacks of parasites than those which remain more constantly underground.

Two species of tachinid flies have been reared from larvæ of *Crambus mutabilis*, namely *Phorocera claripennis* Macq. and *Exorista nigripalpis* Towns., neither of which apparently has heretofore been recorded from this host. Both were reared from larvæ collected by W. H. Larrimer in connection with an outbreak of webworms near La Fayette, Ind., in June, 1920, in which two species of Crambus were concerned, *C. trisectus* and *C. mutabilis*, the former predominating to the extent of about 80 per cent of the total.

Fifteen flies of the species *Phorocera claripennis* Macq. were reared from 14 host larvæ, two of them maturing, in one instance, from one host.

One came from a larva determined before its death as *Crambus mutabilis* and the others all from mixed larvæ specifically undetermined. It is therefore unknown whether this parasite is limited to the single host or divides its attentions between the two. Further rearings are necessary to settle this point. The mature maggots of this species almost invariably issued from their host while it was still a larva and from one to three days after its death, leaving only an empty head shell and a shriveled skin. The adult flies appeared from 11 to 19 (average 13.5) days after pupation. The eggs are white and conspicuous and are attached to any part of the host, most frequently about the thoracic segments. As many as 14 eggs have been seen on one individual, but it seems very unusual for more than one of the parasites to reach maturity, at least on a host of this size. In the one such case observed both of the two flies appearing were very small and one failed properly to expand. In fact, this fly does not seem to have the vigor of *Exorista nigripalpis*, for, although reared under exactly similar conditions, nearly half of them failed properly to expand their wings upon emergence, and some failed even to free themselves from their puparia. Several of the crambid larvæ which yielded these flies did not have eggs on them when collected, but the shells had probably been molted off after maggots had hatched and entered their host.

Only a single fly of the species *Exorista nigripalpis* Towns. was reared from a larva previously determined as *mutabilis*, and as a considerable number were obtained from larvæ known to be *trisectus* this parasite is discussed in a forthcoming paper dealing with *trisectus*.

The writer has records of two species of hymenopterous parasites attacking *Crambus mutabilis*.

Apanteles crambi Weed has been recorded from other species of the genus, and recently (*10*, *p. 546*) from *mutabilis* from South Dakota and Tennessee. When full-grown the grubs of this parasite emerge from their host and spin a mass of white cocoons near by. The host remains alive for several days, but finally dies without moving or feeding. The adult parasites emerge in six or seven days after the cocoon is formed.

Macrocentrus crambivorus Vier. has been reared twice from larvæ of *Crambus mutabilis* at Nashville, Tenn. In both cases the host larvæ were nearly full grown but appeared abnormally pale when collected. In the rearing box each constructed a silken case, and when this was examined a little later the larva had been replaced by a dense mass of elongate yellowish brown cocoons, 20 or 25 in number. The following day the white pupæ could be seen indistinctly through the cocoons. The parasites became adult and active within their cocoons eight or nine days later, but their actual emergence seemed to be entirely dependent on proper conditions of humidity. In one instance the adults, although active within the cocoons, did not emerge until the stopper of the vial was moistened, whereupon they all released themselves within five minutes. Other groups behaved in the same way unless they had been so long confined to their cocoons that they were unable to emerge at all. The adults are slender, yellowish brown in color, and very active. The females trail a long threadlike ovipositor behind them. In the writer's rearings all those emerging from a single mass of cocoons were of one sex. Others, also apparently of this species (determined by S. A. Rohwer as *Macrocentrus* sp.) were reared from two of the undetermined larvæ taken at La Fayette, Ind., in June, 1920. One yielded 14 males and the other 29 females.

ARTIFICIAL CONTROL

Injury to corn by the striped sod webworm can more easily be prevented than remedied. When a field is in meadow or pasture, the moths are attracted to the low, rich portions where the grass growth is most luxuriant. If a sod field be broken and planted to corn, injury is most likely to occur in such portions. To prevent the injury the sod should be plowed as early as possible in the fall, in late July or August if possible. Land plowed after the middle of September generally shows little reduction in infestation compared with that plowed in the spring, because by September the majority of the larvæ have entered their winter quarters, where they are not seriously injured by plowing. If the sod is plowed early enough to deplete seriously the food supply of the fall generation and force the larvæ into winter quarters in an undernourished condition and incompletely protected, the method may prove somewhat beneficial.

Where lands permanently in grass, such as meadows, lawns, and parks, are heavily infested, premature drying and browning of the plants may be caused, becoming especially evident during periods of drought. Little can be done in such cases except to stimulate the growth of the grass by applying a quick-acting fertilizer, such as nitrate of soda. Fortunately, the portion of a meadow most attractive to this insect is the last to suffer from unfavorable moisture conditions and remedial treatment in such places will very seldom be required.

Although poisoned bran bait has been tested in a few instances, for the most part unsuccessfully, no opportunity has as yet presented itself to give this method a thorough trial. It may yet be found feasible for use in heavily infested grasslands.

SUMMARY

Crambus mutabilis is a common species over the eastern half of the United States and as far west as Iowa, Utah, and Texas. Its food plants seem to be limited to the grass family.

It has been known to cause injury to young corn and to grasslands, but in such cases it is usually associated with some other species.

There are three generations a year in Tennessee, the third being the smallest, and gradually diminishing northward until there are only two at the northern limits of the species.

The moths are grass lovers and seek the lower and more luxuriant portions of pastures and meadows. They lay about 500 eggs, dropping them promiscuously as they fly.

The larvæ in the larger instars are distinctly striped and easily recognized. They construct tubular burrows in the earth opening at the surface and feed on near-by grasses, cutting off the leaves and dragging them into the burrows. In the fall they construct tubular nests among the grass stems, either above or partly in the ground, and pass the winter as partly grown larvæ, completing their growth in the spring.

Crambus mutabilis is more specialized than *C. hemiochrellus*, its nearest relative, and is evidently the terminus of one line of development in the genus.

The larvæ seem very susceptible to disease and are also frequently parasitized by both Hymenoptera and tachinids.

Control measures consist of early fall plowing of sod lands intended for corn the following year and rotation of pastures and meadows where the insect is destructive. Ordinarily natural agencies prevent its injurious increase.

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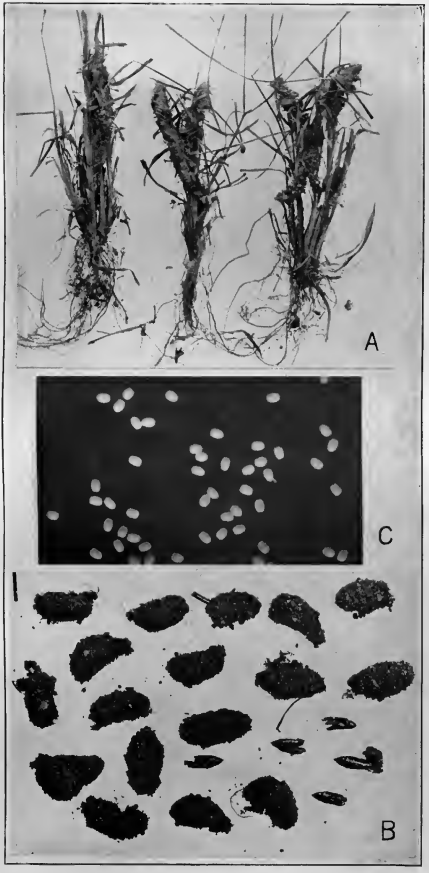
PLATE 1

Crambus mutabilis:

A.—Winter cases of larvæ on blue-grass plants.
B.—Pupal shells and cocoons.
C.—Eggs. Greatly enlarged.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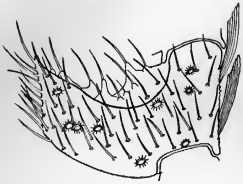

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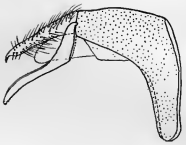

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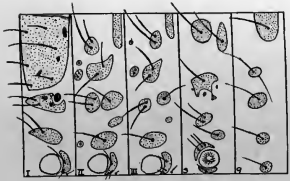

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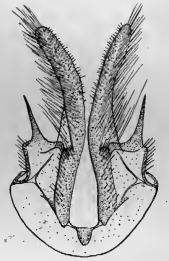

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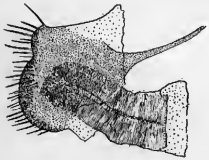

F

G

PLATE 2

Crambus mutabilis:

A.—Male antennal segment (twenty-fifth), greatly enlarged.
B.—Setal map of three thoracic and third and ninth abdominal segments of larva.
C.—Male genitalia: Tegumen and uncus.
D.—Male genitalia: Harpes.
E.—Male genitalia: Aedoeagus.
F.—Female genitalia: Valve.
G.—Tip of pupa, dorsal view.

SILVER-STRIPED WEBWORM, CRAMBUS PRAEFECTELLUS ZINCKEN[1]

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INTRODUCTION

Although of less economic importance than many other members of the genus, *Crambus praefectellus* is so widely distributed that it is sure to be met with by anyone interested in these beautiful little moths. It is one of several species with a longitudinal silvery-white stripe in the forewing (fig. 2). It is most likely to be confused with *C. leachellus* Zincken, which, however, is a larger species with the white stripe running much closer to the costal margin of the wing than it does in *praefectellus*. *Crambus quinquareatus* Zeller and *C. unistriatellus* Packard also resemble it in size and general pattern, but in the former the apex of the wing is acuminate and in the latter the white stripe runs the full length of the wing, characters which easily distinguish their possessors from the species under consideration.

SYSTEMATIC HISTORY

Crambus praefectellus was first described by Zincken (*9, p. 249*)[2] in 1821 from specimens sent him from Georgia. He placed it in the genus Chilo, which at that time was synonymous with what we now know as the subfamily Crambinae. Clemens (*3, p. 203*) redescribed it in 1860 as *Crambus involutellus*. In his revision of the group in 1863, Zeller (*8, p. 18*) placed Zincken's species in its present genus and incorrectly placed *involutellus* as a synonym of *leachellus* Zincken. In this he was followed, with some hesitation, by Grote (*7, p. 77*), but Fernald (*5, p. 45*) corrected the error and first placed *involutellus* Clemens as a synonym of *praefectellus* Zincken, a course approved by all later writers. The synonymy then stands as follows:

Chilo praefectellus Zincken, 1821
Crambus involutellus Clemens, 1860
Crambus praefectellus (Zincken) Zeller, 1863

Although the bibliography of this species comprises some 25 titles, the great majority of these are merely references to the occurrence of the moths in various localities. Felt (*4, p. 85*) figures and discusses the species, but since he did not find it at Ithaca, N. Y., where his work was done, he gives us no biological information. Fernald (*6, p. 31*) figures and describes the adult and concludes with the comprehensive statement, "Early stages and food plant unknown." Since that time Britton's paper (*2, p. 222*) is the only publication that adds to our knowledge.

[1] Accepted for publication July 11, 1922. This paper is the fourth in a series of Contributions to a Knowledge of the Crambinae of North America. I, *Crambus hemiochrellus* Zeller, appeared in Annals of the Entomological Society of America for March, 1918, and II, *Crambus laqueatellus* Clemens, appeared in the June, 1922, issue of the same journal. The third paper, entitled, "Striped Sod Webworm, *Crambus mutabilis* Clemens," precedes this paper in the Journal of Agricultural Research.

[2] Reference is made by number (italic) to "Literature cited," p. 424-425.

GEOGRAPHICAL DISTRIBUTION

Crambus praefectellus is a strictly American species and seems to be limited to the eastern half of the United States. It has been taken in practically every State east of the Mississippi River and also in North Dakota, South Dakota, Minnesota, Iowa, Colorado, Missouri, Arkansas, and eastern Texas. It is reported from Cartwright, Manitoba, and also occurs in eastern Canada, at least along the southern edge. The accompanying map (Fig. 1) shows at a glance its present known distribution. The following list gives the States from which records are avail-

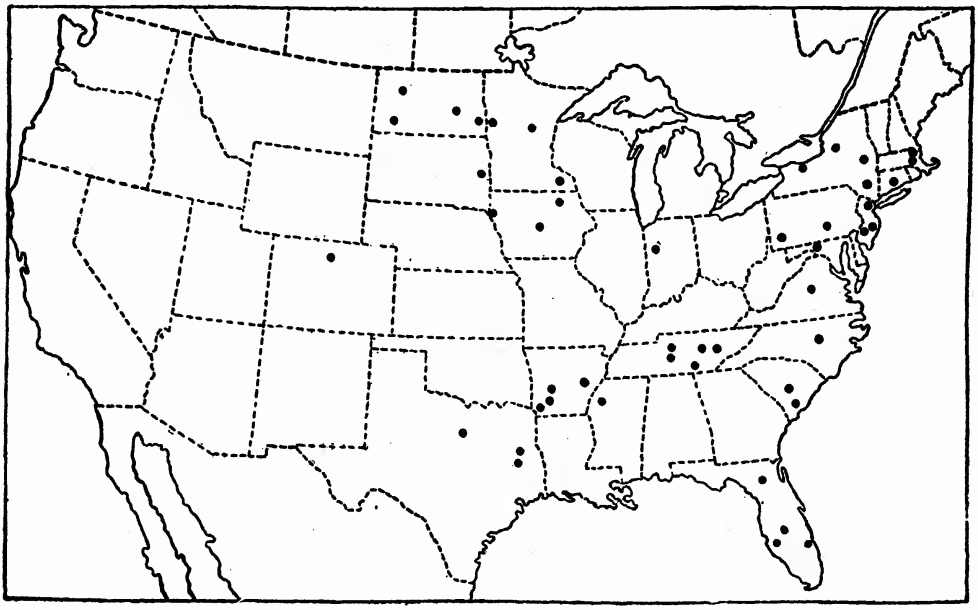

Fig. 1.—Map of the United States showing known distribution of *Crambus praefectellus*.

able and also gives the first and last date and the intervening months when collections have been made:

Arkansas. March 28, June, July 3.
Colorado. (Date uncertain.)
Connecticut. May 26, September 1.
Florida. February 7, March, April 30.
Georgia. (Date uncertain.)
Illinois. May 31, June, July, August, September 3.
Indiana. August 11, 12.
Iowa. July 8, August, September 6.
Kentucky. August.
Maryland. August 30.
Massachusetts. May 31, June, August 22.
Minnesota. June 19, July —.
Mississippi. May 3, June 22.
New Hampshire. August 1 to 7.
New Jersey. May —, June, July, August, September 11.
New York. May 21, June, July, August 18.
North Carolina. June —, July, August 23.
North Dakota. June 12, July 21.
Pennsylvania. May 24, June, July, August 19.
South Carolina. April 1 to 7.
South Dakota. June 24.
Tennessee. April 3, May, June, July, August, September, October 20.
Texas. January 30, March, April, May 22.
Virginia. September 5, 6.
West Virginia. May 30.
Wisconsin. September 10.
Manitoba, Canada. (Date uncertain.)
Ontario, Canada. June 4, July 6.

FOOD PLANTS

Corn, wheat, rye, oats, blue grass (*Poa pratensis*), pigeon grass (*Setaria glauca*), and timothy (*Phleum pratense*) were all used as food plants in the writer's rearing cages and all were accepted readily by the larvæ.

Doubtless this list could be almost indefinitely extended. Judging from the habit of the moths in frequenting weedy waste ground in preference to grassy places, it is very likely that the larvæ also feed on plants other than grasses. The foregoing plants include all on which larvæ have been taken in the field.

ECONOMIC HISTORY

While it can not be regarded as a serious pest, *Crambus praefectellus* in one or two instances has shown that it can cause considerable injury under certain conditions. Single larvæ have been taken destroying wheat at La Fayette, Ind., and corn at Lakeland, Fla., Prescott, Brinkley, and Hot Springs, Ark., and Knoxville and Caney Spring, Tenn. Larvæ were received from Advance, Mo., with the report that they had injured 50 acres of a 300-acre cornfield. Britton's (*2*) recent account is the only published record of injury by these larvæ. In this case a small field of corn in the outskirts of New Haven, Conn., was almost totally ruined. The field had been in grass previously, and was plowed in the spring and planted to corn. The plants were attacked while small, and so numerous were the larvæ that only a very few plants escaped injury and produced grain.

Just what factors in the life economy of this species prevent it from more often becoming destructive can not be stated. Probably parasites, predacious enemies, and disease all play their parts, but nothing is known about these. It is apparent that this insect has not in any special way adapted its life cycle to extremes of climate. It has no definite protective resting period and consequently is overtaken by winter and unfavorable weather in all its different stages, some of them unfitted to resist such conditions. The mortality from such causes must be very great.

If control measures were needed, probably the best would be early fall plowing of land intended for corn the following season. If this were done, and the planting delayed as long as possible in the spring, the ground meanwhile being fallow and free from weeds and grass, there should be very slight possibility of the larvæ surviving until the corn germinated. If the infestation is not discovered until the corn is up, as is usually the case, little can be done but to replant alternately with the old rows, allowing them to stand as long as possible before cultivating them out. This method is described more fully in another paper (*1*, *p.* *15*).

SEASONAL HISTORY

The earliest seasonal record for a moth of *Crambus praefectellus* is January 30, at New Caney, Tex. It has been taken at several points in Florida during February. In Tennessee, where continuous observations have been made for several years, the first moths make their appearance during April, usually toward the end of the month. It is always the first species of the genus to appear. On one occasion a battered male moth was taken at Knoxville on April 3. This is more than two weeks earlier than the moths have been taken in other years, and the pupa from which it emerged may have been formed in a particularly sheltered location.

After their first appearance the moths do not become abundant but are found singly and scatteringly throughout the greater part of the

summer. The generations are not distinct, although at some times the moths appear to average fresher than at others. It is very evident that they breed continuously and that the larvæ do not have any considerable resting period after completing their growth but pupate at once. Judging from laboratory rearing records, there are probably three generations during the year in Tennessee. Farther north this number may be lessened and at the southern limit of its range there are probably more. With a species such as this, in which the generations follow one another without intermission other than the delays due to unfavorable weather conditions, the number of generations in any given season is directly dependent on the length of the growing season and may vary from year to year.

All the available data as to the seasonal appearance of moths in other regions are so scattered and fragmentary that it seems impossible to draw any definite conclusions from them. In the list given under "Geographical distribution," the seasonal records are arranged by States. In the following list the same data are arranged by months, in order to show very incompletely the seasonal trend of the occurrence of the moths.

January	Texas.
February	Florida.
March	Texas, Florida, Arkansas.
April	Texas, Florida, South Carolina, Tennessee.
May	Texas, Mississippi, Tennessee, West Virginia, Illinois, New Jersey, Pennsylvania, New York, Massachusetts, Connecticut.
June	Mississippi, Arkansas, Tennessee, North Carolina, New Jersey, Illinois, Pennsylvania, New York, Massachusetts, Minnesota, South Dakota, North Dakota, Ontario.
July	Arkansas, Tennessee, North Carolina, Illinois, Pennsylvania, New Jersey, New York, Iowa, Minnesota, North Dakota, Ontario.
August	Tennessee, North Carolina, Kentucky, Illinois, Maryland, New Jersey, Pennsylvania, New York, New Hampshire, Massachusetts, Indiana, Iowa.
September	Tennessee, Illinois, Virginia, Connecticut, New Jersey, Iowa, Wisconsin.
October	Tennessee.

THE MOTH

The writer has never found the moths of this species really abundant. Usually they have been taken very sparingly, one or two at a time and very seldom as many as half a dozen in a day's collecting. They were seen most abundantly at Greenwood, Miss., on the night of June 22, 1915, when 34 were taken at electric street lights between 8 and 11 p. m. In the field the moths seem to prefer more or less open, weedy or waste ground, such as neglected strawberry beds or fallow fields, rather than grassy places.

When the moths are flushed during the day, they usually fly only a short distance and may be readily captured with a small vial, but toward dusk they are much more wary, and when disturbed frequently fly 50 feet or more before settling. They alight, apparently without preference, on any part of an object, leaf, grass stem, or very frequently on the bare ground. They seldom rearrange their position after alighting. In the field, the silvery stripe and the brassy shade of the forewing in fresh specimens make them easy to identify at a considerable distance. Around lights at night they can be distinguished from *Crambus teterrellus* Zincken, the only other species of equal size with which they are apt to be asso-

ciated, by their habit of lying closely parallel with the surface on which they are at rest, quite in contrast with the moths of *teterrellus*, which rest with their heads pressed closely to the surface and their bodies elevated at an angle of 25°.

The data on hand show that these moths are not especially prolific, at least compared with some of the other species of this genus. The average number of eggs laid by the 41 females of which the writer has records was 118. This includes moths taken in the field and confined in dry vials and in tin boxes with water and with honey. The largest number laid by a single individual was 533, and only 9 of the entire 41 laid more than 200 eggs. The moths evidently mate immediately after issuing from the pupa, for not one of those collected in the field, some of them very fresh, laid infertile eggs.

There is nothing to indicate that the adults of either sex ingest anything besides water. They do not seem in the least attracted to flowers or other possible food sources. One moth excitedly waved her antennæ when approached with a droplet of honey and when finally induced to taste it, rapidly sucked it up. An attempt was made to determine if food in the form of dilute honey had any effect on the length of life or the egg production. The following table summarizes the results and leads to the conclusion that food has no pronounced effect above that of plain water, on either longevity or fecundity, but that either water or honey appreciably prolongs the life and increases egg production above that of moths confined in dry vials. As the majority of these moths were taken in the field, the averages as given are rather below than above the normal.

TABLE I.—*Relation of feeding to longevity and fecundity of moths of Crambus praefectellus*

	Male.		Female.			
	Longevity.	Number averaged.	Longevity.	Number averaged.	Eggs produced.	Number averaged.
	Days.		*Days.*			
Water	9.07	15	8.9	8	138	8
Dilute honey	7.45	20	11.25	12	134	12
Dry	7.75	4	6.72	18	96	21

DESCRIPTION OF MOTH (FIG. 2)

Wing expanse, 18–25 millimeters. Head, palpi, and abdomen cinereous, the abdomen lighter. Thorax and forewings golden fuscous, the latter with a silvery white stripe bordered with a fine darker line and tapering toward each end, from base to near subterminal line, a tooth near middle of lower side, and a silvery white dash above the tip and often fused with it; from this dash a dark shade with a light costal triangle above it, a light patch below it, and crossed by the plumbeous subterminal line, runs to the apex of the wing. Costal margin wider than in *leachellus*, being more than one-half the width of the white stripe at the middle of the costa. Subterminal space with 5 blackish venular dashes. Fringes white or slightly tinged with ochreous. Hind wings white or slightly cream-colored, fringes white. (Rewritten from Fernald.) The male antennæ are plainly flattened, each segment bearing a wedge-shaped process, which, in the medium segments, is provided with 8 to 10 sensoria (Pl. 1, E). The female antennæ are filiform and are beautifully banded with narrow alternate rings of brown and white (Pl. 1, F).

GENITALIA.—Male: Tegumen (Pl. 1, D) with body very short, about one-third the length of the limbs, which are broad, nearly straight, and almost truncate at the tip. Uncus broad at base but quickly narrowing, slender, and of uniform width for the rest of its length, the distal third dorsad thickly set with short stout spines inclined cephalad, interspersed with a few sparse hairs; gnathos glabrous, its limbs widely separated at their tips but quickly narrowing to the slender body, which considerably exceeds the uncus. Harpes (Pl. 1, B) rather narrow at base, elongate and subfalcate in general shape; costa free except at base but much modified into a short chitinized process, incurved and truncate; cucullus lightly chitinized, strongly concave, widest just above the base and narrowing gradually to the rather obtusely rounded tip, very hairy within, with an especially thick tuft just above the base. Cucullus not sharply separated from the sacculus, which is subquadrate in general outline, with a thickened costal margin and on its disk near the ventral margin a stout, heavily chitinized finger-like spine. The vinculum is much reduced and is merely a band of lightly chitinized tissue connecting the bases of the harpes. Aedoeagus (Pl. 1, C) lightly chitinized, nearly cylindrical, rounded at the base and curved in the shape of an old-fashioned pistol; at the tip truncate and somewhat bell-shaped, the internal lining for half its length roughly tuberculate; just inside the tip is a very short, sharp, chitinized thorn-like cornutus, and about two-thirds toward the base another much larger, acute, oblique spine with a very long narrow base, its tip inclined toward the tip of the aedoeagus. Anellus a mere ventral membrane. Female: Anal plate (Pl. 1, G)

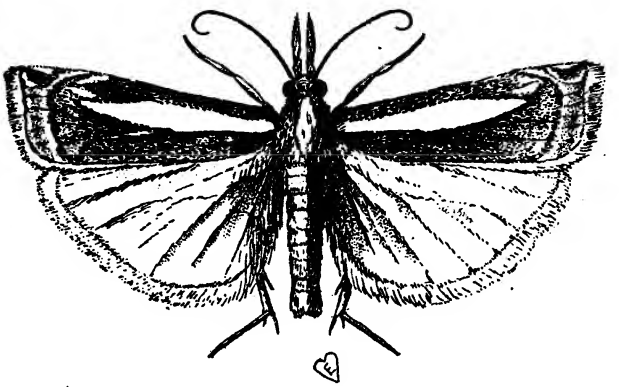

FIG. 2.—*Crambus praefectellus:* Adult. About three times natural size.

two-lobed, the dorsal lobe more feebly chitinized, about one-third the width of the lower, and separated from it by a deep notch, the margins of both lobes thickly set with stout setæ.

THE EGG

As is the case with all other species of this genus so far as known, the eggs are dropped promiscuously by the female during the early evening as she flies about or stands at rest. They are dry and drop down among the grass stems to effectual concealment in the débris beneath.

The incubation period has varied in the writer's experience from 15 days in March in Florida to 5 days in June and July in Tennessee, with all intermediate gradations. During the growing season from 5 to 9 days seems to be about the usual duration.

Almost snow white when first laid, the egg day by day becomes first pink, then flesh color, salmon, and, finally, on about the fourth day, a bright clear coral-red. They remain thus until about 24 hours before hatching, when the head and cervical plate of the contained larva begin to darken, giving the eggs a purplish hue. The larva escapes through a somewhat irregularly cut hole at one side of the larger end of the egg, leaving the empty shell nearly transparent and slightly iridescent.

The eggs of this species are somewhat rounder and with the small end a little more acute than the average for the genus. With this as with

several other species, it is found that the variations among the eggs of different individuals, especially in size, were greater than those existing between this and other species, thus rendering interspecific measurements of very little value. In fact, it holds true throughout the genus that the eggs of the various species are so similar as to render them practically indistinguishable.

To indicate the variation in size of the eggs of this species, the following measurements of two lots of 10 eggs each were made at different times:

TABLE II.—*Egg measurements of Crambus praefectellus*

Lot.	Length.			Width.		
	Maximum.	Minimum.	Average.	Maximum.	Minimum.	Average.
	Mm.	*Mm.*	*Mm.*	*Mm.*	*Mm.*	*Mm.*
1	0.5471	0.5118	0.5207	0.3353	0.3000	0.3106
2	.5736	.4633	.5018	.3442	.2692	.3149

The color is of interest because it has been found that the maximum color attained during the incubation period is very similar in the various subgeneric groups of closely related species, and differs between these groups from a pale straw-yellow to a deep coral-red. Thus the egg colors as well as the head colors of the newly hatched larvæ help to indicate the affinities of the various species.

The chorion is ornamented with acute longitudinal ribs, usually 21 in number, which become obsolete before reaching the poles. The polar areas are covered with scattered oval tubercles of variable size (Pl. 1, H). Between the ribs there are also less prominent cross carinæ, about 17 of these in the length of the egg. The egg is suboval in outline, one end slightly larger and more flattened than the other.

THE LARVA

The writer has never taken larvæ in the field, and the only notes on their normal behavior are those contained in Britton's account (*2*) of the attack on corn in Connecticut. Even here the conditions were not strictly normal, for the grass sod in which the larvæ were living was plowed under in the spring and the field planted to corn, forcing the larvæ onto the young corn plants as the only available food. The larvæ fed in the manner usual to most of the species under similar conditions, cutting a hole into the tender stalk below the ground level and living in a fragile tube of silk and earth particles attached to the stalk and leading off into the ground. Except when actually at work the larva does not remain in the stalk, but in this tube, so that when the plant is pulled the author of the injury, together with most of its domicile, is likely to be left behind in the earth.

In the cages used the larvæ were reared without especial difficulty. For the most part 1-ounce or 2-ounce tin salve boxes floored with damp blotting paper were used and the food was supplied in the form of short sections of the leaves of various grasses, usually blue grass (*Poa pratensis*). Under these conditions the progress through the instars was easily watched. It was not always easy, however, to be sure that a

molt had occurred. The only sure proof was to find the cast of the head, but in this species the first act of the newly molted larva was in most cases to eat the head cast, often leaving nothing but the mandibles as evidence that ecdysis had really occurred.

The first food of the larva consists of the fragment of eggshell consumed in effecting escape from the egg. This particle becomes bright pink or salmon color in the intestinal tract. As soon as it is free from the egg the tiny caterpillar is ready for green food. When placed on a blue-grass leaf it begins operations by cutting a narrow pit lengthwise of the leaf, at first avoiding the veins. This pit soon becomes large enough to contain the entire body of its maker and then a few threads of silk are spun across above it. The excrement is placed on or among these strands and in a few hours the larva is practically concealed from sight by this filthy roof. After the first day or two the larva eats the small veins as well as the tissue between them down to the lower epidermis of the leaf, but not until it reaches the third instar does it consume the entire leaf blade. By this time it has become too large effectually to conceal itself on a blue-grass leaf, and thenceforth seeks the earth, where, for protection, it constructs a tubular retreat of mingled silk and earth particles. From this vantage ground it comes out, usually at night, to cut off and consume one by one the blue-grass leaves.

As stated above, the larvæ of this species do not spend any time in a resting period, but pupate as soon as fully fed, weather conditions permitting. Of course during the cool weather of spring and fall, and in the winter, their activities are much retarded or cease altogether, but as soon as warmer temperatures prevail feeding is resumed and the transformations completed.

The following tables give figures showing the maximum, minimum, and average periods required for the various instars and for the complete life history.

TABLE III.—*Length in days of various stages and instars of Crambus praefectellus*

A. EGGS HATCHING MAY 13

	Eggs.	I.	II.	III.	IV.	V.	VI normal.	VI prepupal.	VII prepupal.	Pupa.
Maximum	9	5	5	4	6	6	6	15	8	12
Minimum	9	3	2	2	3	4	4	7	8	9
Average	9	3.46	3.83	3.00	3.92	5.20	5.00	9.30	8.00	9.87
Number of records averaged		26	29	27	27	25	2	19	1	15

B. EGGS HATCHING JUNE 25

Maximum	5	2	4	4	4	5	5	18	12	11
Minimum	5	2	3	3	3	2	5	11	12	10
Average	5	2	3.50	3.17	3.82	4.25	5.00	13.14	12	10.33
Number of records averaged		14	12	12	11	8	1	7	1	6

TABLE III.—*Length in days of various stages and instars of Crambus praefectellus*—Con.

C. MISCELLANEOUS RECORDS. EGGS HATCHING APRIL 21 TO AUGUST 8

	Eggs.	Larva.	Larva-pupa.	Pupa.	Entire developmental period.
Maximum	15	38	85	18	71
Minimum	5	27	33	9	41
Average	7.73	31.5	51	11.3	51.5
Number of records averaged	22 lots.	14	65	21	.....

In addition to those included in the tables, the writer has records of a few individuals hatching on September 18, which emerged as adults between January 23 and February 26 of the following year, after having been kept in the cool room of the insectary all winter.

It will be noted that most of these larvæ pass through six instars before pupation, but occasionally there is a seventh instar. The last instar before pupation is always much longer than those preceding, so that in order to make the averages correct these records are separated as normal and prepupal.

Through an oversight, detailed color descriptions of the various larval instars except the first were not made. The molted head casts were preserved, however, and the following list of characters is drawn up from them:

TABLE IV.—*Head width of larvæ of Crambus praefectellus*

Instar.	Minimum.	Maximum.	Average.	Number of heads measured.
	Mm.	*Mm.*	*Mm.*	*Mm.*
I	0.229	0.229	0.229	(a)
II	.335	.300	.325	8
III	.529	.459	.512	7
IV	.882	.759	.826	10
V	1.306	.933	1.219	15
VI	1.493	1.306	1.399	2

[a] Large number measured, but exact number not known.

In the first instar the head is shining black, paling somewhat but still fuscous in II. In III the ground color is still paler, with faintly darker areas indicating the later color pattern. In IV these markings have become much darker and more distinct, and the ground color of the head has become pale yellow. In the next two instars the colors remain the same, but become more intense. The color of the body of the larva through most of its growing period is a dull brown with a greenish tinge from the contents. The arrangement of the pinacula and setæ is shown in Plate 1, A.

THE PUPA

The cocoon is a little case about the size and shape of a peanut meat, lined and stiffened with gray silk inside and outwardly covered with particles of earth. It generally lies so near the surface that no "neck" or emergence tube is necessary.

The pupa itself is very similar to others of this genus. Bright yellow when first formed, it soon darkens to a mahogany brown. A day or two before emergence the silvery stripe in the forewing becomes plainly visible through the covering. The

pupa is 9.0 millimeters long and 2.0 millimeters wide. The caudal process is flattened into a broadly triangular plate with sharp margins, its acute tip bent slightly ventrad. Close to the tip of this plate below are two slender bristles with upturned ends, while above, more widely spaced and standing about halfway from the tip to the basal angles of the plate, are two shorter, smaller bristles with down-turned ends. Beneath, the process is flattened but not excavated.

The data as to the duration of the pupa stage are included in Table III. It varies somewhat, depending on the temperature, in the writer's records ranging from 9 to 18 days. The last, however, is very unusual, and the average of all the records puts it at 10.65 days, which is much more nearly correct. Ten days may be taken as the usual duration of this stage during the growing season.

SYSTEMATIC RELATIONSHIPS

Crambus praefectellus is closely similar to *C. leachellus* in structure as in wing markings. The male genitalia differ in that in the former species the cucullus of the harpe is narrower and somewhat more falcate, the free costal margin is shorter and more highly chitinized, and the cornutus of the aedoeagus is smaller. *C. unistriatellus* also undoubtedly belongs to this group. Its harpes and uncus are very similar, but the aedoeagus differs somewhat in its armament. Another species, as yet not definitely determined but externally very similar to *C. leachellus*, has genitalia which place it in this group, though it is certainly specifically distinct from any of the other members. *C. quinquareatus* (considered by Felt as *C. hastiferellus* Walk.) is placed in this group by Felt (*4, p. 85*), but for want of more certain synonymy its position is not discussed.

SUMMARY

Crambus praefectellus is an American species widely distributed throughout the eastern half of the United States. While not often injurious, it has shown itself capable of causing serious damage.

It breeds continuously throughout the growing season. The generations are not distinct, but rearing records indicate that three generations per year is the usual number.

The moths are not often abundant. They prefer waste or weedy land and are seldom found in clean grasslands. The larvæ are readily reared on grasses. Winter is passed by the partly grown or mature larvæ. The moths from the mature larvæ emerge early in the spring and are the first Crambus moths to appear.

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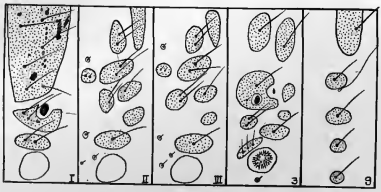
PLATE 1

Crambus praefectellus:

A.—Setal map of larva showing arrangement of pinacula and setæ on the three thoracic and third and ninth abdominal segments.
B.—Male genitalia: Harpes.
C.—Male genitalia: Aedoeagus.
D.—Male genitalia: Tegumen and uncus.
E.—Male antennal segment (twenty-fifth). Greatly enlarged.
F.—Female antennal segment (twenty-fifth). Greatly enlarged.
G.—Female genitalia: Valve.
H.—Polar area of egg. Greatly enlarged.



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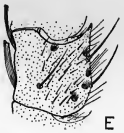

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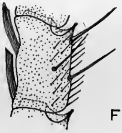

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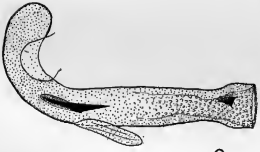

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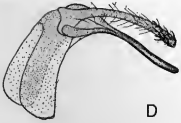

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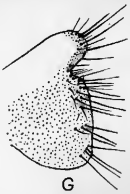

G

MOVEMENT OF SOIL MOISTURE FROM SMALL CAPILLARIES TO THE LARGE CAPILLARIES OF THE SOIL UPON FREEZING[1]

By GEORGE JOHN BOUYOUCOS

Research Professor in Soils, Michigan Agricultural Experiment Station

INTRODUCTION

In conducting various studies upon the temperature[2] and freezing-point lowering[3] of soils, many evidences have been obtained which indicate that the moisture of the soil upon freezing moves from the small capillaries, and probably also from around the particles as thick films, into the larger capillaries of the soil. This accumulation of the soil moisture in the large capillaries takes place especially when the moisture content of the soil is low. When the moisture content is high and under proper conditions the water accumulates as ice capillary columns at the top of the soil surfaces. These results are of considerable interest and importance as they bear on many soil-water relationships, such as the unfree water, available water, freezing-point lowering, vapor-pressure lowering, rate of evaporation, capillary movement, osmotic pressure, aud perhaps others.

EXPERIMENTAL EVIDENCES

In measuring the freezing-point lowering of soils at low moisture content, it was found that upon repeated freezing and thawing the lowering of the freezing point diminished greatly. But upon stirring the soil, even gently, the freezing-point lowering would go back to the original magnitude. If the soil was again subjected to alternate freezing and thawing, the freezing-point lowering would again diminish, and if it was stirred the lowering of the freezing point would become as before. This process could be continued almost indefinitely with practically the same results. Allowing the soil to stand after it was frozen and thawed several times tended to have somewhat the same effect as stirring, that is, it tended to restore the freezing-point depression to the original magnitude. In Table I the results in the case of two soils which will serve as typical examples of the phenomena in question are presented.

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[1] Accepted for publication Aug. 15, 1922.

[2] BOUYOUCOS, George J. SOIL TEMPERATURE. Mich. Agr. Exp. Sta. Tech. Bul. 26, 133 p. 1916.

[3] BOUYOUCOS, George J. and MCCOOL, M. M. FURTHER STUDIES ON THE FREEZING POINT LOWERING OF SOILS. Mich. Agr. Exp. Sta. Tech. Bul. 31, 51 p., 1 fig. 1916. Bibliographical footnotes.

TABLE 1.—*Effect of alternate freezing, thawing, stirring, and standing on the freezing-point depression of soils*

WISCONSIN SUPERIOR CLAY (20 GM. SOIL AND 4.5 CC. WATER).

Treatment.	Freezing-point depression.
	°C.
First freezing	1.115
Second freezing	.955
Third freezing	.645
Fourth freezing	.620
Stirred gently in tube with rod	1.215
Cooled at −10° C. after stirring	.680
Stirred gently in tube with rod	1.215
Cooled at −10° C. after stirring	.700
After standing one day in room temperature	.787
Cooled at −10° C.	.701

MICHIGAN SILT LOAM (20 GM. SOIL AND 3 CC. WATER).

First freezing	0.880
Cooled at −10° C.	.460
Stirred gently in tube with rod	.790
Kept at −10° for several hours after stirring	.360
Stirred gently in tube with rod	.785
Cooled at −10° C. after stirring	.420
Stood in room temperature after the last reading for 10 days	.600
Cooled at −10° C.	.360

BLACK CLAY LOAM (20 GM. SOIL AND 4 CC. WATER).

First freezing	0.760
Cooled at −10°.	.400
Cooled at −10° for several hours	.270
Stood two days at room temperature	.430
Cooled at −10°.	.322
Stirred gently in tube with rod	.760
Cooled at −10° after stirring	.450
Stirred gently in tube with rod	.765

An examination of the results indicated in Table I shows the great influence that different treatments have upon the freezing-point depression of soils at low moisture contents. In the case of the Wisconsin superior clay, for instance, the freezing-point lowering diminished by alternate freezing and thawing from 1.115° to 0.620° C. at the fourth freezing. Upon stirring the soil mass with a rod in the tube, the freezing-point depression increased from 0.620° to 1.215°. When the soil was kept at a temperature of −10° for few minutes the depression fell from 1.215° to 0.680°. As the soil was stirred again the depression rose from 0.680 to 1.215 as before. By leaving the soil to stand for one day at room temperature, after the depression was diminished by repeated freezing, the depression rose from 0.700° to 0.787°. It will be noted that in some cases stirring increases the freezing-point depression even to more than the original extent.

Results of the same type are also obtained on natural field soils. In Table II are shown the results with a silt loam taken from the field when the moisture content was moderately low and the soil had a crumb structure.

TABLE II.—*Effect of alternate freezing, thawing, stirring, and standing on the freezing-point depression of a field soil (silt loam)*

Treatment.	Freezing-point depression.
	° C.
First freezing	1.150
Second freezing	.870
Stirred gently in tube	1.230
Frozen once after stirring	.880
Cooled at −10° C. for several hours	.680

These results, obtained from the natural soil, agree perfectly with and confirm those from the artificially moistened soil. The question is, therefore, what factor is responsible for the great influence on the freezing-point depression of alternate freezing, thawing, stirring, and standing of the soils?

Before the effect of stirring upon the freezing-point depression was discovered, it was thought that the diminution of the lowering of the freezing point was due, at least partly, to the coagulation of the colloids upon freezing, and to the consequent liberation of unfree water from the colloids. The hypothesis [4] advanced was that the unfree water had a lower concentration than the free water, and upon its liberation it went to dilute the free water and thereby increased its freezing-point lowering. In view of the effect of stirring, however, this coagulation theory does not appear to afford the whole explanation for the phenomenon.

The most logical and plausible explanation that now presents itself is the assumption that, upon freezing, the moisture in the small capillaries and that surrounding the particles as thick films accumulates in the larger capillaries of the soil by the force of crystallization. In other words, the water in the larger capillaries, upon freezing, draws upon itself by the force of crystallization the water from the finer or smaller capillaries and films around the soil particles, and grows at their expense. Thus the water in the large capillaries affects the freezing-point depression differently from that in the small capillaries. How this is accomplished will be discussed later. Meanwhile, further evidence is here offered indicating that water moves from the small to the large capillaries upon freezing.

When a soil with low moisture content is frozen, small droplets or particles of ice are formed at different places in the soil mass. These ice particles or droplets occur in soils both under laboratory and field conditions and can be seen very readily and distinctly even with the naked eye. Upon close examination it is found that they occur mainly in the most porous places or in the largest capillary spaces between the soil particles.

However, when the soil is very moist and the freezing process is not too rapid, the moisture freezes at the surface of the soil in the form of

[4] BOUYOUCOS, George J. THE CONCENTRATION OF THE SOIL SOLUTION AROUND THE SOIL PARTICLES. *In* Soil Sci., v. 11, p. 131–138. 1921.

ice capillary columns, or long needle-like crystals. The force of crystallization seems to pull the water from below and bring it to the surface, where it freezes into these massive ice capillary columns or compact needle-like crystals. In Plate I a typical example of this phenomenon is shown. This picture was taken on a muck soil during the latter part of November, when the soil temperature below the surface was still considerably above the freezing point. The ice capillary columns would be formed at the surface of the soil without penetrating the lower depths, growing upward as straight needles or thin capillary tubes massed together. The growth seems to take place at the lower end and push the entire column upward, as the capillary tubes are elongated from below. The ice column shown in Plate I is about 4 inches thick, and was formed during three nights. The formation for each night is indicated by the lines or layers seen in the column.

As previously stated, the water which went to make this 4-inch column of ice came from the capillary water of the soil at a lower depth, and was brought to the surface by the pull or force of crystallization. From these results it is easily understood that it is possible for the moisture to move from the finer capillaries, and from around the particles as films, to the larger capillaries of a soil short of saturation. This phenomenon of the transference of moisture from the smaller to the larger capillaries upon freezing is somewhat analogous to another phenomenon—the tendency of small drops of liquid to unite into a single drop, which is accomplished either by actual contact or by the transference of vapor from the smaller to the larger drops.

In referring again to the effect on the freezing-point depression, another question arises: Why should the water in the large capillaries affect the freezing-point depression differently from that in the finer capillaries?

These differences can be easily explained if the hypothesis[5] previously advanced is correct. This hypothesis assumes that the solution immediately around the soil particles and in the very fine capillary spaces is less concentrated than the mass of the solution. This assumption which accords with the results presented in this paper, holds that the force of crystallization tends to draw the moisture from the finer capillaries and from around the particles as films into the larger capillaries. It is readily seen that during freezing and thawing the dilute solution from the finer capillaries and the films from around the particles go to dilute the solution in the larger capillaries or the mass of the solution. The consequence is that the original freezing-point depression is diminished. When the soil mass is stirred the moisture is again redistributed and readjusted and the freezing-point depression becomes as before.

If the hypothesis of the difference in concentration between solution in mass and that in the finer capillaries and around the soil particles is true, the above explanation is probably the correct one. But Parker [6] has published results to show that when the water is reduced to film or capillary form it has a decided physical effect upon the freezing-point depression. If his claim is true the explanation that immediately suggests itself is that the water in the larger capillaries has less physical

[5] BOUYOUCOS, George J. OP CIT.

[6] PARKER, F. W. METHODS OF STUDYING THE CONCENTRATION AND COMPOSITION OF THE SOIL SOLUTION. *In* Soil Sci., v. 12, p. 209–232. 1921. References, p. 231–232.

effect upon the freezing-point depression than that in the finer capillaries and around the particles as thin films.

It can not be definitely stated now which of these two explanations is correct. If the latter one is true the position or relative distribution of the capillary water as between the finer and larger capillaries affects not only the freezing-point depression but also such factors as osmotic pressure, vapor pressure lowering, rate of evaporation, available water, etc.

Since any treatment of the soil (stirring, breaking up the compound particles or crumbs, freezing and thawing, addition of flocculent or deflocculent agents) would alter the position of the capillary water as between the small and large capillaries and films, and would have a pronounced effect upon the above factors, their determination could not be absolute. Such determinations, for instance, as the vapor-pressure lowering of soils as reported recently by Thomas,[7] could not be considered absolute. If stirring, freezing, etc., affect the freezing-point depression, they will certainly also affect the vapor pressure lowering. The same would be true for several of the other factors.

SUMMARY

Evidence is presented which shows that when a soil short of saturation is frozen, the force of crystallization tends to draw the moisture from the small capillaries and from around the particles as thick films, into the larger capillaries.

However, when the soil is wet or saturated, under proper conditions the moisture freezes at the surface of the soil and forms capillary ice columns or thin needle-like crystals. The force of crystallization draws the water from below, which freezes at the lower end of the column and pushes the entire column upward.

The relative distribution of the capillary water is between the finer and the larger capillaries and may have a very appreciable effect upon such factors as freezing-point depression, vapor-pressure lowering, osmotic pressure, and rate of evaporation. Any treatment of the soil which will alter the relative distribution of the soil moisture as between the finer and larger capillaries would seem to affect these factors.

[7] THOMAS, Moyer D. AQUEOUS VAPOR PRESSURE OF SOILS. *In* Soil Sci., v. 11, p. 409-434, 5 fig. 1921. References, p. 433-434.

PLATE 1

Column of ice composed of thin capillary tubes or needlelike crystals formed at the surface of a wet muck. The force of crystallization brings the capillary water to the surface and, as it freezes into these massive capillary tubes, the whole column is pushed upward as the growth takes place at the lower end of the whole.

Movement of Soil Moisture upon Freezing

PLATE I

NUTRITIVE VALUE OF THE GEORGIA VELVET BEAN (STIZILOBIUM DEERINGIANUM)[1]

By J. W. READ, *Head of Department of Agricultural Chemistry*, and BARNETT SURE, *Associate Professor of Agricultural Chemistry, Arkansas Agricultural College*

This paper is the third (*6,5*)[2] of a series of investigations covering the nutritive value of the Georgia velvet bean as originally planned and outlined by the senior author. It discusses the supplementary relationship of whole and skimmed milk to the hulled seed and the whole plant, and of the leaf and the hulls to the seed. Subsequent papers will deal with the biological analysis of velvet bean meal (ground pods and beans), the dietary deficiencies of some practical rations including the velvet bean as a certain portion of the diet, and the biological evaluation of the whole plant. These investigations are in progress.

In the authors' first paper (*6*) it was shown that the seed of the Georgia velvet bean, unlike most seeds so far studied, has a great abundance of vitamine A, but is deficient in salts, in quality of protein, and in vitamine B; that the raw mature seed is toxic to rats, and that the autoclaved seed, when it is the sole source of food, is inadequate even for maintenance. It was also shown that a ration composed of 60 per cent cooked seed (tough seed coats excluded) and 40 per cent dextrin served for maintenance for eight weeks. During the first six weeks all of the animals were apparently in an excellent state of nutrition, but immediately following this period their coats became rough, with some loss of hair. It was evident that a dangerous point in the maintenance curve had been reached and the ration was changed by the addition of a liberal supply of whole milk. On the modified ration all of the animals made even better than normal growth and three generations were reared successfully. Since the authors' previous work indicated that the seed is rich in the A vitamine, experiments employing skimmed milk instead of whole milk and replacing dextrin by starch were also introduced.

Recently Mattill and Conklin published a paper (*2*) which showed that milk, even when given in the dried form to furnish enough of the solids and fortified with iron citrate, permits no rearing of the young, although it does promote considerable growth. On a ration composed of 99 parts dried milk and 1 part yeast they secured normal growth and partially successful reproduction. For this reason, these authors suggest the possibility of yeast supplying something unique in the ration. On the basis of the results secured on their various milk diet, they also express the opinion that milk may be both quantitatively and qualitatively inadequate for adolescent growth and reproduction, especially in the female, and that it may even contain substances inhibitory to growth in

[1] Accepted for publication Aug. 21, 1922. Published by courtesy of the American Chemical Society; paper read at New York meeting, September, 1921.

[2] Reference is made made by number (italic) to "Literature cited," p. 440.

the third or mature growth cycle. In following up this work, Mattill (*1*) has recently reported that dilution of whole milk powder with lard, starch, and salts in varying proportions did not prevent failure of adolescent growth and reproductive ability in female rats, If, however, a small amount of yeast was added to the milk rations the females cast litters regularly and repeatedly, but the young soon died.

Since the completion of our experiments, a paper by Sherman, Rouse, Allen, and Woods (*4*) has appeared. Using the rat as the experimental animal, they secured practically the normal rate of growth for both sexes on a mixture of equal weights of bread and milk in which white bread furnished four-fifths and milk only one-fifth of the total calories (or a corresponding mixture of dry bread or flour and whole milk powder), but reproduction failed on this simple diet. If ground whole wheat instead of white bread or patent flour furnished four-fifths of the calories in the above ration, young were successfully suckled, though at a considerable loss of weight on the part of the mother, grew to maturity at somewhat less than the average rate, and in several cases have produced and successfully suckled young of the third generation. When the proportion of milk in the diet constituted about two-fifths of the total calories of

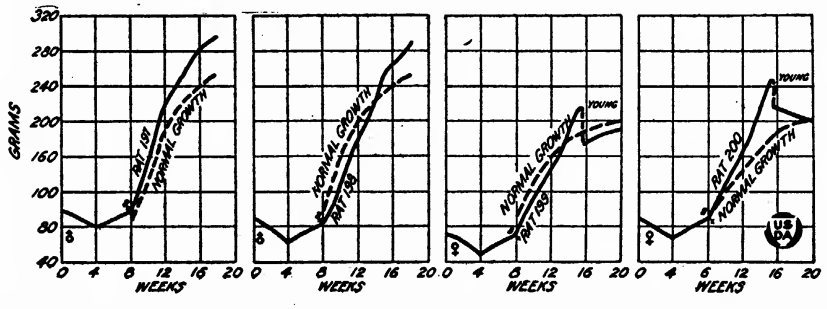

FIG. 1.—Velvet bean, 60 per cent; dextrin, 40 per cent. At point *a* a liberal supply of whole milk was added to the ration. Dotted lines represent normal curves of growth. Y=young.

the food mixture, the rest of which was ground whole wheat, the young were suckled without undue loss of the mother's weight, and these young have grown normally, as have also the young of the third generation. The inference to be drawn from their experiments is that wheat contains some substance or substances deficient in milk which are necessary for reproduction.

In the authors' experiments to determine the supplementary value of hulls (by which is meant the tough outer seed coat) to the seed, these hulls were dried and incorporated into the seed portion of the rations in the same amount as they form a natural part of the seed. The ground seed (hulls excluded) was autoclaved before feeding, but the leaf and hulls were added to the diets in their natural state.

EXPERIMENT I, LOT L.—(Charted in fig. 1.) This ration was started with 60 per cent cooked velvet bean seed (hulled) and 40 per cent dextrin. During the first six weeks of experimentation, the animals were all in a perfect maintenance condition, after which period they began to show characteristic signs of malnutrition, as evidenced by the roughness of their coat, lack of energy, loss of hair, etc. At point *a* a liberal supply of whole milk was added to the ration. From that period a little better than normal growth was obtained. Rat 200 gave birth to eight young

but was allowed only four to rear, in order not to overtax her mammary capacity. These four young were successfully reared to weaning age.

EXPERIMENT II, LOT L.—(Charted in fig. 2.) This experiment shows the successful normal growth of the young of rat 200 to maturity. These animals were permitted to interbreed, and it will be noted that rat 471 reared the third generation successfully. Rat 200 was then bred to one of her sons and successfully reared all the eight young which she brought forth at birth. (Not shown on fig. 2.) Our efforts to substitute the uncooked seed for the cooked met with failure, due evidently to the toxic principle in the raw seed.

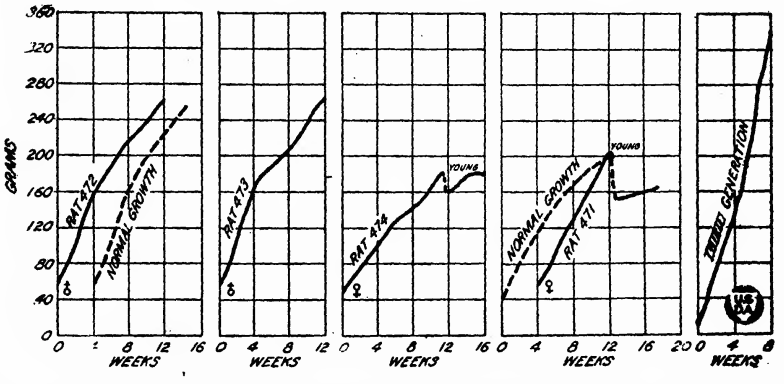

FIG. 2.—Second generation. Velvet bean, 60 per cent; dextrin, 40 per cent, plus a liberal supply of whole milk. Dotted lines represent normal curves of growth. Y=young.

EXPERIMENT III, LOT CXIII.—(Charted in fig. 3.) On a ration composed of 40 per cent velvet bean hay (finely ground whole plant), 60 per cent starch, and a liberal supply of skimmed milk, rat 454 successfully reared the four young to which she gave birth.

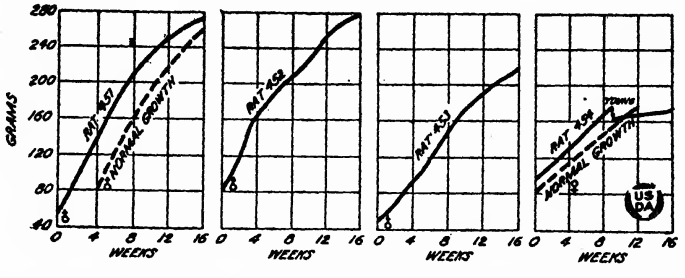

FIG. 3.—Velvet bean (whole plant), 40 per cent; starch, 60 per cent; and a liberal supply of skimmed milk Dotted lines represent normal curves of growth. Y=young.

EXPERIMENTS IV, V, LOT CXIII.—(Charted in fig. 4 and 5.) This experiment showed that quite satisfactory growth was secured through the second and third generations on this simple and monotonous ration of whole plant 40, starch 60, and a liberal supply of skimmed milk. We attribute the poorer showing of the animals on Figure 4 to hot weather and less consumption of milk due to rapid souring. The whole plant and starch mixture was readily eaten by all of our animals on this ration.

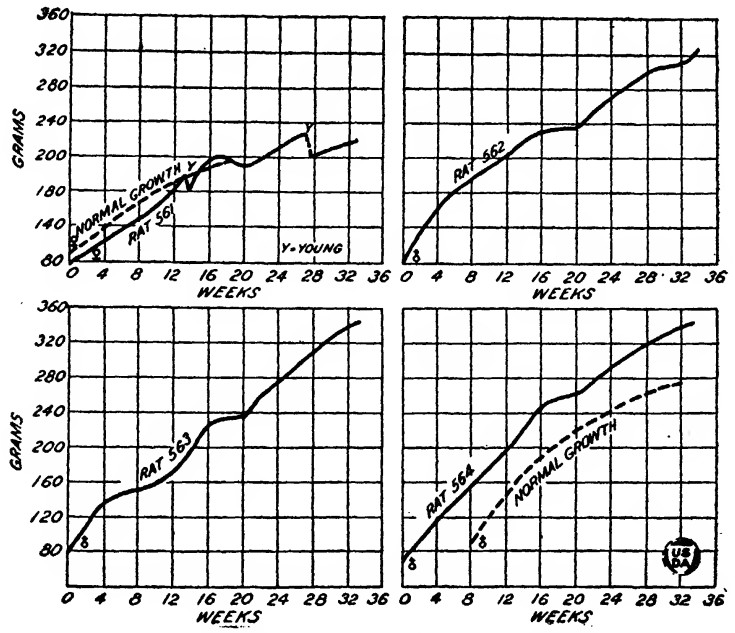

FIG. 4.—Second generation. Velvet bean (whole plant), 40 per cent; starch, 60 per cent, and a liberal supply of skimmed milk. Dotted lines represent normal curves of growth. Y=young.

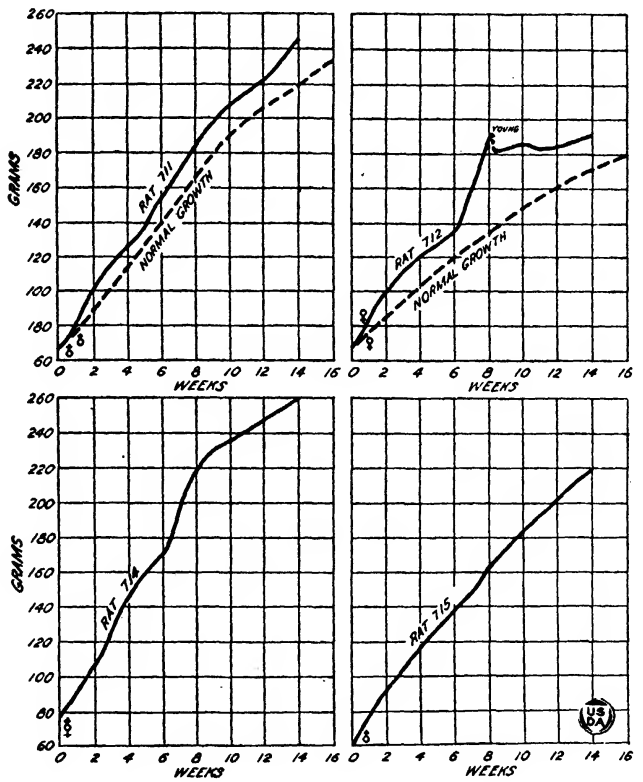

FIG. 5.—Third generation. Velvet bean (whole plant), 40 per cent; starch, 60 per cent, and a liberal supply of skimmed milk. Dotted lines represent normal curves of growth.

EXPERIMENT VI, LOT LXXVIII.—(Charted in fig. 6.) This experiment showed that the velvet-bean hulls offer no supplementing value to the deficient proteins in the seed, neither was there any appreciable change in the character of growth when, at point *a*, 21 per cent of dextrin was replaced by 21 per cent velvet-bean leaves.

EXPERIMENT VII, LOT LXXVI.—(Charted in fig. 7.) It is apparent from this experiment that the hulls in the velvet bean, unlike those in

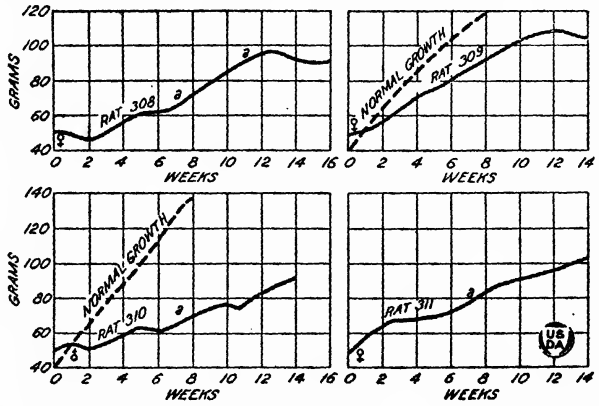

FIG. 6.—Velvet bean, 53 per cent; hulls, 7 per cent; salts (No. 32), 4 per cent; butter fat, 5 per cent; dextrin, 21 per cent. Dextrin carried alcoholic extract of 10 grams ether-extracted wheat embryo. At point *a* the 21 per cent dextrin was replaced by 21 per cent velvet bean leaves to furnish protein. Dotted lines represent normal curves of growth.

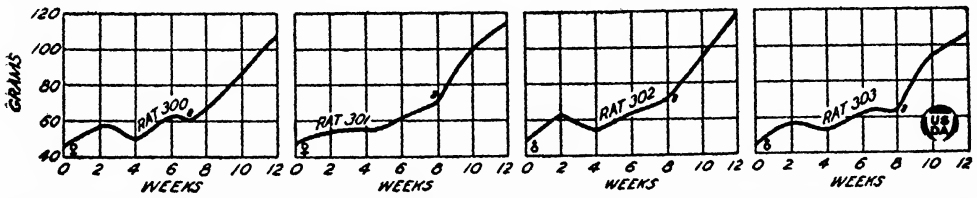

FIG. 7.—Velvet bean, 53 per cent; hulls, 7 per cent; salts (No. 32), 4 per cent; butter fat, 5 per cent; casein, 5 per cent; dextrin, 26 per cent. At point *a* the 26 per cent dextrin was substituted by 26 per cent velvet-bean leaves to furnish the water-soluble vitamine.

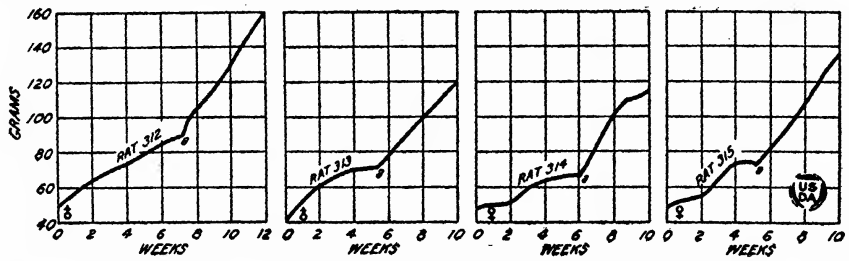

FIG. 8.—Velvet bean, 53 per cent; hulls, 7 per cent; butter fat, 5 per cent; casein, 5 per cent; dextrin, 30 per cent. At point *a* 10 per cent dextrin was replaced by 10 per cent velvet bean leaves to furnish salts. Dextrin carried alcoholic extract of 10 grams ether-extracted wheat embryo.

the rice kernel, are not carriers of the B vitamine. When, however, at point *a* 26 per cent dextrin was replaced by an equivalent amount of velvet-bean leaf a striking change in the character of growth was obtained in all cases.

EXPERIMENT VIII, LOT LXXIX.—(Charted in fig. 8.) The hulls offer very little supplementing value to the seed in so far as salts are concerned, but as low as a 10 per cent concentration of velvet-bean leaves furnishes a very satisfactory source of salts when added to 53 per cent of the seed.

EXPERIMENT IX, LOT LXVII.—(Charted in fig. 9.) This experiment showed that when the velvet-bean seed serves as the only source of the A vitamine fed at a 60 per cent level very good growth is secured.

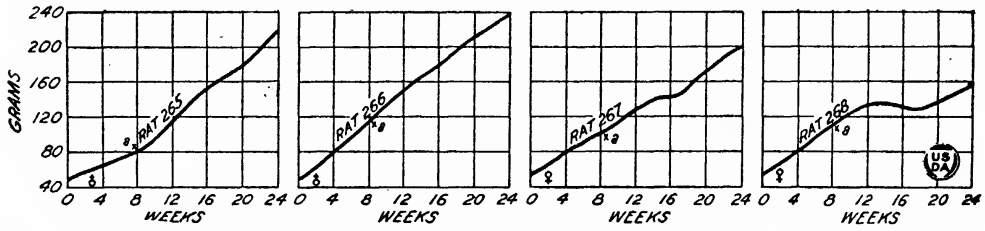

FIG. 9.—Velvet bean, 60 per cent; casein, 5 per cent; salts (No. 32), 4 per cent; dextrin, 31 per cent. Dextrin carried alcoholic extract of 10 grams ether-extracted embryo. At point *a* 4 per cent dextrin was replaced by 4 per cent additional casein.

EXPERIMENT X, LOT LXXX.—(Charted in fig. 10.) It is quite apparent from this experiment that considerable inferior growth is obtained when 7 per cent of hulls is added to a ration containing 60 per cent of the hulled seed, 7 per cent being the concentration of hulls in the whole seed.

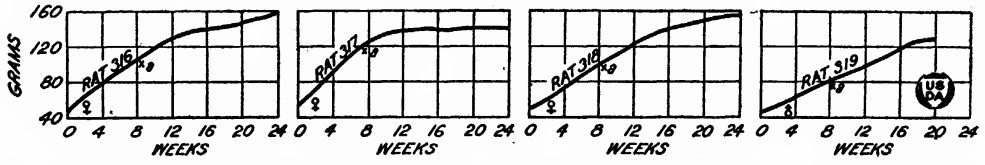

FIG. 10.—Velvet bean, 53 per cent; hulls, 7 per cent; casein, 5 per cent; salts (No. 32), 4 per cent; dextrin, 32 per cent. Dextrin carried alcoholic extract of 10 grams ether-extracted wheat embryo. At point *a* 4 per cent dextrin was replaced by 4 per cent additional casein.

EXPERIMENT XI, LOT CXXIX.—(Charted in fig. 11.) This experiment shows that autoclaving the hulls at 15 pounds pressure for two hours does not remove any apparent toxic substance, and gives additional evidence that the hulls interfere with the utilization of vitamine A.

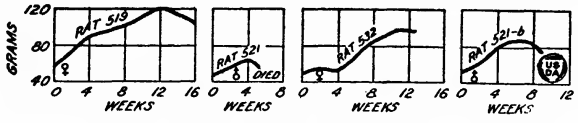

FIG. 11.—Velvet bean, 53 per cent; hulls, 7 per cent; casein, 9 per cent; salts (No. 32), 4 per cent; dextrin, 27 per cent. The hulls in this ration were autoclaved for two hours at 15 pounds pressure. Dextrin carried alcoholic extract of 10 grams ether-extracted wheat embryo.

DISCUSSION

The data for milk presented in this paper are in harmony with the results obtained by Mattill and Conklin (*2*), which show that milk alone, while it allows a certain amount of growth to take place, is inadequate for reproduction in the albino rat. The attempts made by the authors to furnish liquid milk and dextrin with a liberal supply of distilled water resulted in complete failure in most cases, and in only a few cases was some growth obtained. It is quite evident then that milk is lacking in one or more dietary essentials indispensable for satisfactory growth and for reproduction.

From a consideration of the various experiments on nutritive valu: of milk (reported in the literature) and in so far as Osborne and Mendel (*3*) have shown that the amino-acid requirements for growth and for maintenance are different, it seems to the authors that the difficulty may possibly be found with the milk proteins.

It has already been stated that Sherman, Rouse, Allen, and Woods (*4*) found that wheat in certain proportions supplemented milk to the extent of enabling mother rats to rear their young successfully, and on these diets they secured normal nutrition through three generations. The authors found in an extensive study of the nutritive value of the Georgia velvet bean that the velvet bean seed, a legume, supplements whole milk to the extent that three generations have been secured, all of the animals having performed even a little better than normal in their rate of growth. Even on such a simple and poorly constituted physical diet as that composed of 40 per cent velvet bean hay, 60 per cent starch, and a liberal supply of skimmed milk, three generations have been successfully produced by the authors.

The leaf of the Georgia velvet bean is an efficient carrier of salts and vitamine B. Portions of the leaf (10 and 26 per cent) added to 53 per cent of the seed improved the nature of growth considerably from the standpoint of both salts and the B vitamine, respectively. The hulls, however, seem to have no biological value. The authors' results show, however, that they interfere with the utilization of the A vitamine when added to a 60 per cent intake of the seed in the same proportion in which they occur in the seed. It might be argued that the inferior growth obtained by the addition of 7 per cent hulls could be due to a reduction of the total plane of intake from 60 to 53 per cent. However, such is not the case, because comparable growth was obtained when only 20 per cent of the seed, without the hulls, served as the only source of vitamine A.

The composition of velvet bean hulls, as reported by Tracy and Coe (*7, p. 31*), is as follows:

	Per cent
Ash	6.0
Crude protein	5.7
Fiber	30.0
Nitrogen-free extract	57.0
Fat	1.1

It will be noted from the above table that the hulls are very abundant in fiber, or indigestible celluloses. It seems reasonable, therefore, to suggest that the interference of the hulls with the utilization of vitamine A may be attributed to their indigestible celluloses. Autoclaving the hulls for two hours at 15 pounds pressure did not change the nature of their disturbing effect.

SUMMARY

(1) The velvet-bean seed (cooked) when fed at a level of 60 per cent together with 40 per cent dextrin, and velvet-bean hay (whole plant) when fed at a 40 per cent plane of intake together with 60 per cent starch, supplement milk in a manner satisfactory for growth and reproduction.

(2) The Georgia velvet-bean leaf is quite abundant in the B vitamine and contains salts of excellent biological value.

(3) The hulls have no supplementary value, and interfere with the utilization of the A vitamine in the seed. Autoclaving for two hours at 15 pounds pressure did not change their disturbing effect.

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CONTENTS

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SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEET POTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS [1]

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INTRODUCTION

It has been shown (*6*)[2] that the following species of Rhizopus may decay sweet potatoes: *Rhizopus nigricans*, Ehrenb., *R. reflexus* Bainier, *R. artocarpi* Racib., *R. delemar* (Boid) Wehmer and Hanzawa, *R. oryzae* Went. and Pr. Geerligs., *R. tritici* Saito, *R. nodosus* Namysl., *R. arrhizus* Fischer, and *R. maydis* Bruderl. Infection was accomplished by introducing 24- to 48-hour-old cultures grown on sweet potato decoction into "wells" (*5*) made in the sweet potatoes, the "wells" being sealed over with a cover slip set in vaseline. The potatoes were then placed in moist chambers and incubated at temperatures suitable for infection by the species under investigation. Whether or not the capacity on the part of these species to decay sweet potatoes by the above-mentioned method is an indication of the species involved in the decay of sweet potatoes in the storage house at the Government experimental farm at Arlington, Va., and at different temperatures in infection chambers, will be answered, in part at least, in this paper.

The writers [3] have found, by employing the "well" method of inoculation, that the temperature range at which the parasitic species will infect and decay sweet potatoes is nearly as wide as their temperature growth range in artificial cultures. By this method the species employed have an unusual opportunity to cause infection, even to the exclusion of any other species [4] that might be present in the "wells" or on the surface of the potatoes. It would be difficult for any other species present in the "well" to make any headway against the mass of mycelium of a 48-hour-old culture. What might take place under storage conditions may be a different question. Here the inoculum is limited, the species present are in competition with each other, and all the species may not be present. An effort was made, therefore, to determine whether or not the parasitic species infect sweet potatoes over their entire temperature growth ranges, where infection depends upon wounding and the species present on the potatoes.

Hanzawa (*2*) arranged the species studied by him into three groups according to their temperature relations. There was, as is shown by his

[1] Accepted for publication Aug. 25, 1922.
[2] Reference is made by number (italic) to "Literature cited," p. 456.
[3] This statement is based on unpublished data.
[4] There are nearly always some Rhizopus spores on the surface of sweet potatoes and, since the only precaution observed to eliminate them in these experiments was by washing the potatoes, there probably were occasional spores present.

data, considerable overlapping of the temperature range of one group onto that of another. If spores of all the species were present on the sweet potatoes, one might expect the species found within one of these groups to predominate to a greater or less degree in the amount of infection they provided, within the temperature ranges of the particular group, unless the number of spores of some of the species present that belonged to the other groups greatly exceeded that of the particular group under consideration. Within the limits of their temperature ranges one would expect to find some infection by all the species present. The results of the investigation in this field will be discussed later in detail.

APPARATUS

Much of the investigational work was carried out in a series of insulated infection chambers, the construction and dimensions of which are given in figure 1. Heat is supplied by means of a heating coil composed of

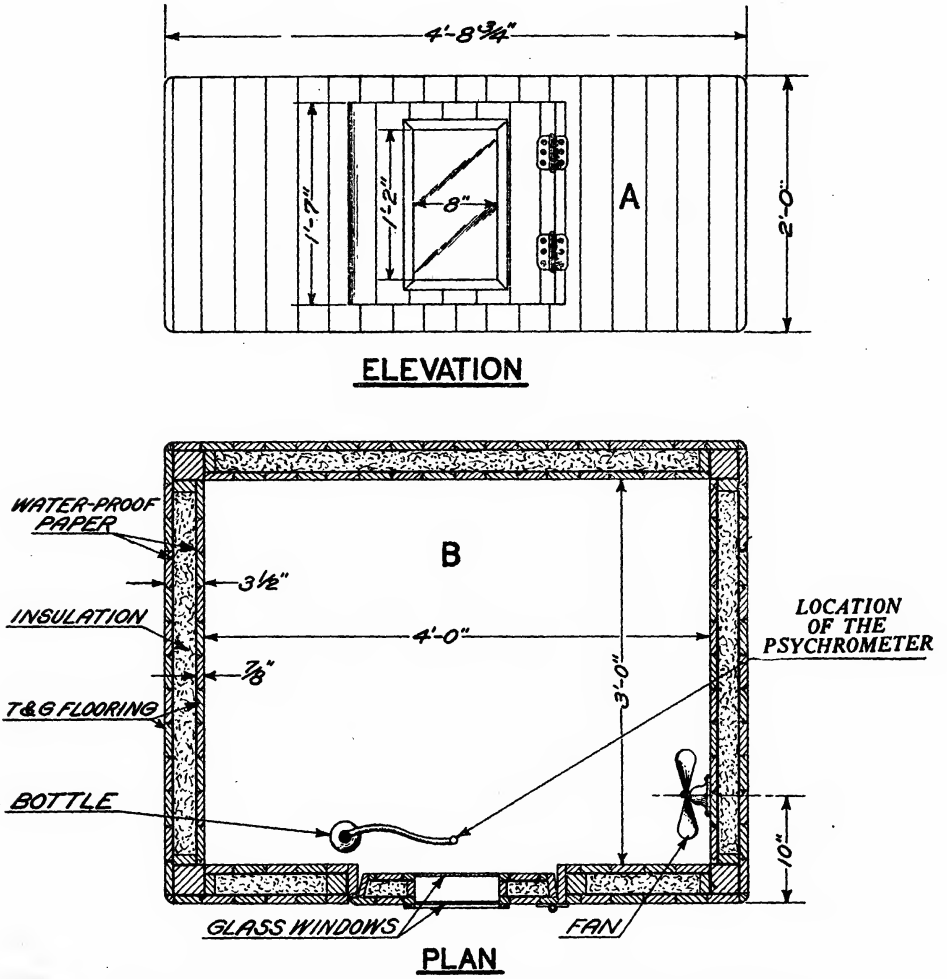

Fig. 1.—Infection chamber. A, front view; B, horizontal cross section.

No. 27 nichrome wire wound around ⅜-inch transite board 2 inches wide and 14 inches long. The floor and end wall immediately adjacent to the heating coils are insulated with transite board. Evaporating pans for the control of the humidity are located one-half inch above the heating coils. Each chamber is provided with standardized dry and wet bulb thermometers, the latter being covered with thin muslin kept wet by a capillary stream of distilled water, the excess of which is drained off by

means of a funnel into a pan located in the door of the chamber. A mercury thermoregulator, which is connected to the heating coils and a 250-ohm relay on a nearby switchboard, is suspended by a rubber band on the back wall. The air within the chamber is kept in motion by means of an 8-inch fan run by a motor located just outside the chamber. The thermoregulator is similar to that designed by Clark (*1*), but differs from it in that there are three arms of mercury instead of one and that one of the electric connections is made directly to one of these arms instead of through a special tube.

The storage house was constructed according to Government plans (*7*). It is provided with two stoves, one at each end, to furnish heat during the curing period, and with steam radiators, one flanking each corner, for the control of the temperature during the winter.

CONTROL OF ATMOSPHERIC ENVIRONMENT IN THE INFECTION CHAMBERS

TEMPERATURE

The thermoregulator used in the chambers controls the temperature within one-half of one degree. The fineness of the control will depend upon the size of the mercury column in the capillary tubing, the amount of mercury used, and its surface area.

The amount of current that passes through the heaters materially affects the control of temperature. Two amperes with a voltage of 110 is all that a 250-ohm relay will handle; in fact, sticking of the relay may be expected at times with this amount. If more current is desired a larger relay should be employed. An ampere and a half is the most that is used in any of the chambers.

To further reduce the danger of sticking, a condenser of 1½ microfarad capacity is employed in connection with each relay. With this type of equipment 20 chambers have been run for 9 months without a single case of sticking.

The capacity of the heaters should be limited to the amount of heat required to heat the chamber to within a few degrees above the desired temperature, otherwise there is a tendency for the temperature to fluctuate or lag.

The amount of current that flows through the thermoregulator should be reduced to a minimum consistent with efficient operation, otherwise the mercury and contact points become insulated and prevent efficient operation.

Direct current is preferable, particularly where mercury thermoregulators are employed. Current with a low number of cycles should not be used when avoidable, since the vibration set up by such a current does not contribute to effective control.

The contact points on the relays should be kept tight, in position, and clean; otherwise they become insulated by the finely divided metal thrown off, especially where a considerable amount of current is used, and thereby reduce the current below that actually required to maintain the desired temperature.

A uniform temperature throughout each chamber was obtained by the use of an 8-inch fan running at about 700 revolutions per minute.

The temperature of the air surrounding a chamber was below that of the chamber, and the desired temperature obtained by heating. The lower temperatures were obtained by placing the chambers in cold storage rooms where the temperature had been reduced by refrigeration.

HUMIDITY

A high relative humidity (about 95 per cent), maintained by means of distilled water in evaporation pans, was used throughout these experiments. The uniformity of the humidity throughout the chambers was maintained by the circulation of the air by means of a fan. In the absence of air circulation there would be, undoubtedly, a higher relative humidity in some parts of the chambers than in others, depending on the vapor pressure in the particular area.

GASES

In order to eliminate any possible effects of gases given off by the sweet potato or other vegetables, provision was made for a constant exchange of air, to be drawn through the chambers slowly by means of a vacuum pump. The amount of air drawn out was roughly determined by drawing it through wash bottles and regulated by stop-cocks.

MATERIALS

The Little Stem Jersey variety of sweet potatoes grown and stored at the Government experimental farm at Arlington, Va., was employed in these experiments. The potatoes were cured for a period of 10 days at temperatures from 25° to 30° C., and held as nearly as possible throughout the season at temperatures between 10° and 14°.

The following species of Rhizopus were employed: *R. nigricans*, *R. tritici*, *R. oryzæ*, *R. reflexus*, and *R. artocarpi*. They were grown on sweet potato agar at a temperature of 20° C.

EXPERIMENTAL DATA

Four types of experiments were used. The methods employed, the purpose of the experiments, and the data obtained will be discussed in connection with each type of experiment.

TYPE ONE

In the first type of experiments the potatoes were merely wounded and placed in the storage house at the Government experimental farm at Arlington, Va., and at different temperatures in the infection chambers. The potatoes were wounded by striking them three or four times on the blunt rim of a wire basket, after which they were placed in wire baskets 12 inches deep and 12 inches in diameter, which were in turn placed in the sweet potato storage house or in the infection chambers.

The purpose of these experiments was to determine what organisms cause decay where infection depends upon the organisms present on the potatoes. The scope of the experiments in this connection was limited to potatoes grown and stored at Washington, D. C. The number of species and the number of spores of each species present on the potatoes might be expected to vary with the locality in which the potatoes were grown, the conditions under which they were stored, and the conditions prevailing in the railway car during transit to the markets and in the markets themselves. The number of spores of a given species also might be expected to vary with the season of the year; in fact, it has been shown that there are fewer spores on the potatoes at digging time and

during the early part of the season than later in the year. There are, however, a sufficient number of Rhizopus spores present during the early part of the season to cause a large percentage of infection when the potatoes are wounded by the method mentioned above.

Fresh wounding contributes materially to infection by Rhizopus.[5] It has been found, as will be shown later in this paper, that fresh wounding is all that is required to permit of infection under the conditions of these experiments.

Table I contains the results of isolations from potatoes that were wounded as mentioned above, but not inoculated. Inspection of the table reveals that two species, *R. nigricans* and *R. tritici*,[6] are probably the species chiefly responsible for the decay of sweet potatoes. The absence of the other species would explain why there was no infection by them, but it is not known whether or not they were present. The presence of *R. tritici* and *R. nigricans* in greater quantity (either as to spores or mycelium) than the other species might explain these results. Later data will indicate to some degree how far this is the case with *R. nigricans*.

TABLE I.—*Isolations*[1] *from uninoculated wounded sweet potatoes held at different temperatures*

Temperatures.	Organisms isolated.	
	Rhizopus tritici.	Rhizopus nigricans.
°C.		
37	60	.....
32	62	3
28.5	8	3
27.5	30	5
26.5	21	19
25	.....	14
23	4	47
20	.....	17
19	.....	6
18	.....	19
15	.....	19
14	.....	78
12	.....	74
11	.....	4
10	.....	20
3-3	.....	4 M.,[2] 2 M. and R. n.

[1] The figures in the columns under the heading "Organisms isolated" represent the number of isolations of the particular organism at the various temperatures. They have no relation to the number of infections, which was always nearly 100 per cent, except between 20° and 30° C., where it was somewhat less. The figures in the succeeding tables under the above heading likewise refer only to the number of isolations.

[2] M.=Mucor. R. n.=Rhizopus nigricans.

Only *R. nigricans* was isolated at temperatures below 20° C. It will be seen, therefore, that *R. nigricans* is the species involved in the decay of sweet potatoes at the usual storage temperatures. The importance of *R. nigricans* on storage will be taken up more fully later.

[5] A paper on wounding as a factor in storage of sweet potatoes is in course of preparation.

[6] Although it is thought by the authors that *R. tritici* is the predominating species at the higher temperatures, it is impossible at present to be certain that *R. nodosus*, *R. oryzae*, and *R. delemar* are not responsible for decay in some instances, and even to the same degree as *R. tritici*. As far as the authors are concerned, there are no characters by which these four species can be definitely separated. *R. tritici*, therefore, will be employed as the name to designate this group of species.

It is interesting to note that *R. tritici* in these experiments did not cause decay of sweet potatoes over its entire temperature range of growth. (Range of growth and infection for *R. tritici* is about 5° to 44–45° C.) Infection by *R. tritici* did not take place by the method employed here, at temperatures at 20° C. and below notwithstanding the fact that it takes place readily where the "well" method of inoculation is used.

The temperature ranges of infection by *R. tritici* and *R. nigricans* overlap between 20° and 30° C. Infection by *R. nigricans* becomes progressively less with the increase in temperature above 20°. *R. tritici* shows a similar decrease in the amount of infection as the temperature passes from 30° to 20°. Infection by the two species becomes nearly equal at 26.5° (Table III).

Results in connection with these and other experiments show that a smaller percentage of the potatoes decay at temperatures between 20° and 30° C. than at higher or lower temperatures. Decay also will start in a large number of cases, then stop. Whether or not these temperatures are more favorable for the potatoes and less favorable for the pathogens, or both, is not clear, except that temperatures near 30° are less favorable for *R. nigricans*.

Table II shows the results of isolations made from wounded but uninoculated potatoes used in experiments in the storage house and in the infection chambers at temperatures at which sweet potatoes are usually stored. The top row of figures represents the average of three experiments, run at different times of the year in the storage house. Most of the isolations proved to be *R. nigricans*. However, in two out of the three experiments, *R. tritici* was isolated in nine instances. Records show that the temperatures were above 20° C. for a few hours during the early part of these experiments. These results indicate that *R. tritici* may be a factor in sweet-potato storage if the temperatures are high enough. *R. nigricans* is, however, the principal cause of decay in the storage house. The four lower rows of figures in Table II represent the results obtained from four experiments run in the infection chambers at temperatures (10° and 14°) corresponding closely to those used in storage. *R. nigricans* was invariably isolated.

TABLE II.—*Isolations from wounded uninoculated sweet potatoes held in the storage house and in the infection chambers at storage temperatures*

Temperatures.	Organisms isolated—Number of cultures isolated.		
	Rhizopus nigricans.	Rhizopus tritici.	Mixed cultures of Rhizopus tritici and Rhizopus nigricans.
°C.			
10–25[1]	140	9	3
14	78	0	0
12	74	0	0
11	4	0	0
10	3	0	0

[1] The figures 10–25 indicate that the temperature varied between 10 and 25°.

TYPE TWO

In the second type of experiments the sweet potatoes were wounded as above and dipped in a spore suspension made by introducing the spores of an equal number of cultures of *R. tritici* and *R. nigricans*, grown under identical conditions, into 10 gallons of water. From 8 to 10 cultures of each organism were usually employed.

It was discovered in the first type of experiments that *R. tritici* and *R. nigricans* were the species chiefly responsible for the softrot of sweet potatoes at Washington, D. C. *R. nigricans* was responsible for all the decay at temperatures below 20° C., quite to the exclusion of *R. tritici*, notwithstanding the fact that *R. tritici*, as shown by unpublished data, has the capacity of causing decay between 6° and 44°, where the "well" method of inoculation is used. Both *R. tritici* and *R. nigricans* decayed sweet potatoes between the temperatures of 20° and 30° and *R. tritici* above 30°. It is possible that the amount of infection by these species, as well as their temperature ranges, may have been influenced by the number of spores present on the potatoes. The number of spores present of both species, in turn, may have been influenced by local conditions and the conditions under which the potatoes were stored. The storage temperatures were nearly always below 20°, except during the curing period of from 10 days to 2 weeks. Since *R. nigricans* is essentially a low-temperature form, these temperatures are more favorable for its development than for *R. tritici*, which grows better at high temperature. The second series of experiments, therefore, were designed to determine whether or not the range of infection discovered in the first series would be altered in any way, especially the lower limit at which *R. tritici* caused infection, when the sweet potatoes were inoculated with approximately the same number of spores of each species.

Table III shows the results of 920 isolations made during two years in connection with 15 different experiments, most of which were designed to determine the distribution of decay by *R. tritici* and *R. nigricans* at different temperatures. In 12 of these experiments, wounded potatoes were inoculated with a spore suspension of *R. tritici* and *R. nigricans*. In the other 3 experiments the potatoes were wounded but not inoculated, and the data include all the isolations made from potatoes held at 14° C. and 74 out of the 81 isolated from potatoes held at 12°. Some of the experiments in which the isolations were made from potatoes held at low temperatures (7° and below), were designed to determine the lower limit at which these organisms would decay sweet potatoes. In the latter experiments the potatoes were wounded and inoculated as described in the first part of this paragraph. In these experiments the two species are distributed according to temperature, in much the same manner as shown in Table I. The upper and lower limits for infection by *R. tritici* are approximately 44° and 20°, respectively. The highest and lowest temperatures at which *R. nigricans* caused infection were 27° and 3.5°, respectively.

These results tend to indicate that the range of temperatures at which these species will infect sweet potatoes is so wide as to leave little possibility of adjusting temperatures in which to store the potatoes which will be outside the range of infection by these organisms. Although it has been found that these organisms do not cause any appreciable amount of decay at temperatures below 6° C. it was shown by Harter, Weimer, and Adams (5), and later verified by the authors, that if potatoes were

held for a few weeks below this temperature they almost invariably decayed with *Mucor racemosus* Fres. There is nearly always 100 per cent infection by *M. racemosus* under cold storage conditions and also in the infection chambers below 6°.

TABLE III.—*Isolations from wounded sweet potatoes inoculated with R. tritici and R. nigricans and held at different temperatures*

Temperatures.	Organisms isolated.					
	Rhizopus tritici.	Rhizopus nigricans.	Mucor.	Botrytis.	Bacteria.	Mixture of organisms.
°C.						
44	3				12	19 P. and[1] bact.
40	10					
39	15					
38	15					
35.4	10					
33.5	39					
32.7	10					
31.4	10					
30.8	33					
30	48					
29.5	25					
28.9	5					
27	35	4				
26	16	14				
23	34	23				2 R. n. and R. t.
20	2	48				1 R. n. and R. t.
19		27				1 R. n. and R. t.
15		21				
14		78				
12		81				
11		46				
10		14				
9		19				
7		10			1	20 M. and R. n.
6		29	24			31 M. and R. n.
5.5			5	22		
3.5		3	15			3 M. and R. n.
2.7			27			
2			10			

[1] P.= Penicillium; bact.= bacteria; R. n.= Rhizopus nigricans; R. t.= Rhizopus tritici; and M=Mucor.

If this method of wounding is employed, whether the potatoes are inoculated or not, the percentage of infection by *R. tritici* and *R. nigricans* is nearly always 100 at temperatures above 30° and below 20° C., respectively, the percentage being lower between these temperatures.

Sweet potatoes are sometimes injured by heat at 40° C. if the humidity is high, and badly injured at 44°, the highest temperature at which *R. tritici* caused infection.

TYPE THREE

The third type of experiments was designed to determine how effectively other species compete with *R. nigricans* in producing infection at the usual storage temperatures. The potatoes were wounded as in previous experiments, some having been previously washed in tap water to reduce if possible the number of spores present on their surfaces and

others were inoculated while in the condition in which they came from the storage house. Washed and unwashed potatoes, wounded in the same manner as those inoculated, were used as controls in all the experiments.

The following species were employed in these experiments: *R. nigricans*, *R. reflexus*, *R. artocarpi*, *R. tritici*, and *R. oryzae*. Washed and unwashed potatoes were inoculated with each of the species alone, except *R. nigricans*. Inoculation in each case was by dipping them into a spore suspension. Washed and unwashed potatoes were inoculated with a mixed spore suspension of *R. reflexus* and *R. nigricans*. Washed potatoes were inoculated with a mixed spore suspension of *R. artocarpi* and *R. nigricans*, and unwashed potatoes with *R. tritici* and *R. nigricans*.

In the preceding experiments *R. nigricans* was shown to be the principal organism causing decay at the usual storage temperatures. This species has also been shown to cause decay of many fruits and vegetables. (*4*) It was found in the second type of experiments that when the sweet potatoes were inoculated with *R. tritici* and *R. nigricans*, *nigricans* caused all the decay below 20° C., notwithstanding the fact that *R. tritici* is not only capable of infecting potatoes at these temperatures, but apparently [7] to the same degree where the "well" method of inoculation is employed.

In view of these facts it was thought desirable to make competitive tests between *R. nigricans* and some of the other species as to their capacity to infect sweet potatoes.

R. tritici was selected, first, because it is the species most commonly isolated from sweet potatoes next to *R. nigricans*, and, second, because with the exception of *R. nigricans* it is the species most commonly received in response to requests to other investigators for cultures of Rhizopus. It has been sent in, on a number of occasions, as *R. nigricans*.

R. oryzae was selected because Hanzawa (*2*) placed it in the high-temperature group (*R. tritici* falling into the intermediate and *R. nigricans* into the low-temperature group) with the idea of studying one organism of each thermal group.

R. reflexus was chosen because it belongs to the same thermal group as *R. nigricans*, its upper temperature limit being slightly higher than that of *R. nigricans* and its lower temperature limit slightly lower. Furthermore, it decays sweet potatoes (where the "well" method is used) fully as rapidly as *R. nigricans*.

R. artocarpi was likewise chosen because it belongs to the same thermal group as *R. nigricans* and is able to decay sweet potatoes over much the same temperature range, where the "well" method of inoculation is used.

The spore suspensions employed in these experiments were made by introducing spores from cultures of the respective species into battery jars containing water. In every case except that of *R. nigricans* the concentration was so great as to render the water almost black.

The reason for using such highly concentrated spore suspensions was to insure ample competition for *R. nigricans*, which, in the first and second series of experiments, successfully excluded *R. tritici* from infecting potatoes at temperatures below 20° C. and which in the course of these experiments proved to be able to compete against odds with the other species.

[7] It is not practical to directly compare the amount of decay caused by one species with that of another because of the indeterminable factors involved, but the indications are that the rate of decay by *R. tritici* is as rapid as that of *R. nigricans* at most temperatures below 20° C.

The spore suspensions of *R. nigricans* used in connection with some of the other species was not so highly concentrated, because it so happened that there was not a sufficient number of cultures of *R. nigricans* available.

Table IV shows the results of competitive tests of infection by *R. tritici* and *R. nigricans*, first when potatoes were washed and inoculated with *R. tritici*, second, when unwashed potatoes were inoculated with *R. tritici*, and third, when unwashed potatoes were inoculated with a spore suspension of *R. tritici* and *R. nigricans*. Controls of both washed and unwashed potatoes were also included.

R. nigricans alone was isolated from the unwashed and control potatoes and greatly predominated when either the washed potatoes were inoculated with *R. tritici* or unwashed potatoes were inoculated with a mixed spore suspension of *R. tritici* and *R. nigricans*. These results indicate that *R. tritici* can not compete with *R. nigricans* and is not much of a factor in the decay of sweet potatoes at these temperatures. The number of spores of *R. nigricans* on the potatoes as they were obtained from the storage house or when the potatoes were washed, was sufficient to cause nearly all the infection, even when the potatoes were inoculated with a highly concentrated spore suspension of *R. tritici*. Washing reduces infection by *R. nigricans* but slightly.

Table V shows that *R. artocarpi* can compete more successfully with *R. nigricans* than *R. tritici*. When *R. artocarpi* is used alone as the inoculum, the percentage of infection is fairly high, especially at 14° C. The washing of the potatoes seems to have no effect. When the inoculations were made with *R. nigricans* and *R. artocarpi* together, no infection by the latter organism took place. *R. nigricans* alone was isolated from the controls.

Although these results show that *R. artocarpi* may cause considerable decay if spore suspensions of high concentration are used, it seems probable that under storage and transit conditions the number of spores of *R. artocarpi* would rarely if ever be sufficient to be a factor in the decay of sweet potatoes, because *R. nigricans* seems to be universally present and would likely grow and produce spores as readily as *R. artocarpi* under most, if not all, conditions.

R. reflexus, as is shown in Table VI, was capable of causing considerable decay when it was used alone as the inoculum.

TABLE IV.—*Organisms isolated from sweet potatoes inoculated with Rhizopus tritici or with a mixed spore suspension of Rhizopus tritici and Rhizopus nigricans, or not inoculated and held as controls*

Temperature.	Inoculated with Rhizopus tritici.					Inoculated with Rhizopus tritici and Rhizopus nigricans (unwashed).		Control.	
	Washed.			Unwashed.				Washed.	Unwashed.
	Rhizopus tritici.	Rhizopus nigricans.	Rhizopus tritici and Rhizopus nigricans mixed.	Rhizopus tritici.	Rhizopus nigricans.	Rhizopus tritici.	Rhizopus nigricans.	Rhizopus nigricans.	Rhizopus nigricans.
°C.									
14.....	2	13	2	0	13	2	6	14	17
12.....	1	25	0	0	13	0	3	12	16

TABLE V.—*Organisms isolated from sweet potatoes inoculated with Rhizopus artocarpi or with a mixed spore suspension of Rhizopus artocarpi and Rhizopus nigricans, or not inoculated and held as controls*

Temperature.	Inoculated with Rhizopus artocarpi.						Inoculated with Rhizopus artocarpi and Rhizopus nigricans (potatoes washed).		Control.	
	Washed before inoculation.			Unwashed.					Washed.	Unwashed.
	Rhizopus artocarpi.	Rhizopus nigricans.	Rhizopus artocarpi and Rhizopus nigricans. (mixed).	Rhizopus artocarpi.	Rhizopus nigricans.	Rhizopus artocarpi and Rhizopus nigricans. mixed.	Rhizopus nigricans.	Rhizopus tritici and Rhizopus nigricans.	Rhizopus nigricans.	Rhizopus nigricans.
°C.										
14.....	11	11	3	16	9	2	13	2 R.t and R.n and 2 R.t.	16	16
12.....	9	16	1	7	26	.....	16	.....	15	17

TABLE VI.—*Organisms isolated from sweet potatoes inoculated with Rhizopus reflexus or with a mixed spore suspension of Rhizopus reflexus and Rhizopus nigricans, or not inoculated and held as controls.*

Temperature.	Inoculated with Rhizopus reflexus.						Inoculated with Rhizopus reflexus and Rhizopus nigricans.		Control.	
	Washed before inoculation.			Unwashed.			Washed.	Unwashed.	Washed.	Unwashed.
	Rhizopus reflexus.	Rhizopus nigricans.	Rhizopus reflexus and Rhizopus nigricans (mixed).	Rhizopus reflexus.	Rhizopus nigricans.	Rhizopus reflexus and Rhizopus nigricans mixed.	Rhizopus nigricans.	Rhizopus nigricans.	Rhizopus nigricans.	Rhizopus nigricans.
°C.										
14.....	12	17	0	10	16	2	22	15	14	17
12.....	17	14	2	16	15	1	8	15	12	30

This species caused no infection, however, when *R. nigricans* was included in the inoculum. Washing the potatoes did not influence the results. These results indicate that *R. reflexus* probably is not normally a factor in the decay of potatoes at storage temperatures.

The results recorded in Table VII show that *R. oryzae* [8] falls into the same group as *R. tritici* with regard to the amount of infection at these temperatures. The only infection that took place was when the potatoes were washed. This fact may have had no relation to washing and may have been accidental, for in the cases of *R. artocarpi* and *R. reflexus*, washing had no effect upon the amount of infection. The effects of

[8] It is assumed for two reasons that *R. oryzae* was the organism that caused the infection, first, because such a highly concentrated spore suspension was used in the inoculation of the potatoes, second, because *R. tritici* or the other species belonging to this group never have been known to cause infection when it was not used in the inoculum, except in the one instance shown in Table V in the case of *R. tritici*.

washing in connection with these experiments and those with *R. tritici* are so small as to fall within the limits of experimental error.

The results recorded in Tables IV, V, VI, and VII show: First, that *R. tritici* and *R. oryzae* can not compete with *R. nigricans* at temperatures of 12° and 14° C. in the infection of sweet potatoes, even where high concentrations of spores of these organisms are used in the absence of *R. nigricans* from the inoculum; second, although *R. artocapi* and *R. reflexus* are more successful than *R. tritici* and *R. oryzae* in competition with *R. nigricans*, they can not compete when the latter is included in the inoculum; third, *R. tritici*, *R. oryzae*, *R. artocarpi*, and *R. reflexus* do not infect sweet potatoes at these temperatures when infection depends upon the organisms present on the potatoes, while *R. nigricans* does.

TABLE VII.—*Organisms isolated from sweet potatoes inoculated with Rhizophus oryzae, or not inoculated and held as controls*

Temperature.	Number of cultures isolated.			Remarks.
	Potatoes inoculated with Rhizopus oryzae.			
	Washed before inoculation.		Unwashed.	
	Rhizopus nigricans.	Rhizopus oryzae and Rhizopus nigricans mixed.	Rhizopus nigricans.	
°C.				
14	14	2	13	Same control as in Table V.
12	13	.....	14	

When comparing the results from experiments with *R. tritici* and *R. oryzae* with those obtained with *R. artocarpi* and *R. reflexus* some reservations must be made because it is not known that the concentration of the spore suspensions are equal or comparable The differences in the amount of infections are so great that they would seem to be due to differences in capacity to infect, rather than to differences in concentration. The concentration was so great in every case that it would seem that a small variation in concentration would alter but little the amount of infection by the particular species used in the inoculum. In fact, very little infection occurred under any circumstances with *R. tritici* and *R. oryzae*. It is reasonable to expect that *R. reflexus* and *R. artocarpi*, especially the former, would cause more infection at these temperatures than *R. tritici* and *R. oryzae*, since the former are low, while the latter are high temperature forms.

It will be seen from Tables V and VI that it may be important in studies of resistance and susceptibility to take into consideration the temperatures at which comparisons are made. For instance, *R. artocarpi* is more successful in competition with *R. nigricans* at 14° than at 12° C., while *R. reflexus* is more successful at 12° than at 14°. These results are consistent with other temperature relations of these organisms, which show that the lower temperature limit for infection with *R. reflexus* is lower than for *R. artocarpi*.

In only one instance has *R. tritici* (Table V) been isolated from sweet potatoes at storage temperatures when the potatoes became infected, in the absence of this organism in the inoculum. It is to be expected that *R. tritici* would be responsible for a small percentage of infections,

since it can infect at these temperatures and probably is nearly always present on the potatoes. (Table I.)

The fact that *R. nigricans* was always obtained from the controls as well as inoculated potatoes in previous experiments when the temperature conditions were right indicates that this species is usually, if not always, present on the potatoes.

TYPE FOUR

In the fourth type of experiments the potatoes were wounded as in the preceding experiments, but inoculated differently. The first inoculation was made with a suspension of spores of *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarpi*, taken from two cultures of each species of the same age and grown under identical conditions, suspended in 3 liters of water; the second inoculation was with a supension of spores of *R. tritici*, *R. oryzae*, *R. reflexus*, *R. artocarpi*, and *R. nigricans*, two cultures of each of the first four species and one of *R. nigricans*. The same amount of water as in the first case was used in making the inoculum.

The object of these experiments was to determine which species would infect sweet potatoes when several species were in competition with each other at storage temperatures. The number of spores of each species present in the inoculum only approximated that of the other species, and in the case of *R. nigricans* was probably much less. Of course, as has been shown in previous experiments, *R. nigricans* is usually, if not always, present, but it is believed that, at least when the potatoes were not inoculated with *R. nigricans*, the number of spores of this species present per unit area was less than that of the other species, because the concentration of the spores in the inoculum of the other species was fairly high.

It did not seem practical at the time to attempt to use exactly equivalent concentrations because of the difficulties involved. The most serious difficulties were, first, to obtain an equal number of spores of each species, second, the number of spores may not be a measure of the germinating and infecting capacity of the spores, which in turn may vary with the species, and, latterly, some variation in the germinating and infecting capacity of the spores of the different species may be expected under different conditions, as, for example, at different temperatures, and yet a comparison between species must be made under identical conditions to be valid. This is shown to be the case by the fact that, although the temperature ranges of *R. reflexus* and *R. artocarpi* run almost parallel, *R. reflexus* is the more successful in competition with *R. nigricans* at 12° C., while at 14° *R. artocarpi* is the more successful (Tables V and VI). It is true that the temperature range of *R. reflexus* is wider than that of *R. artocarpi*, but its maximum is about the same number of degrees above that of *R. artocarpi* as its minimum is below. This difference in temperature ranges is, perhaps, sufficient to account for their difference in infective power. It probably would be difficult to find two fungi of identical temperature ranges. To make a quantitative comparison these factors must be taken into consideration.

It is believed, however, that the results from the methods employed in these experiments will show which species of Rhizopus are important in the decay of sweet potatoes.

Table VIII shows the number of isolations obtained from potatoes inoculated with *R. tritizi*, *R. oryzae*, *R. reflexus*, and *R. artocarpi*, and from potatoes inoculated with the same organisms plus *R. nigricans*, at the temperatures 14° and 18° C. It will be noted that at 18° *R. artocarpi*

was isolated from 17 potatoes as compared with *R. nigricans* from 4, when *R. nigricans* was not included in the inoculum. On the other hand, *R. artocarpi* was obtained from only 6 potatoes as compared with *R. nigricans* from 11 (and in 3 of these cases the isolations were a mixture of *R. artocarpi* and *R. nigricans*) when *R. nigricans* formed a part of the inoculum. At 14° quite a different relationship was found. When *R. nigricans* was excluded from the inoculum, *R. artocarpi* was isolated from 6 potatoes (4 of these isolations were a mixture of *R. artocarpi* and *R. nigricans*), *R. nigricans* from 29 and *R. reflexus* from 3. When *R. nigricans* was included in the inoculum, *R. artocarpi* was isolated once, a mixture of *R. artocarpi* and *R. nigricans* twice, and *R. nigricans* 33 times. These results show, first, that the percentage of infection by *R. artocarpi* was higher when *R. nigricans* was excluded from the inoculum, although at 14° the difference was small; second, that 18° was more favorable for infection by *R. artocarpi* than 14°. This was especially evident when *R. nigricans* was excluded, in which case the infection by *R. artocarpi* greatly exceeded that of *R. nigricans;* third, that a temperature of 14° was more favorable for *R. reflexus* than one of 18°; fourth, that *R. artocarpi* showed a slightly higher percentage of infection at 14° than *R. reflexus* (these results correspond to those recorded in Tables V and VI); fifth, that *R. tritici* and *R. oryzae* at these temperatures infect less readily than either *R. artocarpi* or *R. reflexus;* and sixth, that *R. nigricans* infects more readily than any of the other species. This is shown, even when infection by *R. nigricans* is compared with that of *R. artocarpi* at 18°, because although *R. artocarpi* caused the greater part of the decay when *R. nigricans* was excluded from the inoculum, the reverse was true when *R. nigricans* was included. This was true notwithstanding the fact that two cultures of *R. artocarpi* were used as compared with one of *R. nigricans*. Even assuming that spores of *R. nigricans* on the potatoes were equal in number to the *R. artocarpi* spores in one culture and the number of spores in the cultures of the two were equal, the margin is much in favor of *R. nigricans*. It is believed that there is a greater number of spores in a culture of *R. artocarpi* than in a culture of *R. nigricans*.

The percentage of infection by *R. artocarpi* and *R. reflexus* as compared with *R. nigricans* is not as high in these experiments as is shown in Tables V and VI at 14° C., but the concentration of the spores in the inoculum was not as high in the latter as in the former case.

TABLE VIII.—*Organisms isolated from wounded sweet potatoes inoculated with a mixed suspension of spores of a number of species of Rhizopus*

Organisms employed.	Temperature.	Number of isolations.	Organisms isolated.
	°C.		
Rhizopus tritici, oryzae, reflexus and artocarpi.....	18	21	17 R. artocarpi. 4 R. nigricans.
	14	38	2 R. artocarpi. 4 R. artocarpi and nigricans, mixed. 29 R. nigricans. 3 R. reflexus.
Rhizopus tritici, oryzae, reflexus, artocarpi and nigricans.....	18	17	3 R. artocarpi. 3 R. artocarpi and nigricans, mixed. 11 R. nigricans.
	14	36	1 R. artocarpi. 2 R. artocarpi and nigricans, mixed. 33 R. nigricans.

DISCUSSION OF RESULTS

R. tritici and *R. nigricans* are the two species chiefly responsible for the decay of sweet potatoes, known as softrot. The former is responsible for decay at the higher and the latter at the lower temperature, while the two overlap between 20° and 30° C. Although other species are capable of causing softrot, they do not seem to do so under the storage conditions at Washington, D. C., and in the infection chambers at the different temperatures. *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarpi* cannot compete successfully with *R. nigricans* at temperatures of 12° and 14° when sweet potatoes are inoculated with any one of these organisms along with *R. nigricans*. Even though high concentrations of spores of *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarpi* are used alone, *R. nigricans* nearly always causes more decay than any of them, and *R. tritici* and *R. oryzae* cause very little under such conditions.

When a mixed spore suspension of *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarpi* is used in inoculating the potatoes held at temperatures of 14° and 18° C., *R. artocarpi*, *R. reflexus*, and *R. nigricans*, with *R. nigricans* greatly predominating, were isolated at 14°, and *R. artocarpi* and *R. nigricans* at 18°, *R. artocarpi* greatly predominating. When *R. nigricans* was added to the inoculum just mentioned, *R. nigricans* and *R. artocarpi* were the only species isolated, with the former greatly predominating.

These results show that *R. nigricans*, except in the case of *R. artocarpi* at 18° C. (when *R. nigricans* was not included in the inoculum), was the chief agent of decay, even in the presence of the highly concentrated spore suspension of the other species used in these experiments.

Why *R. nigricans* should cause so much decay when such high concentrations of spores of the other species were employed, especially in the cases of *R. reflexus* and *R. artocarpi*, cannot be explained now. This seems rather strange when it is considered that *R. reflexus*, in particular, seems to decay sweet potatoes fully as rapidly as *R. nigricans* when the "well" method of inoculating is employed at the same temperatures. The two cases are hardly comparable, however, since in the present experiments we are dealing with the percentage of infection, while in the case cited we have to do with the rapidity of decay after infection has occurred. When the "well" method is used there is nearly always 100 per cent infection, irrespective of the species employed. This method is not at all comparable, however, to the one employed in these experiments.

When these results are compared with those obtained by Harter, Weimer, and Lauritzen (*6*), who showed that several species of Rhizopus have the capacity to decay sweet potatoes, one should take into consideration the methods employed in the two cases. In the former case the particular species involved in infection was not only present as a concentrated mass of active mycelium, but had the additional advantage of having access to the available food still remaining in the decoction. The species concerned also had access to the food rendered available soon after inoculation by the action of the pectinase, present in the decoction, upon the middle lamellae (*3*). In the latter case, infection depended either upon the spores present on the potatoes as they were removed from storage, or upon the spores introduced as a suspension in water. Where the potatoes were not inoculated, the species present may have been limited to those (*R. tritici* and *R. nigricans*) isolated in the first type of experiments. If other species were present, they were

unsuccessful in competition with these two species. When the potatoes were inoculated as above, the competition between the species present, whether introduced or occurring normally, was more nearly equal than it was between the species present on the potatoes as they came from storage and the inoculum used in connection with the "well" method.

It should be realized from a comparison of the results obtained by the two methods, that because a fungus produces a disease under one set of experimental conditions, it does not necessarily do so under another. The factors involved in determining infection under experimental conditions may vary greatly from those under normal conditions, depending upon, first, whether or not the experiments are designed to approximate normal conditions, and, second, whether or not the circumstances and our knowledge enable us to recognize and control the factors involved.

There seems to be some unknown factor or factors, either as a part of the capacity to decay, or associated with it, on the part of *R. nigricans* that enables it under normal conditions or the conditions of these experiments to infect sweet potatoes to the exclusion of the other species.

SUMMARY

(1) *Rhizopus nigricans* and *R. tritici* are the species primarily responsible for the decay of sweet potatoes known as softrot, *R. nigricans* at temperatures between 6° and 20° C., and *R. tritici* at 30° and above, the two overlapping between 20° and 30°.

(2) The temperature range of infection by *R. tritici*, *R. nigricans*, and *Mucor* is so wide as to exclude the possibility of storing sweet potatoes beyond the limits of this range.

(3) *R. tritici*, *R. oryzae*, *R. reflexus*, and *R. artocarpi* can not compete successfully with *R. nigricans* at the temperatures of 12°, 14°, and 18° C.

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THE INHERITANCE OF GROWTH HABIT AND RESISTANCE TO STEM RUST IN A CROSS BETWEEN TWO VARIETIES OF COMMON WHEAT [1]

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INTRODUCTION

There are three general methods by which the tremendous destruction of wheat by black stem rust may be reduced. These are the eradication of the common barberry (*13*),[3] the growing of rust-resistant varieties (*1*, *10*), and the use of improved methods of field culture (*14*). The losses have been especially severe in the hard red spring wheat area. No hard red spring wheat of high quality and yield, which is generally resistant to stem rust in the hard spring wheat belt, is now grown on a large acreage. Kota, apparently, is highly resistant to stem rust in those districts in which it has been grown, but on account of weak straw it is likely to lodge in the more humid sections. Therefore, the importance of developing other rust-resistant hard red spring wheats is quite apparent.

The production of varieties of wheat resistant to stem rust is a complex problem. Until recently it was supposed that only one form of stem rust caused the epidemics on wheat, although some of the early workers believed that the parasitic capabilities of the rust were easily modified (*2*). However, Stakman and Piemeisel (*17*), followed by others (*8*, *9*, *15*) have shown that *Puccinia graminis tritici* Erikss. and Henn. in reality consists of many biologic forms which differ in their pathogenicity for certain varieties of wheat.

This discovery explains why the same variety of wheat may be resistant when grown in one locality and susceptible when grown in another, or why a variety may be resistant in the same locality in one year and susceptible in the next. It is obvious that if a wheat is to be resistant in the field it must be resistant to all of the biologic forms present in the locality in which it is to be grown.

Both Kanred and Marquis are good milling and high yielding wheats. Kanred, however, is practically immune from several biologic forms of stem rust to which Marquis is susceptible. The purpose of this paper is to present data regarding the mode of inheritance of growth habit (winter versus spring habit) and resistance to stem rust in a cross between Kanred and Marquis wheats.

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[1] Accepted for publication Aug. 21, 1922.

[2] This work on the development of rust-resistant varieties of wheat was under the direction of Dr. H. K. Hayes and Dr. E. C. Stakman. The cross was made in 1918 by Carl Kurtzweil and others. The seed of the first generation was furnished by Doctor Hayes. The biologic forms of rust were supplied by Doctor Stakman and Mr. M. N. Levine.

[3] Reference is made by number (italic) to "Literature cited," pp. 468–469.

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Hayes, Parker, and Kurtzweil (7) recently studied the inheritance of resistance and susceptibility, to what apparently was a single biologic form, in crosses between common and durum wheats and common wheat and emmer. They showed that the inheritance of resistance and susceptibility to stem rust was not the same in different crosses. In the durum-common cross, susceptibility appeared to be dominant while in the emmer-common cross the F_1 was resistant, but not as resistant as the emmer parent. There was some linkage in transmission between the emmer and durum types and resistance to stem rust. Resistant emmer and durum types were very common, while it was quite difficult to obtain resistant common wheats.

Puttick (*11*) reported the results of a study on the reaction of the F_2 plants of a cross between two varieties of wheat which react reciprocally to two biologic forms of stem rust. Many gradations in reaction to both forms of rust appeared, varying from complete susceptibility to complete immunity.

Extensive rust surveys have been made during the last few years. The prevalence, distribution, and virulence of the various biologic forms of *Puccinia graminis tritici* are now being ascertained and, for all practical purposes, the parasitic effect on the hosts seems to be constant (*16*). The plant breeder now has a definite basic foundation for the development of varieties resistant to stem rust.

Twenty-one biologic forms [4] of rust have been found in the upper half of the Mississippi Valley. As the winter wheat, Kanred, is resistant to 2 of these forms and immune from 11, the value of this variety in breeding a rust-resistant spring wheat is apparent. If there could be isolated from the progeny of a Kanred-Marquis cross, a spring wheat which would be resistant to some or all of the forms to which the winter parent is resistant, one step would be accomplished of the many needed to produce a generally resistant variety.

Kanred is a true winter wheat which, when sown in the spring, at University Farm, St. Paul, Minn., produces only an occasional head late in the season and does not set seed. Marquis is a true spring wheat, which, when sown in the fall, seldom, if ever, lives through the winter. Growth habit, as used in this paper, is meant to indicate that general difference which exists between true spring and true winter varieties in their ability to produce heads normally when sown in the spring of the year (Pl. 1, A).

Apparently few investigations have been made on the inheritance of growth habit. Innumerable observations have been reported in literature on the differences in heading period and maturity between varieties in both spring and winter groups. These differences, while they may be of the same general nature as winter habit, are comparatively very minute, but they are constant, as was shown by Fruwirth (*3*) in 1918.

A single head of wheat was selected and divided into two parts. One-half of this seed and its progeny was constantly sown in the fall and the other half in the spring. This process was continued for 8 years. The two lots of seed were then sown together in both the fall and spring and the growth habits compared. The period of blossoming and ripening was the same for all plots, showing that selection within a pure line was of no value.

[4] Unpublished data furnished by E. C. Stakman and M. N. Levine.

The nature of the processes involved in bringing about the heading period has been a matter of speculation for some time. The climatic units which control these processes and permit winter grains to produce seed are finely interwoven. Although temperature and moisture are very important factors in the growth and maturing of plants, it is quite evident from the plant life about us that the time of flowering and fruiting of most plants is definitely connected in some way with the advance of the season. Garner and Allard (*6*) have clearly demonstrated this third factor to be the change in length of day and night. They found that certain plants which ordinarily require a short day for flowering and fruiting could be induced to flower and fruit in the middle of the summer by shortening the length of day to that which was normal for the regular flowering season. This was done by placing the plants in a dark-house for a certain number of hours each day.

In contrast to this group of plants which require a short day for flowering and fruiting, is that group of plants which require a long day of light. These plants flower regularly in the late spring or early summer. Garner and Allard place our small grains in this group.

As regards the inheritance of the growth-habit character, the results reported by previous workers do not appear at first to be in full agreement. Spillman (*12*) reported in 1909, that the winter character was dominant over the spring character in a cross between a winter common wheat and a spring club wheat. Fruwirth (*4, p. 176*) in 1910, cites Tschermak as having reported that the winter type was dominant over the spring type. When sown in the fall, the first generation of hybrids wintered over somewhat better than the true winter forms; but when sown in the spring, they remained dormant through nearly the whole summer. Single shoots appeared and began to blossom, but they produced no seed.

Gaines (*5, p. 42–45*) in 1917, reported that he obtained a segregation of spring and winter types from a cross between 2 spring varieties of barley, Rice and Beardless. The F_1 when sown in the spring, headed normally. In the F_2 there was a ratio of 3 winter plants to 13 spring plants. The plants in the third generation bred fairly close to expected ratios. He found that seasonal variations influenced the heading periods and consequently the ratios. The segregation in the F_2, however, indicates a dominance of the spring type over the winter type.

A complete and detailed study of the genetic nature of growth habit in wheat varieties has been made recently by Vavilov and Kouznetzov (*18*). They crossed a common winter wheat with a club spring wheat and found a clear dominance of the spring character over the winter character. There was a complicated segregation in the F_2, and some of the segregates (including many intermediates) were homozygous in the F_3. Of the 552 F_2 plants, 500 were early or late spring plants and 52 were typical winter plants. The results obtained by the writer on the inheritance of spring and winter habit are quite in accord with those of Vavilov and Kouznetzov.

MATERIALS AND EXPERIMENTAL METHODS

Marquis, a hard red spring common wheat of high quality, was crossed with Kanred in the summer of 1918. The latter, which is immune from several different biologic forms of stem rust, is a high yielding selection from Crimean hard red winter wheat.

The crossed seed was sown in the fall of the same year and produced 80 plants, 2 of which were winterkilled and 5 of which were not crosses. The remaining plants were harvested individually and the seed sown in the spring of 1920.

In the second generation a population of approximately 5,000 plants was grown. In order to facilitate observations the seeds were sown at intervals of 3 inches, in rows 1 foot apart. The date of emergence of the first head on each plant was noted. The hybrids formed a continuous series for date of head emergence, beginning with those which came out at the same time as the spring parent, to those which did not emerge at all. In this way they resembled the winter parent. (Pl. 1, B.)

The time of heading was divided into weekly periods. One week from the day on which the first plant headed, tags were placed on plants on which one or more heads had emerged. These comprised the first class. All of the Marquis control plants headed during the same period as did those plants included in the first class. One week later, tags were attached to all plants which had headed since those of class 1. These constituted the second class. This process was continued for 8 weeks, after which period no more plants headed. The plants which did not head were classed as true winter types. The winter parent controls failed to head, thereby falling into the same class as the winter hybrids.

From the first 7 heading classes in the F_2, 65 families were grown in the F_3. The plants from the seventh class produced only a few seeds, while those of the eighth class headed so late that no seed was produced at all. In sowing, the seed was again spaced as in the second generation so that a study could be made of the individual plants.

Several of the families were uniform for heading period in the F_3. In these cases the entire plot was given a general heading date, as in a varietal test. Others of the families were heterozygous for date of head emergence. In those plots a final count was made at harvest time of the number of plants which failed to head and the number which produced heads.

The rust studies were made on plants growing in the field under an artificial epidemic produced with several different biologic forms of stem rust and also on inoculated seedlings in the greenhouse.

In the second generation all the plants were grown in the field under an artificial epidemic produced by spraying the plants with a suspension of urediniospores of several different biologic forms. Both parents were susceptible to some of the rust forms which were used. All of the hybrid material was as susceptible as either parent in this epidemic and therefore a detailed genetic study of the inheritance of rust resistance under field conditions could not be made. Studies on the inheritance of resistance and susceptibility were made in the greenhouse by inoculating the F_3 seedlings with cultures of urediniospores of known biologic forms.

The seedlings in the greenhouse were grown in 4-inch pots and inoculated when they were 1½ to 2 inches tall. After inoculation they were placed in a glass-topped chamber and incubated 48 hours. The notes on infection were taken 12 to 14 days after the date of inoculation. Some of the plants were completely susceptible and the others were immune since there were no intermediate types of infection. The uredinia were large, coalescing, and normal in every respect on the seedlings both of Marquis and of susceptible hybrids, while the seedlings of Kanred and

of the resistant hybrids were immune. There were, therefore, only two classes, i. e., immune and susceptible.

An intensive study was made by testing a large number of F_3 plants with a single known biologic form to which the Marquis parent is susceptible and the Kanred parent is resistant. Two questions then naturally arise: How is the reaction tendency of the host to all of these biologic forms inherited? Is the reaction due to the presence of a single genetic factor, or, if several factors are concerned, are they linked in the process of segregation? An attempt was made to solve these questions by inoculating various F_3 selections, which were homozygous in their reaction to the first form studied, with 12 other biologic forms of stem rust.

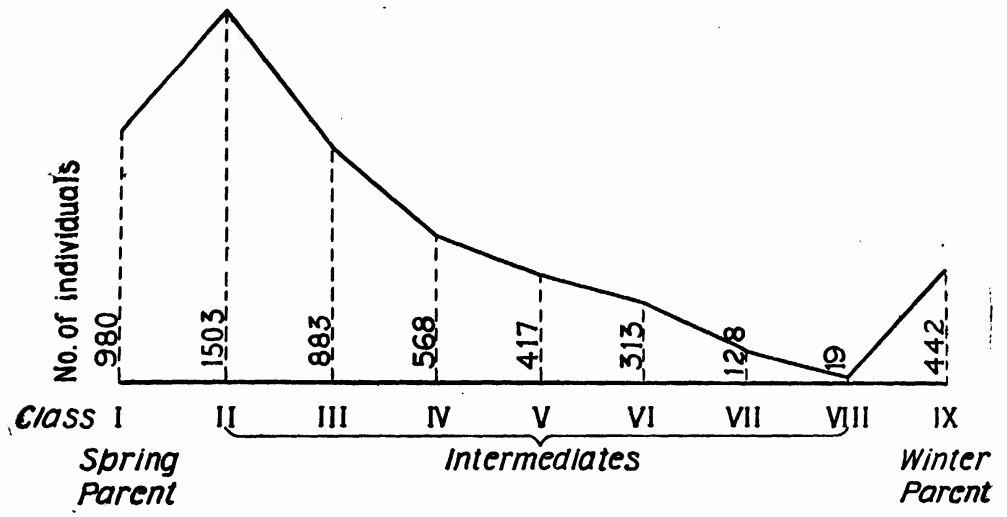

Fig. 1.—Diagram showing the segregation for time of heading in the spring-sown F_2 of a Marquis × Kanred hybrid.

EXPERIMENTAL RESULTS

THE MODE OF INHERITANCE OF GROWTH HABIT

The first generation material was sown in the fall. Because of the mildness of the season, only 2 out of 75 plants were killed during the winter. Had the winter been severe, it is possible that a higher percentage of the first generation plants would have been killed. No F_1 plants were grown from spring-sown seed.

All of the seed from the F_1 was sown in the spring of the following year, producing an F_2 population of 5,250 plants. Of this number 4,808 plants headed during the summer and 442 plants did not. Of the former, 980 plants headed at the same time as did the spring parent. In the second weekly period 1,502 plants headed, which is the greatest number to fall into any one heading period. From the second period on to the eighth, there was a gradual decrease in the number of plants heading. In the eighth period there were but 19 plants. The total number for each class is shown in figure 1.

It is very evident from the chart that the segregation of the plants for growth habit characters in the F_2 is of a complex nature. All of the plants in the first 5 classes matured like any ordinary spring wheat variety. It would be quite fair, then, to assume that in general, the plants in these

first 5 classes could represent reasonably well the true spring type. If such were the case, there would be 4,350 plants of the spring type and 900 of the winter, a ratio of approximately 5 spring plants to 1 winter plant. If it were assumed that all plants which headed during the summer were of spring type, there would be a ratio of approximately 11 spring to 1 winter. In either case there is a partial dominance of the spring over the winter habit.

A study was made to ascertain whether any correlation existed between the inheritance of growth habit and the presence of awns. A total of 432 plants were tabulated according to their growth habit in relation to this character. The awn characters were recorded from the F_2 individual plants and controlled by the progeny performance in the F_3. The results are presented in Table I.

TABLE I.—*The relation between awns and growth habit in the F_2 progeny of a Kanred × Marquis hybrid*

Heading period.	Number of plants with long awns.	Number of plants with intermediate awns.	Number of awnless plants.
1	18	25	14
2	17	29	11
3	14	21	16
4	21	21	24
5	23	26	16
6	29	41	38
7	6	9	11
8	1	.....	1
Total	129	172	131

The results presented in Table I show that there is a lack of correlation in the inheritance of awns and growth habit characters. While the numbers are not very great for each separate heading period, there is a total of 129 bearded plants compared to 131 beardless plants for the first 8 periods.

The segregation of plants for growth habit in the F_3 families was in accord with the segregation obtained in the F_2. The growth habit of the plants belonging to the various F_2 groups is shown in Table II.

TABLE II.—*The growth habit in F_3 of plants belonging to separate F_2 heading groups in a Marquis-Kanred hybrid*

Heading period.	Number of families.			
	Grown.	Homozygous for spring habit.	Heterozygous for growth habit.	Homozygous for winter habit.
1	10	10	0	0
2	10	6	4	0
3	10	7	3	0
4	10	5	5	0
5	10	2	8	0
6	9	0	9	0
7	6	0	4	2

The plants selected from the first heading period in the second generation were homozygous for the spring habit of growth in the third generation. Beginning with the plants in the second heading period, there was a gradual decrease in the number of families which were homozygous for the spring character and a corresponding increase in the number of families heterozygous for the same character as the seventh heading period was approached. There was also a gradual change in the ratios of spring to winter types progressing from the first to the seventh heading period. In the seventh period, 2 families were homozygous for winter character. If the plants in the eighth class had produced seed in the second generation, there undoubtedly would have been a still larger number of homozygous winter types in proportion to the number of spring types.

One can readily see from the data presented that there is a great difference in degree of heterozygosity of the plants in the various heading periods. In the first heading period all 10 of the F_3 families are homozygous for spring type; in the second, 6 of 10; in the third, 7 of 10; in the fourth, 5 of 10; in the fifth, 2 of 10; in the sixth, all 9 of the families are heterozygous for growth habit, and in the seventh period 4 families are heterozygous for growth habit and 2 are homozygous for winter type. With the exception of those from the sixth heading period, homozygous forms were obtained in all classes grown. A number of F_3 families were homozygous spring types comparable to our ordinary hard red spring wheat varieties. In addition to these early-heading plants, a few families were obtained which were homozygous for a heading period much later than that of the Marquis parent.

From Table III it will be noted that there is a correlation between the F_2 heading period and the percentage of spring types produced by the various F_3 families.

TABLE III.—*Showing the growth habit of the F_3 from F_2 plants heterozygous for growth habit*

Heading period.	Number of types.		Spring types.
	Spring.	Winter.	
			Per cent.
1	All.	None.	100
2	154	13	92.2
3	121	14	89.6
4	158	39	80.2
5	236	46	83.7
6	187	69	73
7	12	6	66.7

With the exception of the very slight increase in the fifth heading period, there is a very regular decrease in the percentage of spring types to winter types as one proceeds from the first to the seventh heading period. Here also there undoubtedly would have been a more complete reversal of the ratios of spring types to winter types in plants of the eighth and ninth heading periods, had it been possible to grow these plants in the third generation.

THE MODE OF INHERITANCE OF RUST RESISTANCE

The F_1 plants grown in the field during the summer of 1919 were not infected, since no rust developed in the field where they were grown. Consequently no determinations of resistance or susceptibility could be made under field conditions.

The F_2 plants were extremely susceptible when grown in the field under an epidemic produced artificially by inoculation with several biologic forms. The Marquis control plants, which are completely susceptible to all of the forms used in producing the epidemic, had an average rust infection of 87.7 per cent. The hybrids growing under the same conditions had an average rust infection of 80 per cent. This high susceptibility of the hybrids growing in the field was to be expected, because both the Marquis parent and the Kanred parent are susceptible to some of the forms used in producing this epidemic. These results very clearly demonstrate how a general field epidemic may fail to differentiate the segregation for resistance and susceptibility in the progeny from a given cross. The most accurate and reliable method of determining the resistance and susceptibility of hybrid progeny to any given number of biologic forms is to grow the plants under controlled conditions and to inoculate them with single known biologic forms.

The plants of 10 families from each of the first six classes, and 5 families from the seventh class, for growth habit (fig. 1), were tested for their reaction to Biologic Form I. This form is one which has been carried in pure culture since 1916 and has remained constant in its reaction on various host plants throughout this period. Of the 65 families tested, 23 were pure for resistance to this form of rust, 10 were susceptible, and 32 were heterozygous. The ratios are not very significant when taken from such a small number of families. There is a numerical ratio of 23 resistant families, 32 heterozygous, and 10 susceptible. The ratio of homozygous to heterozygous families is a very close approximation to the expected 1:1 ratio. The inoculation results are given in Table IV.

TABLE IV.—*The reaction of Marquis and Kanred, and various families of the F_3 from a cross between Kanred and Marquis, to Biologic Form I of stem rust* [1]

Class.	Homozygous for resistance.		Heterozygous for resistance.			Homozygous for susceptibility.	
	Number of families.	Number of individuals.	Number of families.	Number of individuals resistant.	Number of individuals susceptible.	Number of families.	Number of individuals.
1	3	102	4	96	26	3	82
2	3	75	7	184	54	0	0
3	3	108	4	102	35	3	88
4	1	21	8	239	59	1	26
5	4	111	4	89	28	2	73
6	6	140	3	65	18	1	24
7	3	50	2	23	2	0	0
Total	23	607	32	798	222	10	293
Marquis	.....	0	.....	0	0	.....	83
Kanred	.....	83	.....	0	0	0	0

[1] A total of 2,086 individual plants were inoculated. The resistant hybrid plants were just as free from lesions as the resistant Kanred parent. The susceptible hybrid plants were completely susceptible, producing large, vigorous, and confluent uredinia. This type of infection was like that obtained on the Marquis parent (Pl. 2).

There were 1,020 individuals in the 32 families which were heterozygous in their reaction to Biologic Form I. Of this number, 798 were immune, while 222 plants were clearly susceptible, an approximate ratio of 3 resistant plants to 1 susceptible. In some cases there were a few plants which failed to become infected in families which, judging from the majority of the plants inoculated, should have reacted as homozygous for susceptibility. Upon reinoculation it was found that the plants really were susceptible and had merely escaped infection. In the case of the heterozygous families it is not surprising, therefore, that the number of resistant plants is a little larger than expected. Apparently, there should have been a simple ratio of 3 resistant plants to 1 susceptible. There is a deviation from the expected of 33 ± 9.33.

From these data it is very evident that the segregation for resistance and susceptibility to this one biologic form of stem rust is very simple. Many desirable types were obtained in the F_3 which are homozygous for spring habit or growth and are immune from Biologic Form I.

Several F_3 selections, homozygous in their reaction to Form I, were inoculated with 12 other biologic forms. The results obtained were very striking and consistent, and are presented in Table V.

TABLE V.—*The reaction of Marquis, Kanred, and F_3 families of the cross between Kanred and Marquis, to 13 biologic forms of stem rust*

Biologic form.	Variety or hybrid family number.																
	Marquis.	Kanred.	29	30	31	41	42	43	47	48	54	55	60	79	80	205	181
I	[1] S	I	.....	.....	I	I	.....	.....	.....	I	I	I	I	I	I	I	S
III	S	S	.....	.....	.....	S	S	S	S	S	.....	.....	S	S	S	S	S
IX	S	I	I	I	.....	I	.....	.....	.....	.....	.....	I	.....	.....	I	I	S
XIV	R	I	I	.....	.....	I	I	I	I	I	.....	.....	.....	.....	I	.....	.....
XVII	S	I	I	.....	I	I	.....	I	.....	.....	.....	.....	I	.....	.....	I	S
XVIII	S	S	S	.....	S	S	.....	.....	.....	S	.....	.....	S	S	S	S	S
XIX	R	I	.....	.....	I	I	.....	.....	.....	I	.....	.....	I	I	I	I	.....
XXI	S	I	.....	.....	I	I	I	.....	I	I	.....	.....	I	I	I	I	S
XXIX	S	I	I	I	I	I	.....	I	.....	I	I	I	I	I	I	.....	.....
XXXII	S	S	.....	.....	.....	S	.....	.....	S	S	S	S	S	.....	.....	.....	.....
XXXIV	S	S	.....	S	.....	S	.....	.....	S	.....	S	S	S	S	S	.....	.....
XXXVI	S	S	.....	S	S	S	S	.....	S	.....	.....	.....	.....	.....	.....	.....	.....
XXXVII	S	I	I	.....	I	I	.....	.....	I	I	I	I	I	.....	I	.....	.....

[1] S=Completely susceptible; I=immune; R=resistant, a type of infection intermediate between that of S and I.

It will be noticed in Table V that as far as the reaction of the two parents is concerned, the 13 biologic forms of rust may be placed into 2 groups. The first group is typified by Form I, to which Marquis is susceptible and from which Kanred is immune.

The second group is represented by Form III, to which both Marquis and Kanred are susceptible. Here it will be seen that all of the progeny are as susceptible as either parent and identical in their reaction in this respect.

The hybrid families may also be placed in two groups; (*a*) those whose rust reactions are similar to the Marquis parent, and (*b*) those that are similar to the Kanred parent. Family 41, for example, is identical with Kanred in its rust reaction to all of the forms used, and family 181 is identical with Marquis in its rust reaction to all forms used.

These results very definitely demonstrate that resistance and susceptibility to several biologic forms of stem rust may be carried either in a single genetic factor or in different factors linked in the process of segregation.

With this fact established, it has been possible to inoculate the progeny from this cross with a single biologic form of rust from which the Kanred parent is immune. By their reaction to this one form it is clear that without inoculating they will react similarly to the other biologic forms from which Kanred is immune. In this manner, numerous F_3 selections have been obtained which are pure spring types immune from all of the known biologic forms of stem rust from which the Kanred parent is immune.

DISCUSSION OF RESULTS

The results show very clearly the complicated nature of the genetic difference between a spring and a winter variety of wheat. The results could not be explained very well on a simple monohybrid or dihybrid basis. The segregation indicates very minute differences for this character between the individual plants. While the time of heading was divided only into weekly periods, it was very evident during the progress of the experiments that the differences between the individual plants could be determined within a few days. For practical purposes and convenience in gathering the data, however, it was decided that heading periods, one week in duration, would be sufficient to indicate accurately the nature of the segregation for this character.

The results presented in Table IV show that there was no correlation between the growth-habit character and reaction to rust. The same numeric relations exist between the susceptible and resistant plants regardless of their respective times of heading. This transfer of the rust resistance of the winter parent to the progeny having the growth-habit character of the spring parent, attained the initial objective of the experiment. Several thousand rust-resistant families were obtained in the F_3 and F_4 which are being studied and tested for desirable agronomic characters in general.

These results are of particular interest not only in that they contribute to the solution of the general problem of breeding varieties of wheat resistant to rust, but also from the biological viewpoint. It is a common opinion that winter forms are more ancient or primitive than spring forms. Vavilov points out that this opinion is based on the fact that the so-called wild progenitors of our cereals are winter forms. Upon closer investigation of these wild species, however, he discovered the existence of spring as well as winter forms. He states (*18*):

> As a matter of fact, spring races in natural conditions have originated as a result of hybridization of different varieties of winter plants, and, vice versa, spring varieties could give origin to winter varieties. Both kinds of plants can be obtained synthetically one from another.

The wide genetic variability which different varieties of wheat may have for growth habit explains why spring character may be dominant over winter character and vice versa. The segregation which one would expect in a cross depends upon the factors present for the growth-habit character in the parental material. One might cross two varieties of winter wheat which from all appearances seemed to be homozygous for winter habit and in the progeny obtain some plants with the spring habit of growth. It has been shown (*18*) that spring types have appeared in the F_2 of such a cross. Likewise two spring types may be crossed and some progenies which are winter types will be obtained. This has been shown (*5*) in the case of two varieties of spring barley, in the F_3 and F_4 of which appeared several winter forms.

In view of the preceding facts and the data presented in this paper, it is not at all surprising that various workers have drawn different conclusions regarding the dominance of spring and winter characters in wheat and other cereals. The large number of F_3 selections, homozygous for heading period and varying all the way from the pure spring character to the pure winter character, demonstrates how finely the differences for growth habit may be divided. Each F_3 family (or selection) with a different heading period is of a different genetic nature. And as soon as these types become fixed, one ought to be able by intercrossing the types, to produce varying degrees of dominance of spring and winter characters.

Probably one of the most important principles that this study has demonstrated is the necessity for using known biologic forms of rust in the determination of the resistance or susceptibility of any given host. Attention was called to this principle by Stakman, Levine, and Leach (*15*) in 1919; by Hayes, Parker, and Kurtzweil (*7*) in 1920; and again by Puttick (*11*) in 1921. It has already been pointed out that previous to the discovery of the existence of biologic forms of stem rust of wheat, the breeding of resistant varieties met with failure time and again. Simply because a variety of wheat is resistant in a given locality for a period of years, there is no assurance that it will always be resistant in that locality or in any other locality in which it may be grown. And for the same reason one can not expect to prove conclusively, in an experimental plot, the resistance of any given variety, unless it is tested for resistance against all of the rust forms which exist in the area in which it is expected to be grown later. One can readily see the difficulty of producing an artificial epidemic in the field with certainty that a number of forms are present in sufficient quantity to have equal opportunity to attack all plants. Even if this were possible in a practical way, it would bring about difficulties in the synthetic production of a resistant variety. This point was clearly demonstrated in the work from which the data presented in this paper was taken.

The F_2 population was grown in the field under an artificial epidemic produced with several different biologic forms of rust. One of the parents and many of the hybrid plants were resistant to some of these biologic forms, as was proved when they were inoculated with the pure culture in the greenhouse. But in the field, all of the plants were equally susceptible, for all practical purposes. There was no method by which one could differentiate the plants one from another under this general epidemic. If a genetic study was to be made of a given number of biologic forms in their reaction on certain host plants it was evident that the work must be carried on under controlled conditions.

After the susceptible individuals have been eliminated in this manner, the general selection for desirable agronomic characters can be carried on in the field. As soon as this desired agronomic type is obtained, it will be crossed again with other varieties or selections which are resistant to other biologic forms and this process will be continued until a desirable agronomic type has been obtained which is resistant to all of the biologic forms of stem rust.

The fact that resistance and susceptibility of the host to several different forms of rust are inherited as a single genetic factor makes this cross very valuable. Kanred is known to be immune from at least 11 different biologic forms of stem rust, and, as far as tested, the immune progeny possesses all the immunity of the Kanred parent. This has further demonstrated that a variety of common wheat may be produced synthetically which will be resistant to a large number of the biologic forms of stem rust.

SUMMARY

(1) The segregation for growth-habit characters in the progeny of a cross between a winter and a spring wheat accords with Mendelian laws. The results indicate the presence of multiple factors for this character.

(2) Homozygous types for both spring and winter characters were obtained in the F_3. In the F_2, some plants appeared which were homozygous in F_3 for a heading period which was 5 weeks later than that of the Marquis (spring) parent.

(3) Kanred is an awned winter common wheat, while Marquis is an awnless spring common wheat. In the progeny of this cross there was no correlation between growth habit and presence of awns.

(4) The segregation for rust reaction to Biologic Form I in the progeny of a cross between Kanred and Marquis wheats shows a simple Mendelian ratio of approximately three immune plants to one susceptible plant. There is a clear dominance of immunity over susceptibility, since there are no intermediate types of infection with this rust form.

(5) The progeny of this cross were inoculated with several biologic forms of stem rust and show that the reaction of the host to all of these forms is inherited as a single genetic factor.

(6) In the F_3 some of the hybrid families were homozygous for the spring habit of the Marquis parent and for the rust resistance of the Kanred parent.

(7) There was no correlation between the inheritance of growth habit and the manner of reaction to stem rust.

(8) These facts further demonstrate that varieties of common wheat may be produced synthetically which will be resistant to a large number of the biologic forms of stem rust, *Puccinia graminis tritici.*

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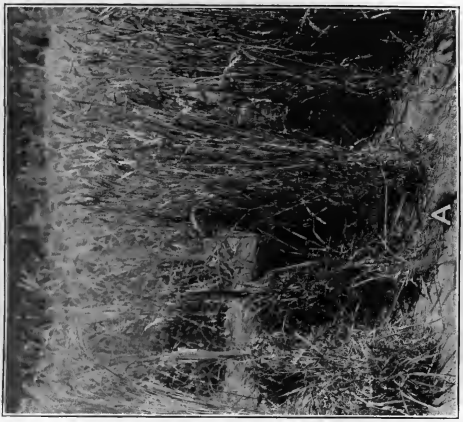
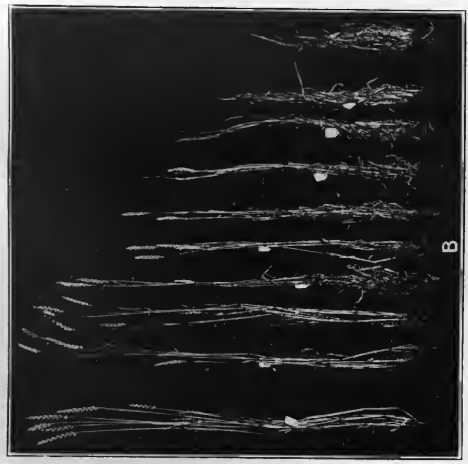
PLATE 1

A.—Showing the difference in growth habit between Kanred winter wheat (left) and Marquis spring wheat (right) at harvest time, when both are spring sown.

B.—Showing the segregation for time of heading in the F_3 plants from the Marquis×Kanred cross, when seed from F_2 heading classes was spring sown. Left, Marquis; center, F_2 hybrids, arranged in order of heading dates, from left to right; right, Kanred.


A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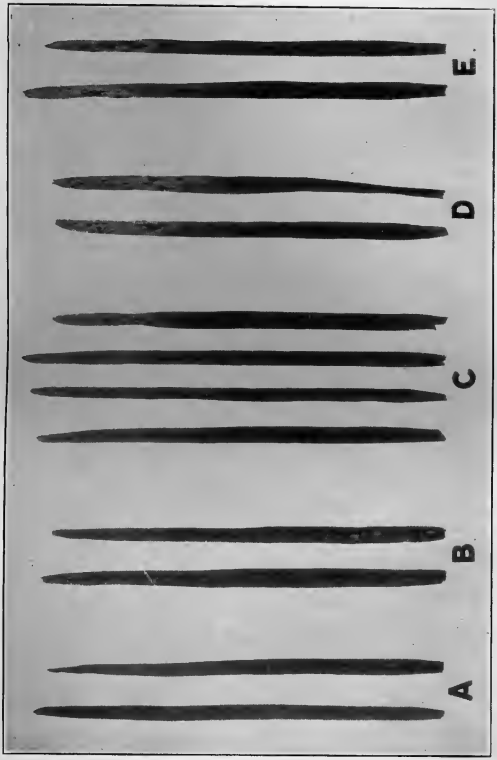
E
D
C
B
A

PLATE 2

Seedlings of Kanred, Marquis, and F_3 families of the cross between Kanred and Marquis, inoculated with a rust form from which Kanred is immune and to which Marquis is susceptible. A, Kanred, immune; B, F_3 generation, immune; C; F_3 generation, segregating for susceptibility to rust; D, F_3 generation, susceptible, E, Marquis, susceptible.

EFFECT OF ORGANIC DECOMPOSITION PRODUCTS FROM HIGH VEGETABLE CONTENT SOILS UPON CONCRETE DRAIN TILE [1]

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PREFACE

The work which is covered in the following report was begun purely as an engineering study. It arose out of the fiduciary duty of the engineer to protect the interest of his client, namely the man who ultimately pays the bill and upon whose prosperity the permanence of the whole business rests. Necessity made the scope of the investigation much broader than was at first expected. Accident drew attention to the apparent failure of a structural material under certain limited conditions which opportunity gave a chance to investigate. This does not, however, mean that the material will fail under any other conditions than those investigated. Nor does it mean that material not investigated is therefore exempt from criticism. It is believed that this investigation very materially aided a movement for the general improvement of the material investigated, not only for limited but, also, for the general application of the material.

Other material, not covered in the scope of these investigations, but used for the same purpose, is, under certain conditions, as liable to failure as is the material investigated. A general movement for its improvement is very much to be desired. A recognition of the special adaptability of each material to its own particular class of work would aid materially in stabilizing the business.

PART I. GENERAL INTRODUCTION

The extremely rapid development, particularly in the last few years, of the drainage of wet farm lands and the increasingly large investments in drainage that are being made by the farmers of this country are making necessary an intensive study of both materials and methods. Burned clay was the first material to be used extensively in this work, and by its use the early science of land drainage was developed.

The adaptability of concrete for making drain tile soon became obvious and the manufacture of concrete drain tile is now one of our well established industries. A very large proportion of the lands which may be profitably drained are either in large alluvial deposits or in immense plains left by glacial action. Under each of these conditions the formations which would carry good industrial clays are in many instances deeply buried and the farmer may have to go a considerable distance for his supplies, and pay heavy freight charges on them.

Another factor of very considerable importance is that the efficient handling of clay products requires a very considerable outlay for plant, necessitating, in its turn, a market, developed before the establishment of the plant and of sufficient size to keep the plant busy. This is impossible in the newer districts.

[1] Accepted for publication Aug. 15, 1922.

Concrete tile, on the other hand, may be made in small and inexpensive plants, adapted to seasonal business of various sorts, and the bulk of the material used is available at a comparatively low cost.

The formation of drainage districts and the digging of large outlets is of little value without complete interior drainage of the wet lands by the construction of laterals, spaced as the soil condition may require. In outlying districts the excessive cost of clay tile due to long haul prohibits its use. With this in mind it may be said that concrete, if it can be made durable, is potentially the controlling factor in the drainage development of the newer parts of the country.

Unfortunately, when first manufactured, concrete tile made a bad start. It was claimed that as most of the water got into the tile through its walls and not at the end of the tile, the more porous those walls were, the more efficient the drain would be. This erroneous idea was honestly promoted by engineers and was used as a sales argument by the manufacturers of concrete. Standard specifications at the present time permit an absorption of 10 to 12 per cent and unscupulous manufacturers have been making a product that merely holds together long enough to get it into the ground. Failure due to the breaking down of the tile has been common, but the firms manufacturing poor material have generally been of short life while those who turned out a good product have usually grown and increased their business. The result is that the average product of today is far superior to that of 10 or even 5 years ago.

It has frequently been noted that tile made by certain manufacturers, or, more correctly, the tile made by manufacturers in certain districts did not last as well as tile manufactured in other districts. The manufacturer was invariably blamed for any failure.

INVESTIGATIONS

In most average soils, well-made concrete tile appears to be permanent. As the vegetable and mineral matter of soils varies, however, the concrete tile disintegrates.

In some of the arid and semi arid districts of the West and Middle West, for instance, concrete has broken down in wet soil containing a high percentage of some alkalies, particularly alkaline sulphates. The cause is becoming fairly well understood, but the cure is still under investigation. This disintegration appears to have first been reported officially by Headden (*7*)[2] and Tannatt (*14*) in 1908. Since that time the subject has been very carefully investigated, notably by Bates, Phillips and Wig (*2*) in 1912; Wig and Williams (*16*) in 1915; Wig, Williams and Gates (*18*) in 1916; Steik (*10*) and Wig, Williams and Finn (*17*) in 1917; Steik (*11*) in 1919, and Miller (*8*) and Williams (*19*) in 1922.

The outstanding fact developed by this work is the destruction of concrete by alkaline sulphates of a comparatively high concentration in the soil water of alkaline soils.

Winter and Musselman (*21*) in 1915, and Winter (*20*) in 1917, working at the Michigan Agricultural College, investigated the solubility of concrete in water and in various acids.

The University of Washington has made tests covering the destruction of concrete by carbonic acid.

In Europe several marsh investigators have noted the disintegration of concrete in "Hoch moor." This corresponds to our "muskeg," which is

[2] Reference is made by number (italic) to "Literature cited," pp. 499-500.

composed almost entirely of moss and other low order plant accumulations, without inwashed soil or lime.

Vogler (*15*) in his "Grundlehren der Kulturtechnik," published in 1909, draws a strong distinction between low moor and high moor, repeatedly affirming the resistant quality of cement tile in low moor and its destruction in high moor.

Tacke (*13*), in 1910, published a paper on the substances in peat which would probably be destructive to concrete. He mentions the disintegration of concrete in a moor containing as high as 17 per cent of iron pyrite. He also discusses the probable destruction from humic acids of concrete in high moor and its permanence in low moor.

Bersch (*3*) in his "Handbuch der Moorkultur," published in 1909, makes the same distinction between low and high moor as does Vogler, and draws the same conclusions regarding the durability of the concrete tile in the two classes of peat.

None of these authorities followed up his observations by any published research results, but the behavior of concrete tile in peat was considered of such importance that a subcommittee of the German Committee on Reinforced Concrete was assigned to the task of making investigations. Their work was interrupted by the war.

In this country previous to 1921, if there had been any suspicion of destructive effect on concrete tile due to weak soil acids, no report had been made of any investigations induced by such a suspicion. Wig, Williams and Finn (*17*) in 1917, while investigating the effect of alkali on tile, noted a disintegration that took place on one of their tile lines at Columbia, Mo., where tile were laid in supposedly neutral soil as a control against the disintegration of similar tile laid in alkaline soil. They say in their report:

> An exceptional condition has arisen at this project, presumably caused by some local action, in that the tile of series 1 to 16, excepting series 5 (tar coated), showed evidences of disintegration on the lower outer surface, indicated by the apparent dissolving away of cement leaving the sand grains coated with a brown stain.

The tile at Columbia were laid in mineral soil overlaid by 12 to 15 inches of black soil.

Williams (*19*) in 1922, reports on the same tile that in all cases except those which had been in the ground only one and two years, and those which were tar coated, the tile were stained and pitted.

Alway (*1*) in 1922, refers to the work of Tacke at some length. He questions the probabilities that the breaking down of the concrete tile is due to the action of organic acids or that concrete tile break down in peats containing a high percentage of lime. He says:

> Humic acids are not carried in the bog water of high lime peats. These acids are more or less soluble in pure water and in weak mineral acids, as phosphoric and boric, but insoluble in hydrochloric and sulphuric acids. With soluble compounds of calcium, magnesium, iron and aluminum, they form insoluble compounds, so called humates which are insoluble in water as well as in moderately concentrated alkali solutions.

Soluble sulphates, particularly those caused by the breaking down of iron pyrite (a common condition in acid peat bogs) seem to him to be the most probable cause of disintegration, but he also includes hydrogen sulphid and alkali waters.

Stewart (*12*), collaborating with Doctor Alway, and writing in the same publication, makes a direct statement along the lines suggested by Doctor Alway's paper.

In February, 1921, the writer (*4*) offered the theory that concrete tile were liable to disintegration in any soil carrying a high percentage of organic matter. This was followed in July of the same year by a further statement (*5*) to the same effect.

In addition to the authorities quoted, numerous observers in the last two years have reported a breaking down of concrete tile lines in soils high in organic matter. None of the authorities quoted, however, either in this country or Europe, have followed up their observations with exhaustive research which they have later reported. Nor have any of them considered it probable that concrete tile would break down in soils carrying a high percentage of lime. Consequently in the work covered by these investigations, this point has been especially recognized and covered.

UNIVERSITY MARSH

A tract of 110 acres of peat marsh is located on the experimental farm of the University of Wisconsin. For the past 10 years this land has been used for experimental purposes in drainage. This area, which at one time was a swampy bay of Lake Mendota, is generally known as University Marsh. Most of its surface is a little above the lake and the soil consists of fibrous peat 2 to 6 feet deep, underlain by sand and clay. In the deeper parts of the marsh there is a bed of marl between the peat and the mineral soil below. The marl bed varies considerably in thickness up to a maximum of about 18 inches, and around its margin frequently blends with, and is interlaid by sand. Since diking, various types of drainage have been worked out, improvements and changes being made as their value was demonstrated.

On the higher side of the marsh are a number of high morainal hills from which the surface water of about 400 acres drains down. Heavy seepage also works into it from below, expecially in the sandy portion. This is sufficient if the stream is concentrated into a pipe to lift the water 2½ feet above the marsh surface.

From the beginning, this experimental work on the marsh has been under the direction of E. R. Jones, professor of agricultural engineering at the University of Wisconsin and State drainage engineer. During the fall of 1919, and the season 1920, the work was under the direct charge of the writer, whose principal function was a determination and compilation of data on the physical change in the marsh brought about by the various stages of drainage and cultivation.

Part of the work consisted of an examination of the tile lines themselves. This was done by groups, taking as a group a series of tile having a common outlet and laid at the same time and under similar conditions. On each group several pits were dug for the purpose of examination. If no unusual conditions were disclosed, it was assumed that none had developed. If, however, any unusual condition were disclosed, more pits were dug for the purpose of determining whether or not the unusual conditions were inherent in the group.

DISINTEGRATED TILE

Seven lines of tile, two of which were concrete, were laid by students in May and June, 1914. Lines 5 and 7 were of concrete. In December, 1919, on opening these two lines, the tile were found to show signs of failure.

More pits were opened, and it was discovered that the entire lines were badly disintegrated, decay being greatest at the upper end and farthest away from the pump house. It was at first thought that the tile might have broken down, owing to bad workmanship, but this was disproved when it was found that at the extreme upper end of the tile lines, where rotted muck had flowed in and partially filled the tile, the tile had been protected, and had not decayed. It still bore the marks made by the mold, and, upon breaking, the interior of the tile wall appeared to be in perfect condition. The surface of the tile, both inside and outside, was rough, due to the grains of sand and gravel falling away. Inside the disintegrating layer, the tile was discolored for about $\frac{3}{32}$ of an inch, but appeared to be dense, while a thin layer in the center of the wall was unaltered. This last fact shows that the tile had not broken down due to the passage of waters through the walls, but to solvent action upon the walls themselves. Disintegration was greatest on the outside of the tile and at the ends and much greater on the top than on the bottom. Disintegration on the inside of the tile was greater at the top than on the bottom, and was not greater at the water line, except at the joints, where it met heavy disintegration from the outside.

Since the tile showed signs of good workmanship, it was assumed that the disintegration must have been due to the solvent action of some substance present in the soil.

Correspondence was begun and the literature searched to secure information which would throw some light on the reason for the disintegration. The most probable solution of the difficulty was first presented by Prof. H. B. Roe, of the University of Minnesota, who cited the destruction of concrete tile in his State by the action of sulphate salts. This possibility was further investigated and talked over with chemists. The conclusion tentatively arrived at was that the disintegration was caused by sulphates produced by the decomposition of proteins in the peat. This solution seemed satisfactory.

Work on the marsh was discontinued at this point because of cold weather. The compilation of data could not go ahead until more field work was done and opportunity was taken to test out the assumed presence of sulphates in the marsh and their effect on new tile.

PART II. PRELIMINARY STUDIES

LABORATORY INVESTIGATIONS

With the advice and assistance of Prof. E. Truog and E. J. Graul of the Soils Department, laboratory studies were made, beginning in February, 1920. A cement tile company furnished new, uncontaminated tile for the tests. These tile were machine made, very dense, steam cured, and three months old at the time of the tests.

MARSH WATERS

No sulphur in the form of sulphates could be detected in water from the marsh by the addition of barium chlorid to a hot solution slightly acid with hydrochloric acid. The assumption that the disintegration was due to the presence of sulphate salts was therefore unfounded, and the whole cause of the destruction still remained obscure.

The next step was to find actually what was in the marsh waters and if those marsh waters would dissolve the tile. Iron and aluminum were

thrown down in considerable quantity by ammonia. Lime magnesia was also thrown down by ammonium carbonate.

Lime in the water was determined by the following method: To 100 cc. of the water a few drops of HCl were added to dissolve anything which might be held in suspension, and then 5 gm. of ammonium chlorid. Ammonium hydroxid was added in sufficient quantity to make the solution strongly alkaline and acetic acid to make the solution just slightly acid. After boiling, the iron, aluminum, and phosphorus were filtered off and the precipitate carefully washed. To the boiling filtrate and washings, 10 cc. of 10 per cent solution of ammonium oxalate were added, then dilute ammonia until the solution was slightly alkaline. After digestion the solution was allowed to stand over night, then filtered on ash-free filter papers and washed with hot water.

The precipitate on the filter paper was then dissolved with 10 per cent solution of sulphuric acid. Next, 2 cc. more of H_2SO_4 was added, plus enough water to bring the volume to 200 cc. The solution was then warmed and titrated with $\frac{1}{10}$ normal solution of potassium permonganate. Lime was calculated on the basis of 1 cc. of solution (.0028 gm. of CaO). The results of these tests are given in Table I.

TESTS WITH CARBONIC ACID

Carbonic acid was next suspected as being the solvent. A quantity of distilled water was saturated with pure carbonic acid. Weighed fragments of tile of approximately the same shape and size and from which all loose particles were removed were placed in 500 cc. bottles and 475 cc. of the various waters added. The bottles were then tightly corked, placed in a revolving shaker machine and agitated 24 hours intermittently for 3 days. Duplicate samples of each were run. It was attempted to determine the solubility of the tile by determining the amount of lime originally in the water and in the sample run. It was found that lime was actually thrown out of solution from the waters taken from the bog and that the carbonated water did not contain nearly as much lime as did the distilled water. The results are shown in Table I.

TABLE I.—*Grams of CaO per liter, in marginal and bog waters in their original condition, as collected in February, and in these and in distilled and carbonated waters after agitating 3 days with similar pieces of new tile*

Test No	Original water.		Waters agitated with tile.			
	Marginal.	Bog.	Distilled.	Carbonated.	Marginal.	Bog.
	Gms	*Gms*	*Gms*	*Gms*	*Gms*	*Gms*
1	0.0896	0.0854	0.2156	0.1120	0.0651	0.0945
2	.1120	.0857	.2226	.0476	.0588	.0756
3	.1106	.0868	.1365	Broken.	.0525	.0357
4	.1036	.0784	.....	.....	.....	.....
5	.1008	.0770	.....	.....	.....	.....
Average	.1033	.0826	.1918	.0798	.0588	.0686

In the agitated waters it will be noted that some of the tests in a series show a marked variation from others in the same series. This was attributed to the fact that the samples of tile were probably far from uniform.

Two outstanding facts were indicated. The first was a greater solubility of the tile in distilled water than in either the carbonated or bog water. The second was that though pieces of tile had been agitated for 3 days in water from the margin of the marsh, the quantity of lime actually in the water was less than in the beginning.

This result showed that the original premises were not well founded. They were either incorrect or something had been omitted from them. Within the power of the test to show it, carbonic acid was not causing the destruction of the tile; neither was the marsh water, apparently.

TESTS FOR ALKALI

There appeared to be no explanation for the results given in the Table I until it was suggested that perhaps the marsh waters did not actually contain free carbonic acid and that the tile, even after a lengthy curing, might contain free alkali. To test this, a quantity of tile was powdered and to it a small amount of distilled water was added which was immediately poured off and filtered. It gave a powerful reaction with phenolphthalein, showing that the tile was not only alkaline but that a considerable quantity was soluble in water. A quantity of the powdered tile was boiled in distilled water, filtered, and while still hot 100 cc. of the filtrate measured. This gave 3.582 gm. of residue on evaporation, a surprising percentage considering the age of the tile.

The marginal and bog water was then tested on the assumption that they contained free organic acids. Methyl orange showed that they did not. They were then titrated with standard sulphuric acid solution, $\frac{1}{14}$ normal strength, using methyl orange as an indicator. The test disclosed strong alkaline reactions, showing unneutralized alkalies of the strength indicated in Table II.

TABLE II.—*Showing the amount of $\frac{1}{14}$ normal acid solution required to neutralize the free alkali in marginal and bog water and its equivalent in CaO per liter of water*

Equivalents.	Marginal water.	Bog water.
Cubic centimeters of one-fourteenth normal solution used...	15.30	14.80
CaO equivalent per liter, gm.....	.1530	.1480

The contradictory results obtained were now explained on the basis of the precipitation of calcium carbonate formed from the more soluble calcium bicarbonate by the free alkali of the tile taking away one-half of the carbonate radicle.

However, this did not make the explanation of the disintegration of the tile any clearer; instead, it became still more obscure.

COMPARISON OF OLD AND NEW TILE

The results of the tests using the new tile being apparently so different from what had actually happened in nature, it became necessary to compare the old and new tile in order to determine whether or not the new tile differed in any material degree from that which originally went into the drains.

One entire new tile was taken from the marsh, wiped with a towel, and weighed while still wet. It was then dried to constant weight in an electric oven at 110° C. and again weighed. The loss was 11.90 per cent, representing the proportion of water contained.

Similarly two new pieces of new tile were weighed in their natural air-dry condition, dried in an electric oven at 110° C., weighed again, boiled for two hours in water, and weighed again. The results are tabulated in Table III.

TABLE III.—*Percentage of water absorbed by old and new tile*

Samples.	Weight at 110° C.	Water absorbed in ground.	Water absorbed from air.	Water absorbed on boiling.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Tile from marsh	100	11.90	.....	.....
New tile, sample (a)	100	.....	1.551	6.631
New tile, sample (b)	100	.....	1.545	7.095

From this table it will be seen that the tile which was taken from the marsh had nearly double the porosity of the new tile. How much this porosity was altered during the time it was buried in the ground it is impossible to say. From the maker's statement there is no doubt that the tile was less carefully made than that now turned out by the same manufacturer. An absorption of 12 per cent would indicate a good average tile at the time these were made, and is about the average of material turned out by the less careful manufacturers at the present time.

COMPARATIVE ALKALINITY OF TILE

The next step was to compare the alkalinity of the old and new tile. It would be natural to assume that any free alkali would be very quickly neutralized after the tile was placed in the ground. A quantity of the old tile and of the new tile were pulverized and 2 gm. taken from each. Then 200 cc. of distilled water was added to each sample and phenolphthalein used as an indicator. The solution was titrated by adding at successive intervals $\frac{1}{14}$ normal solution of sulphuric acid, extreme care being used, particularly toward the end of the operation, not to permit the solution to contain any free acid and so expel carbonic acid. The operation was carried on over 2 days, titrating about every half hour with results as shown in Table IV.

TABLE IV.—*Amounts of $\frac{1}{14}$ normal acid solution required to neutralize free alkali in 2 gm. each of old and new tile, and percentages of free alkali indicated*

Item.	Old tile.	New tile.
Grams of tile used	2.00	2.00
Cubic centimeters of $\frac{1}{14}$ H_2SO_4 solution used	17.70	40.50
Equivalent grams free CaO or equivalent	.0354	.0810
Per cent free CaO or equivalent	1.770	4.050

The results given in the preceding table are very remarkable. Not only did the new tile after a presumably thorough curing still contain 4 per cent of free alkali, but the old tile after 5 years in the ground, during which time it was partially destroyed, still contained nearly half as much. The importance of the fact indicated can hardly be overestimated. It will be discussed later.

SOLUTION IN HCl

New tile in concentrated HCl was then tested. Strong evolution of bubbles showed the presence of a considerable percentage of carbonate. The dried filtrate was ignited and treated again with acid. The exact percentage of solubility is shown by Table V.

TABLE V.—*Percentage of new tile soluble in concentrated HCl*

Constituent.	Percentages.
Insoluble silica	47.42
Total soluble	52.58
Soluble silica	3.40
Total solubles other than silica	49.18

ULTIMATE SOLUBILITY IN ORGANIC ACIDS

In order to ascertain the percentage of the tile which might be ultimately soluble in organic acids and to establish a relation between it and the aggregate used, an acid of low hydrogen-ion concentration was chosen, namely, quarter strength acetic, and samples of the old and the new tile and the aggregate from which the tile were made were treated with it. The acid used was supposed at the time to be full strength, but was later found to be about 85 per cent. From each sample previously dried in the electric oven, 5 gm. was taken and boiled for 10 minutes in the dilute acid and the residue weighed after drying in the electric oven at 110° C. The results are given in Table VI.

TABLE VI.—*Percentages of old tile, new tile, and aggregate soluble in 21 per cent strength acetic acid*

Item.	Old tile.	New tile.	Aggregate.
Grams taken	5.00	5.00	5.00
Residue	3.3090	3.2777	3.5152
Loss	1.6910	1.7223	1.4848
Per cent soluble	33.82	34.45	29.69

ANALYSIS OF AGGREGATE

A screen analysis of the sand and gravel aggregate used in the manufacture of the tile gave:

Grade:	Per Cent of Total.
Coarser than 10 mesh	46.6
10 to 20 mesh	13.4
20 to 40 mesh	18.2
40 to 60 mesh	12.2
60 to 80 mesh	4.8
80 to 100 mesh	1.6
100 mesh	2.4
	99.2

Tomlinson[3] has calculated the mineralogic proportions in Waukesha sand by relative density and miscroscopic count, proportions of carbonates and of quartz in different sizes of Waukesha sand, as follows:

Group:	Carbonates.	Quartz.
Coarser than 10 mesh	72.1	1.5
10 to 20 mesh	72.1	7.0
20 to 40 mesh	55.7	35.4
40 to 100 mesh	36.9	58.1

Proportion of Mineral groups in Waukesha sand

Group:	Per Cent.
Igneous rock	0.42
Shale group	....
Quartz group	32.31
Dolomite group	65.37
Feldspar	....
Heavy minerals	.83
Not sorted	1.07
	100.00

TESTS OF CEMENT

Both the old and the new tile had been made with a slag Portland cement of average chemical composition. This cement was also treated with acetic acid to ascertain its solubility. It showed a trace of carbonic acid bubbles.

A quantity of the cement was mixed into a thick paste with water, thoroughly worked and rolled into balls by hand, then flattened on glass, made into patties and allowed to set one day under a moist cloth. At the end of that time, the patties had set firmly and showed no signs of malformation, swelling, or shrinkage cracks. Boiled one hour in water, they showed no signs of failure.

Some of the patties were allowed to set three days in water and at the end of that time appeared to be in perfect condition. They were then powdered, and 2 gm. dried at 110 C°., were treated with 21 per cent strength acetic acid. Other patties, made in a similar way at Minnesota, were allowed to set in water for three months, and then five months in air at a temperature averaging 75° F. These were powdered and treated like the others. The first test showed no evolution of bubbles, but the second gave bubbles in considerable amount, showing that the cement had carbonated during the eight months set. The result of the two tests are tabulated in Table VII.

[3] TOMLINSON, C. W. UNPUBLISHED THESIS. Univ. of Wis., Col. of Engin., 1915.

TABLE VII.—*Solubility of cement set 4 days and 8 months in acetic acid of 21 per cent strength*

Item.	4-day set.	8-month set.
Weight of sample (gm.)	2	2
Weight of residue (gm.) (a)	.559	0.4290
Weight of residue (gm.) (b)		0.4665
Percentage of solubility (a)	72.02	78.55
Percentage of solubility (b)		76.68

The two tests show an average increase in solubility of 5.50 per cent. If this is to be explained on the basis of calcium hydroxid becoming carbonated during the eight months it would indicate a proportion of unneutralized lime in the freshly set cement equal to 25.8 per cent.

ANALYSIS OF CEMENT

A cement company very kindly furnished the following analysis of their cement and a report of physical tests.

Chemical analysis of cement, average of daily analyses, March, 1920

	Per cent.
Silica (SiO_2)	22.04
Oxid of Iron (Fe_2O_3)	3.30
Alumina (Al_2O_3)	5.70
Lime (CaO)	62.64
Magnesia (MgO)	3.73
Sulphuric Acid (SO_3)	1.23
Loss on Ignition	.50

Physical tests of the same cement, average for February, 1920

Fineness:
- Passing 200-mesh sieve ..... 81 per cent.

Setting time:
- Initial set ..... 2 hrs. 45 min.
- Final set ..... 5 hrs. 5 min.

Tensile strength:
- 1 part cement to 3 parts standard Ottawa sand—
- 3 days (1 day in moist air and 2 days in water) ..... 239 lbs.
- 7 days (1 day in moist air and 6 days in water) ..... 322 lbs.
- 28 days (1 day in moist air and 27 days in water) ..... 420 lbs.

RESULTS OF PRELIMINARY STUDIES

Up to this point the work done may be considered as preliminary studies, though that was not the intention when some of the tests were begun. The principal points brought out may be enumerated as follows:

(1) The alkalinity of the marginal and bog waters in February.
(2) The powerful alkalinity of the tile even after 5 years in the drain.
(3) The similarity of the old and new tile except in the matter of density.
(4) The solubility of the thoroughly matured tile in weak organic acid.
(5) The absorption of CO_2 during long a period of curing.

Analyses of American Portland Cements (from "Portland Cement")

Made from—	Where made.	SiO_2.	Fe_2O_3.	Al_2O_3.	CaO.	MgO.	K_2O.	Na_2O.	SO_3.	Loss.
Cement rock and limestone.	Nazareth, Pa.	19.92	2.28	7.52	62.48	3.19	0.52	0.66	1.51	1.46
	.....do.	21.14	2.30	6.94	63.24	3.26	.36	.51	1.12	1.24
	Bath, Pa.	19.64	2.80	7.52	62.31	3.04	n. d.		1.60	1.48
	Alpha, N. J.	21.82	2.51	8.03	62.19	2.71	n. d.		1.02	1.05
	Northampton, Pa.	21.94	2.37	6.87	60.25	2.78	.61	.87	1.38	3.55
	Coplay, Pa.	22.26	2.10	5.36	63.32	3.81	n. d.		.89	1.24
	Omrod, Pa.	22.20	2.27	6.69	62.61	3.00	.32	.61	1.32	1.56
	Martins Creek, Pa.	20.23	2.50	7.12	62.94	3.38	n. d.		1.45	1.25
	Reading, Pa.	24.16	1.45	5.10	62.95	3.12	.21	.50	1.35	1.40
Limestone and clay or shale.	Ban City, Mich.	20.72	2.85	7.17	62.64	1.97	.48	.12	1.42	2.58
	Wellestone, Ohio.	21.84	5.05	6.77	62.66	.80	n. d.		1.24	.....
	Chanute, Kans.	20.74	3.72	7.06	62.76	1.78	.41	.23	1.12	1.40
	Ada, Okla.	12.28	3.20	6.36	59.66	3.11	.80	.25	1.40	2.82
	*Stroh, Ind.[1]	21.78	2.65	7.31	62.35	2.88	0.47		1.78	.78
	*Glens Falls, N. Y.	21.50	10.50		63.50	1.80	.40		1.50	n. d.
	Alsen, N. Y.	23.94	3.20	5.62	62.32	1.77	n. d.		.90	1.68
	Fordwick, Va.	21.31	2.81	6.54	63.01	2.71	n. d.		1.42	2.01
	Davenport, Calif.	25.38	1.20	3.34	62.96	1.20	n. d.		.35	4.58
	Cement, Calif.	22.34	3.30	7.00	60.72	1.30	n. d.		1.05	2.54
	*Baker, Wash.	24.63	8.56		62.88	1.60	n. d.		1.33	n. d.
	St. Louis, Mo.	23.12	2.49	6.18	63.47	.88	n. d.		1.34	1.81
	Demopolis, Ala.	19.36	4.10	9.18	63.20	1.16	n. d.		1.18	1.12
	*Portland, Colo.	21.88	2.85	7.14	64.94	Trace.	1.18		.73	1.08
Marl and clay.	*Middlebranch, Ohio.	21.24	4.14	7.85	63.22	.28	.68		1.11	1.32
	*Coldwater, Mich.	21.22	3.83	7.51	63.75	.82	n. d.		1.58	1.02
	Sandusky, Ohio.	21.93	2.35	5.99	62.92	1.10	.63	.27	1.55	2.92
	*Bronson, Mich.	22.90	3.60	6.80	63.90	.70	1.10		.40	.60
	*Harper, Ohio.	21.30	2.00	6.95	62.50	1.20	n. d.		.98	4.62
	*Warners, N. Y.	22.04	3.41	6.45	60.92	3.53	n. d.		1.25	.....
Limestone and blast furnace slag.	Chicago, Ill.	22.41	2.51	8.12	62.01	1.68	n. d		1.40	1.02
	.....do.	23.06	2.88	8.16	62.10	1.88	.36	58	1.57	.....

[1] Analyses made by Richard K. Meade, with the exception of those designated *.

The average is represented by the following:	Per cent.
Silica	22.0
Alumina	7.5
Iron oxid	2.5
Lime	62.5
Magnesia	2.5
Sulphur trioxid	1.5

PART III. DEFINITE RESULTS

In the preliminary work where tests were made with tile, the proportion of tile to water was far in excess of the proportion between the tile in a marsh, and the marsh itself was always sufficiently high to make the combined solution strongly alkaline. In all later work the aim was to reverse the proportions so as to make the influence of the marsh predominate. The marsh water, too, taken in the month of February, was alkaline. It became necessary to determine if the water in the marsh remained alkaline under the influence of warmth and if the tile were acted upon by a large excess of solutions produced under warm conditions.

TEST WITH SURPLUS OF BOG WATER

The results of the tests with a comparatively large piece of tile in a small quantity of water being unsatisfactory, due to the excessive alkalinity of the tile, another scheme was devised which was intended to subject tile to a large quantity of water. In order to increase the rapidity of action, the surface of the tile was increased as much as possible by powdering the tile. From this powdered material 50 gm.

was placed in a 1-inch glass tube, 10 inches long, stoppered at each end, a glass tube giving ingress and egress at bottom and top. The inlet tube at the bottom was curved downward on the inner end to prevent the tile grains from escaping downward through the tube. Peat taken from a depth of 5 feet in the same pit from which the bog water was taken was mixed with distilled water in a 2-gallon glazed earthen jar having an orifice near the bottom. A glass tube led through this orifice and connected on the inside with a long inverted "U" on the bottom of which was a glass funnel covered with muslin as a filter. The tube through the orifice connected on the outside with a filter made of a 1-inch stoppered glass tube in which was asbestos pulp packed above glass wool. The object of the filter was to prevent the escape of any trace of solid matter into the powdered tile. At the upper end, the tube containing the powdered tile connected with a glass tube which led downward into another weighed filter made of a porcelain "gooch" in which ⅛-inch of shredded asbestos had been packed. A pump was connected on below the "gooch" to draw the water through the system from the mixture of peat and water in the earthen jar. The last filter was carefully weighed after drying it in an electric oven at a temperature of 110° C. In action the pump sucked the water from the jar through the first filter, through the powdered tile, and through the second filter, which caught all fine sediments carried upward by the descending current. It was the intention to weigh the filter and the contents of the tube to ascertain the percentage of loss in weight, if any.

SETTING OF POWDERED TILE

The experiment as outlined was a failure, but several most interesting facts were discovered. At the end of the first day the apparatus began to work badly and more force had to be put on the pump to draw the water through. At the end of two days, the apparatus refused to work at all. It was taken apart and in the "gooch" was found a considerable thickness of brown semigelatinous rubbery mass completely clogging it. Where the water from the peat had passed upward through the powdered tile, that also had been stained brown. The "gooch" was disconnected and the water from the tile was led downward through a long tube acting as a siphon into a large glass container. At the end of another 24 hours the flow had entirely stopped and the powdered tile had "set" firmly.

A sample of powdered tile was moistened with water and allowed to stand overnight, and found to be set firmly in the morning. The same was done with the old tile, powdered, with like result, though the set was not so strong. This was repeated with both tile, using a large excess of carbonated water, the powdered tile being shaken up in the water. Both, on settling, set much more strongly than before.

10-DAY PEAT CULTURES

To overcome this difficulty of the powdered tile "setting" another scheme was devised. Peat from the top of the University Marsh, which gave a slightly acid reaction, peat from the pit, which was alkaline, and peat from north central Wisconsin, which was strongly acid, were placed in glazed earthern jars and distilled water added. The jars were then allowed to stand 10 days in a warm room and the water tested for acidity. All were acid, and in the following proportions when titrated with $\frac{1}{14}$ normal hydroxid solution, using phenolphthalein as an indicator.

TABLE VIII.—*Showing amount of $\frac{1}{14}$ normal alkaline solution required to neutralize acidity of 100 cc. water from 10-day cultures of surface and deep peat from University Marsh, and northern acid peat; also its equivalent in grams of free lime per liter*

Water sample (10-day culture).	$\frac{1}{14}$ normal solution used.	Equivalent per liter to CaO.
	cc.	*Gm.*
Surface peat (a)	0.55	0.0110
Do (b)	.60	.0120
5-inch peat (a)	.45	.0090
Do (b)	.50	.0100
Northern acid peat (a)	6.75	.1350
Do (b)	6.80	.1360

TESTS WITH 10-DAY CULTURES

Duplicate bottles were prepared containing 2 gm. each of new tile and to each was added 400 cc. of distilled water. The same was done with carbonated water, marginal water, bog water, surface, deep peat, and northern peat culture. These were immediately tightly stoppered, placed in a shaker machine, and agitated continuously 54 hours.

It will be noted that only the northern peat water was in sufficient quantity to neutralize the free alkali in the 2 gm. of tile. This was further indicated by the rate of settling of the solids held in suspension. While all the other bottles, even the carbonated water, settled overnight, the acid peat water was still turbid after 3 days and showed distinct layers of colloidial material slowly settling in the liquid above the sediment in the bottom.

Hall (*6*) has shown that the alkali earth, particularly the carbonates, have a strong flocculating action assisting in the deposition of sediments. The results just obtained indicate that the products of organic decay have an even more powerful effect in retaining them in suspension.

TABLE IX.—*Residue left after treating 2 gm. of tile with 400 cc. of water as in Tables I and VIII (agitated 54 hours and settled 14 hours)*

400 cc. of—	Weight of tile used.	Weight of residue.	Soluble.	Increase.
	Gm.	*Gm.*	*Per cent.*	*Per cent.*
Distilled water (a)	2	1.8796	6.02	.....
Do (b)	2	1.8532	7.34	.....
Carbonated water (a)	2	1.8983	5.08	.....
Do (b)	2	1.8727	6.36	.....
Winter waters:				
Marginal water (a)	2	2.0270	.....	1.35
Do (b)	2	2.002	.....	.01
Bog water (a)	2	2.0100	.....	.50
Do (b)	2	2.0119	.....	.559
10-day cultures:				
Surface peat (a)	2	2.0965	.....	4.82
Do (b)	2	2.0960	.....	4.80
Peat 5 feet deep (a)	2	2.0090	.....	.45
Do (b)	2	1.8948	5.26	.....
Northern peat [1] (a)	2	2.2007	.....	10.03
Do (b)	2	2.0885	.....	4.12

[1] The duplicates of the last two pairs showed distinct differences in the color of their filtrates; the darker filtrate passing through much more rapidly, possibly due to a thinner filter, and carrying colloidal material with it.

SOLUBILITY OF TILE IN DECARBONATED PEAT WATER

Next, 400 cc. of the most acid peat water was taken, from which all carbonic acid had been removed by drawing through it air passed through sodium hydroxid, and 2 gm. of powdered tile were added. The solution was then agitated in the shaker machine, the residue filtered out on a dried, weighed, ashless filter, dried in an electric oven to constant dryness, weighed, and then residue and filter agitated for 14 hours in 400 cc. of carbonated water.

TABLE X.—*Results of agitating 2 gm. of tile with acid peat water and treating the residue with carbonic acid water* [1]

Item.	Weight after treating with acid peat water.	Weight of residue treated with carbonated water.
Original sample.....(gm.)..	2. 0000	2. 1288
Residue.....(gm.)..	2. 1288	1. 9129
Per cent increase.....	6. 44	.....
Per cent decrease from sample treated with peat water....	.....	10. 14
Per cent decrease from original.....	.....	4. 25

[1] The filtering of the solution of tile with peat water was carried on with considerable difficulty, owing to the colloidal nature of the products, yet the solution showed only a slight turbidity.

INCREASING ACIDITY OF ROTTING PEAT

In order to ascertain whether the process of decay increased the percentage of acid in solution or not, the acidity of the water on the peat cultures was tested after 20 days and compared with the 10-day culture. Conditions in the northern peat culture had been altered by the addition of more water, so that was excluded from the calculations.

A titration of 100 cc. of the water with $\frac{1}{14}$ normal solution of sodium hydroxid was made, using phenolphthalein as an indicator.

TABLE XI.—*Amount of $\frac{1}{14}$ normal alkaline solution required to neutralize the acidity of 100 cc. of water from 20-day cultures of surface peat and deep peat from University Marsh and the equivalent in grams of free lime per liter*

Sample taken.	Amount of $\frac{1}{14}$ solution used.	Equivalent to CaO per liter.
	cc	*Gm.*
Surface peat (a).....	2. 95	0. 0590
Do (b).....	3. 15	0. 0630
5 feet peat (a).....	3. 50	0. 0700
Do (b).....	3. 30	0. 0660

TABLE XII.—*Summary of Tables X and XI, showing acidity of 10-day cultures equivalent to grams of free lime per liter of water*

Sample taken.	Original state.	CaO equivalent.	
		10-day culture.	*20-day culture.*
Surface peat (a)	Faintly acid	0. 0110	0. 0590
Do (b)	....do	. 0120	. 0630
Peat 5 feet deep (a)	Alkaline	. 0090	. 0700
Do (b)	....do	. 0100	. 0660
Northern peat (a)	Acid	. 1350	.....
Do (b)	....do	. 1360	.....

During the late winter of 1922, with the assistance of Mr. R. C. Reck, chemist of the drainage laboratory, University Farm, St. Paul, a series of determinations were run, using peat solutions derived from Coon Creek peat, which was alkaline. Distilled water was put on a quantity of the deeper peat from the Coon Creek experimental tracts. The water was tested one-half hour after being put on the peat, again at 10 days, and again after 30 days, the 30-day test water being free from carbonic acid. The results of the tests, in which methyl orange was used as an indicator, are given in Table XIII.

TABLE XIII.—*Determinations of relative alkalinity or acidity of water from Coon Creek peat at different ages*

Age of test water.	Test No.	Amount taken.	$\frac{N}{20}$ solution used.	CaO equivalent per liter.
		cc.	*Gm.*	*Gm.*
1st day	1	25	0. 52	0. 0292
Do	2	50	1. 04	. 0292
10th day	1	Neut.	.....	.....
Do	2	Neut.	.....	.....
30th day	1	25	. 40	. 0224
Do	2	25	. 35	. 0196

In order to ascertain if any effect were produced on the concrete tile by a weak solution of organic acid free from carbonic acid alternating with carbonic acid, 2 liters of the peat solution were taken and air passed through for 24 hours. The air was first drawn through moist sodium hydroxid to eliminate CO_2. The water was then tested for acid, with results given in Table XIII. Two flasks were then taken and 400 cc. of the peat water placed in each. One gram of oven-dry, powdered tile was then added to each bottle. The bottles were then corked and agitated for 48 hours. The solutions were then filtered on weighed ashless filters and the residues dried and weighed. The residues, which were not removed from the filter, were replaced in the flasks and to each was added 400 cc. of distilled water containing pure carbonic acid. This carbonic acid water was tested and found to be 0.016 normal strength. The solutions were then agitated for 24 hours and the residue dried and weighed. The residue was then treated again with peat water and carbonic acid water, agitating 24 hours. The results are tabulated in Table XIV.

TABLE XIV.—*Effect on concrete by treating repeatedly with weak peat water and with CO_2 water*

Item.	Sample 1.	Sample 2.
Original weight (gm.)	1.0000	1.0000
Residue after treatment with 400 c. c. of peat water (gm.)	.9684	1.0464
Residue after treatment with 400 c. c. of CO_2 water 0.016 normal strength (gm.)	.8367	.9120
Residue after second treatment with peat water (gm.)	.7879	.9167
Residue after second treatment with CO_2 water (gm.)	.7843	.8890
Total net loss (gm.)	.2157	.1110
Total net loss (per cent)	21.5700	11.1000

These experiments gave the first really constructive information. They showed that the powdered tile was powerfully acted upon by the peat waters, the amount of action depending upon the acidity of the peat water. In this action a semigelatinous mass was precipitated out of the solution, increasing the weight of the total solids. On treating with carbonic acid water, this gelatinous mass was removed and about as much tile dissolved as would have been the case had the tile not been previously treated with peat water.

ACTION ON NEAT CEMENT

In order to ascertain if the decaying peat had any action on "neat" cement a small quantity of the same cement as used in Table VII was mixed with water to make a stiff mortar. With this mortar small patties were made by first rolling the mortar into a ball by hand and working it thoroughly together. These balls were then flattened on glass into patties which were 2 inches across, one-half inch thick in the middle, and tapering to a thin edge. The upper surface was troweled with a spatula until the "laitance" was brought to the surface. The patties were then allowed to set in warm water for a week.

After removal from the glass the patties were placed in a jar of peat and kept moist for three months in a warm place. At the end of that time the patties were broken and the fracture examined. It was found that on the lower side where the cement had been against the glass the peat solution had penetrated to about three thirty-seconds of an inch and the surface could be easily scratched with a knife. On the upper side, however, which had been packed by hand and then troweled, there was no sign of any acid penetration, and the surface was very hard and dense.

There was no experimental work done to determine whether the resistant quality of the cement was due to the greater density of the mass, to the bringing to the surface of the "laitance," or to some other cause not indicated.

RESULTS

In addition to the five principal points brought out in the preliminary studies, five additional ones are indicated in the later work. They are:

(6) The setting of the partially decomposed tile on grinding and moistening.

(7) The gelatinous compounds of organic matter and concrete.

(8) The solubility of this compound in carbonic acid.
(9) The action of the organic compounds on neat cement.
(10) The increase in quantity of acids as decomposition proceeds.

PART IV. FIELD WORK

Before drawing a definite conclusion from the results of these laboratory investigations it was decided to make most careful field observations to ascertain if the laboratory findings were borne out by actual conditions. These observations have covered two years and during that time the subject has attracted considerable public attention. Reports have come in, notably from the muck lands of the South, which considerably broaden the scope of the investigations.

REPRESENTATIVE LOCALITIES

The field work was planned so that it should cover the greatest possible range of soil conditions. No attempt was made to get mere numbers of observations. On the other hand, an attempt was made to investigate the peats overlying dissimilar geological formations, first in Wisconsin and later in Minnesota.

LIMESTONE SOIL

In Wisconsin, eastern Dane County was taken as typical of the glaciated limestone country. This includes University Marsh, where the peat was underlain by marl. This has been described earlier in this paper. During 1920 a considerable amount of the tile in the eastern part of the marsh was relaid at a greater depth. Among these were alternate lines of Group VII. They included the lines opened up in 1919 from which the samples were taken. It was found that about half the tile were either collapsed or were not fit to be put back in the ground. This is well illustrated in Plate 3, A. This rapid final collapse, after disintegration was once well under way, later proved to be characteristic.

A large marsh of the alkaline type south of Madison, Wis., was also investigated. It lay in the bottom of a stream valley and in its natural condition would be subject to more or less periodic overflow and seepage and wash would be heavy. Water would either be standing or running in the tile the greater part of the time. A portion of this marsh was drained in 1914. The tile were examined in the spring of 1920. A pile of unused tile showed that the quality was not of the highest, as judged by present standards, but was a very good average at the time it was made. On opening up the tile lines an interesting condition was disclosed. At the outlet of the lines fresh drainage water did not enter the tiles in large quantities but found its outlet into the main ditch. The inside of the tile was covered with sediment and the concrete thereby protected. Under these conditions there was little disintegration. Farther up the lines, where water either stood or ran a larger part of the season, the cement of the tile had almost completely disappeared. On account of their fragility, there was considerable difficulty in taking out samples. The tile apparently retained their perfect shape and were functioning quite properly as long as they were not disturbed. The act of digging, however, caused their complete collapse. At the upper

end of the line, where the lower part of the tile was entirely protected by soft mineral soil, little disintegration had taken place, but the upper part was badly pitted where peat had lain against it (Pl. 2, A).

RECENT LIME DRIFT

Walworth and Ozaukee Counties were taken as representative of the heavy limestone drift area of recent formation and rich in lime. The peat swamps in this area are very narrow, have been subject to heavy wash, and are high in mineral matter. The soils are comparatively tight clays and loams and seepage is negligible. Consequently, the ground water in summer descends below the tile lines and, except for short intervals, usually remains there throughout the growing season (Pl. 2, B). Tile No. 2 came from near the mouth of a short drain where it was not subjected to standing water. No. 3, however, came from opposite the mouth of a low draw where there would be considerable seepage from the surrounding highland. The peat areas in this formation are comparatively small, and as the rate of disintegration is slow it is not expected that it will cause serious difficulty.

OLD RESIDUAL SOILS

Richland County was taken as representative of the unglaciated area, and here it was found that in the acid residual soils the tile broke down with comparative rapidity.

ACID SANDY SOILS

Wood and Juneau Counties were taken as representative of the sandstone area. These soils are for the most part very strongly acid. In this district concrete tile set in peat broke down within a year after they were laid. The sewer tile shown in Plate 4, B, was laid in clay soil high in lime. The outside gave no sign of disintegration but the inside was somewhat pitted. This area appeared to be the most destructive to concrete of any that were investigated. Throughout this whole sandy district the use of concrete in marshes appears to be only a temporary expedient. Opportunity was offered to examine the concrete culverts on the roads through the cranberry marshes of Wood County. Conditions here offer the most severe test that could be devised, for the acid waters of the cranberry marshes and reservoirs remain against the concrete throughout the season. All of the culverts and bridges examined showed signs of disintegration, the destruction having penetrated into the solid concrete. Plate 3, B, shows this very strongly.

GRANITE SOILS

Waupaca County was considered typical of the granite soils. An observation made at Wyanwega was extremely interesting, for the tile were laid not in peat, but in sand underlying muck. An area of some 53 acres was tiled in 1915. In 1917 the tile system began to give trouble. In 1918 several breakdowns occurred, owing to the complete collapse of the tile at those points. In 1919 there were over 20 breakdowns, one of which was about 100 feet long. Strange to say, in spite of its thicker wall, the 8-inch outlet main seemed to suffer the most. Plate 4, A, shows samples from this system. In no case except No. 4 did the peat come in contact with the tile. No. 3 was taken from a tile line on a flat piece of high ground. There was no peat on this line.

MINNESOTA CONDITIONS

In the late fall of 1920, the writer removed to Minnesota. This State does not have drained peat bogs or swamps on the wide range of soils that occur in Winconsin. Practically all of the Minnesota peats that have been drained are of the "high lime" type. A large proportion of them are in sloughs and depressions of the low ground of the northwestern glacial drift whose soil is composed almost entirely of ground-up shale and limestone. The peats themselves are built up mostly of plants of a high botanical order.

The low lime peats of Minnesota lie largely to the north and northeast overlying the more acid, northeastern drift and the rock outcrops of that part of the State. They are derived mostly from the remains of plants of a low order, in which the mosses predominate. Low lime peats in Minnesota have not yet proved their economic importance. Underdrainage of any soils is not far advanced in the northeastern part of the State and the farming of peat can be said to have not yet begun.

GRAND RAPIDS STATION

LOW LIME PEAT

Opportunity was offered to investigate only one tract in Minnesota where a low lime bog had been drained for a number of years. In fact, it is the only one known in the State where concrete tile has been used. This was on the State experimental farm at Grand Rapids, Minn. The tract was tiled in 1910 under the direction of the State experiment station at St. Paul, Minn., Prof. J. T. Stewart being directly in charge of the work. The tile lines were laid an average of about 3½ to 4 feet deep. Part of the area tiled was cultivated experimentally. The rest was left in its natural condition. In 1918 the system showed signs that it was not functioning properly. Water did not drain away after storms as it should. In 1918 almost the entire system was taken up and relaid. It was found that about one-fourth of the tile had completely collapsed or were not fit to put back in the ground. The design of the system was somewhat altered. The 75 per cent of the tile that were in fair condition were used a second time and the balance was replaced with clay tile.

Plate 5, A, B, and C, shows different views of one of the better tile that was stock piled and kept for use. Disintegration was not serious on the outside at top and bottom, but was considerable on the sides. The top inside shows no signs of disintegration, but the bottom inside is very badly eaten.

In June, 1921, the lines were examined. Plate 6, A, shows the specimens taken. No. 1 was from the cultivated area on the central line which was relaid. The specimen came from 50 feet south of the fence. No. 2 was taken from the same line 50 feet north of the fence in uncultivated peat. It is interesting to note the greater disintegration of the tile in the raw peat. The peat at the Grand Rapids station is very fibrous and was cut out by the tilers in large pieces. When these were thrown back into the trench they frequently formed an arch over parts of the tile without touching it. The results of this are shown in No. 2, Plate 6, A, and the actual conditions in the ground in Plate 5, D. On all of the body of the marsh the ground water would drop below the tile lines throughout the greater part of the season. No. 3, Plate 6, A, was taken from mineral soil in a part of a line that was not relaid. This was

a seepage line that ran along the western edge of the bog but, at the place where the sample was taken, cut across a point of high ground about 200 feet wide on which there was no peat.

Crumbs were broken off by hand from the rotted portion of all of these tile, powdered, shaken up with distilled water for one minute and tested for free alkali. All gave a violent alkaline reaction.

The strength and absorption of these tile were tested in the drainage laboratory of the Minnesota Experiment Station by D. G. Miller, senior drainage engineer of the United States Department of Agriculture, and J. A. Wise of the Minnesota Experiment Station. The difference in porosity between the decayed tile and that which was not decayed is not strongly brought out. The fact is indicated that the tile were of high grade for the time they were made.

TABLE XV.—*Results of physical tests of concreate tile in peat (11 years) at Grand Rapids, Minn.*

Item.	No. 1.	No. 2.	No. 3.
Internal diameter (in.)	5	5	6
Weight (lbs.)	8. 72	8. 81	12. 02
Breaking load pounds (per linear ft.)	819	783	851
Absorption, bone dry and boiling method (per cent)	.....	.....	.....
Top in mold, lower side in marsh	11. 8	13. 6	11. 7
Center piece, upper side in marsh	12. 0	.....	10. 2
Center piece, side in marsh	.....	13. 7	.....
Bottom in mold, upper side in marsh	11. 3	11. 9	.....
Bottom in mold, lower side in marsh	.....	.....	10. 5

COON CREEK HIGH LIME PEAT

Early in 1921 a movement was started to show that concrete tile would not disintegrate in peats carrying a high percentage of lime. This claim was supported by most eminent authorities and there was no published work to contradict it. As the high lime peats include all the peat areas of Minnesota that are yet of economic importance and the greater part of those of southern Wisconsin, the point raised was paramount. For the investigators to be able to limit the destruction of concrete to the low-lime peats would be of immense benefit to the States affected, for it would reduce the problem to the proportions of an academic study. It would, however, necessitate a classification of the peat areas before drainage. The results of the observations on high-lime peat are therefore given in some detail.

A peat area was selected that was assumed to offer ideal conditions for the preservation of the tile, i. e., the Coon Creek experimental tract. Here a peat area 3 to 6 feet deep is underlain in its deeper portion with marl of unknown depth and extent but sounded to a thickness of 17 feet.

The tract was selected by Dr. F. J. Alway, chief of the soils division of the Minnesota Agricultural Experiment Station, for experimental work in the agricultural utilization of high lime peats and is maintained for that purpose. On the south, the tract is bounded by low hills of wind-blown sand, derived from the limestone drift of the northwest glaciation. This sand extends out underneath the peat some 300 to 400 feet, where it drops off quite sharply, marl occupying the depression between the sand and the peat above. The part under cultivation extends a little beyond the shoulder where the sand drops away, and the outer lines of tile (laterals

1 and 2 on the map, 1 to the westward and 2 to the eastward of the main) have a few inches of marl under them below the peat.

There is very heavy seepage from the sand hills down through the sand underlying the peat. Natural escape of the seepage water at the main channel of Coon Creek is blocked by the comparatively impermeable marl. The main outlet drain of the tract cuts across the marsh and supplies the necessary outlet. The laterals at the time they were laid were from 3½ to 4 feet deep, and are now 3 to 3½ feet below the surface of the peat. There is from 1 to 1½ feet of peat between the bottom of laterals 1 and 2 and the sand. The main outlet, however, since it was first dug has always been about a foot below the outlets of the laterals. There has been a tendency, therefore, in times of little flow for the seepage water to escape directly from the sand into the main outlet without passing upward into the laterals. This condition would be accentuated both at the lower end of the laterals near the main and at the upper end of the laterals where their grade carries them above the almost flat ground water table. Lateral 1 carries heavy seepage from a bay indenting the high land, and the tendency for the ends of the lateral to get above the ground water level would be partially neutralized. In lateral 2, however, the tendency would be most marked at all low water stages.

The tile system was laid out and installed in 1918 under the direction of Mr. H. B. Roe, drainage engineer of the experiment station. Alternate lines were of clay and concrete. When the concrete tile arrived they were freshly cured and did not meet the strength requirements. As they also appeared to be quite uniformly porous the entire lot was condemned by Mr. Roe. Another test of the tile was made 13 days after the first, which indicated a much greater strength. Under the circumstances it was decided by Doctor Alway that it would be better to use the tile than lose a season. The tile were therefore installed.

TABLE XVI.—*Tests of Coon Creek tile Oct. 2, 1918, one week after delivery (5 and 6 inch tile tested)*

Test No.	Wet weight.	3-point breaking load.	Calculated breaking load.
	Lbs.	*Lbs.*	*Lbs.*
1	9¾	375	562
2	10	375	562
3	10¼	413	620
4	10	325	488
5	10⅛	425	638
Average	10. 025	382. 6	574

TABLE XVII.—*Tests of Coon Creek tile Oct. 15, 1918, three weeks after delivery (5-inch tile tested)*

Test No.	Dry weight.	3-point breaking load.	Calculated breaking load.
	Lbs.	*Lbs.*	*Lbs.*
1	8¾	538	807
2	8⅜	675	1, 013
Average	8. 56	606. 5	910

From Tables XVI and XVII it would appear that the average cold absorption of the air tile was approximately 1.465 pounds, or 17.1 per cent, and that Mr. Roe's condemnation of the tile was well founded.

In June, 1921, an inspection of the tile was arranged for, to take place, on August 1, Doctor Alway acting as intermediary. There were present the local manager of a cement association, the director of the publicity department of the same cement association, the engineer for a Minnesota concrete pipe and tile association, Dr. F. J. Alway, of the soils department of the agricultural engineering branch of the Minnesota Experiment Station, and the writer.

Altogether, five pits were opened, the location of the first three being chosen by the representative of the cement association and the location of the last two being chosen by the writer. The first three pits were all opened on lateral 2 at 265, 450, and 600 feet from the main. This lateral, as previously described, is underlain by marl, at a depth of about 1 foot below the grade of the tile. Two tiles were taken from each pit and were numbered in duplicate 1A, 1B, and 1C. At the writer's suggestion branch a of lateral 1 was opened 20 feet from its outlet and again at 735 feet from its outlet. The first of these two pits was underlain by marl. The second was in uncultivated peat and had no marl below it. Two samples were taken from each pit and numbered in duplicate 2A and 2B. The samples were set up in order on the running board of one of the cars and photographed by members of the party. One set was then chosen by the publicity director of the cement association and the other taken by the writer. The upper and lower sides of the samples taken by Elliott are shown in Plates 7, A and B. It will be noted that the tile which remained above the water table for long periods of time are very little attacked, while the tile which kept moist without much flow even though close to the marl is more disintegrated than those which carried a heavy flow of high ground water. Of the two from branch a, the tile taken from above the sand is more disintegrated than the one from above the marl. Samples of water were taken from the pits by the publicity man. On digging holes to the grade of the tile for the collection of water no water appeared. The holes were then deepened still more. At hole 1 B, the shovel handle was thrust down through the marl to the sand underneath. The water then rose in the hole. At pit 2 B, the hole was deepened almost to the underlying sand, permitting the water to collect. What was done with these samples is not known to the writer, but they can not be taken as representative of the water in the peat. They would be representative of the ground water in the sand subsoil, probably contaminated by the marl and peat.[4]

The samples of the tile taken by Elliott were tested on October 25 in the drainage laboratory of the experiment station. Crumbs were broken by hand from 1B, 2A, and 2B. All showed a strong alkaline reaction. The results of the physical tests are given in Table XVIII.

[4] Since the above was written the 1922 report of the American Society for Testing Materials has come to hand. It is believed that the samples tested by Dr. Witt and referred to in paragraph 3, p. 262 of that report, are the samples just mentioned.

TABLE XVIII.—*Results of physical tests on samples* [1] *2¾ years in peat taken from drains at Coon Creek Aug. 1, 1921 (5-inch tile tested)*

Physical tests.	Sample. 1A.	Sample. 1B.	Sample. 1C.	Sample. 2A.	Sample. 2B.
Weight after 3 months drying (pounds).	9. 70	9. 02	9. 19	9. 27	9. 53
Breaking load (pounds)	826	804	1007	810	1016
Absorption, bone dry and boiling method;					
Top piece in mold	12. 9	15. 6	13. 7	13. 8	13. 6
Center piece in mold	14. 3	17. 9	15. 1	16. 2	15. 4
Bottom piece in mold	14. 1	13. 1	13. 3	17. 4	15. 8
Average	13. 8	15. 5	14. 0	15. 8	14. 9

[1] A considerable number of tile had been left over when the job was finished, and had been stock piled on high ground at the edge of the marsh. Of these, 10 were taken as a sample, 5 being given to the soils department and 5 tested on October 25 in the drainage laboratory.

TABLE XIX.—*Results of physical tests on concrete tile from stock pile at Coon Creek (5-inch tile tested, age 3 years)*

Physical tests.	Sample 1-6.	Sample 1-7.	Sample 1-8.	Sample 1-9.	Sample 1-10.
Weight after 3 months drying (pounds.)	10. 27	9. 30	10. 25	9. 61	10. 00
Breaking load (pounds)	1152	983	1196	830	1265
Absorption:					
Top piece in mold	12. 2	13. 3	11. 8	15. 1	13. 4
Center piece in mold	12. 2	15. 6	13. 1	14. 1	16. 3
Bottom piece in mold	13. 2	14. 2	13. 0	14. 5	14. 3
Average	12. 5	14. 4	12. 6	14. 6	14. 7

A comparison of the figures in these tests discloses some interesting facts that are tabulated in Table XX.

TABLE XX.—*Comparison of physical tests on Coon Creek tile at different ages and under different conditions*

Physical tests.	1 week after delivery.	3 weeks after delivery.	3 years old in stock pile.	2¾ years old in peat.
Weight, air dry average	.....	8. 56	9. 89	9. 34
Wet weight	10. 025	.....	.....	.....
Breaking load, dry	.....	910	.....	.....
Breaking load, wet	574	.....	1085	893
Absorption, cold, per cent (estimated)	17. 1	.....	.....	.....
Absorption, boiling	.....	.....	13. 8	14. 8
Absorption, top piece in mold	.....	.....	13. 2	13. 9
Absorption, center piece in mold	.....	.....	14. 3	15. 8
Absorption, bottom piece in mold	.....	.....	13. 8	14. 7

TABLE XXI.[1]—*Comparison of tile in peat with tile from stock pile at Coon Creek, tested Oct. 25, 1921*

Item.	Per cent.
Loss in weight	5.5
Loss in strength	17.8
Increase in porosity:	
Ends	5.2
Middle	10.4

[1] Summary of Table XIX

PEAT IN LAKE PRAIRIE DISTRICT

On September 19, 1921, a concrete tile line was examined on the NW. ¼ and SW. ¼, sec. 29, township of Lake Prairie, about 10 miles northwest of St. Peter, Minn. In 1919, an 8-inch tile had been laid a short distance into a peat pocket about 1,200 feet long. The line was not completed. After eight months in the ground the tile was taken up and found to be very badly disintegrated on the bottom, as shown in Plate 6, B. New tile was laid in the fall of 1920 and the main completed. At the time of the examination it also showed signs of serious disintegration. No samples of the new tile were taken. Tests of the old tile showed physical conditions as summarized in Table XXII.

Table XXII.—*Absorption tests, bone-dry and boiling method, of concrete tile in peat 8 months, and on bank 1 year, near St. Peter, Minn.*

Sample taken.	No. 1.	No. 2.
Top piece in mold	13.0	25.3
Center piece in mold	16.6	19.3
Bottom piece in mold	14.6	14.6
Average	14.7	19.8

All of the fragments of tile No. 1 were from the upper side. The upper and center fragments of tile No. 2 were from the partially disintegrated portion. The tile were apparently of about the same character originally, a rather low grade without sufficient coarse aggregate.

RESULTS

In addition to the 10 points brought out in the laboratory, the field studies indicate the following:

(11) Concrete tile, as at present made, break down in all peat soils, no matter what the underlying mineral soil may be.

(12) A high percentage of lime, even the presence of marl, is no guaranty of immunity.

(13) A high percentage of lime delays but does not stop the process of disintegration.

(14) An acid subsoil aids in the disintegration.

(15) The more porous the tile the more rapid the disintegration.

(16) The presence of water is necessary for disintegration to take place.

(17) It is not necessary for the tile to be actually in the peat for disintegration to take place, but merely that the peat waters shall have free access to it.

(18) The violent alkalinity of the tile, continuing even to the time of complete disintegration.

(19) The destruction of solid concrete if the acid waters lie against it continuously.

PART V. DISCUSSION AND CONCLUSIONS

The mechanical adaptability of such a material as concrete to the manufacture of drain tile and the aid which it can give to drainage work is very great. Its permanence in ordinary soils seems to be all that can be desired. That, however, is not a part of the discussion of this paper. It is with the suitability of concrete for peat soils or soils high in organic matter with which this paper deals.

From the facts brought out in the investigation it would seem that there are some things which should be remedied before concrete tile, as now made, can be said to be suitable for use in soils high in organic matter. The first of these difficulties is the presence of free alkali. The observations of the past year seem to indicate that this is a characteristic of all concrete tile, retained even to the time of complete collapse. If it is true that free alkalinity is an inseparable characteristic of concrete as now made, and if water is present as a conveying medium, then the ultimate destruction of the tile in the presence of organic acid seems inevitable. The free alkali and the free acid are incompatible and must react against one another. This might not be serious if the products of the reaction were insoluble in water. The investigation has shown that they are highly gelatinous and are very readily soluble in water carrying carbonic acid.

That carbonic acid is absorbed in large quantities by the concrete is indicated by the tests with the cement patties. It may also have had considerable to do with the increase in weight and density of the tile which lay in the stock pile at Coon Creek.

Destruction of the concrete by water carrying carbonic acid must eventually take place exactly as the lime is leached from the surface of any soil by the formation of the double carbonates of calcium, but this process probably would be extremely slow and would not be of economic interest to the engineer. It is the rate of decomposition that is the controlling factor.

DISINTEGRATION IN LOW-LIME PEATS

On this rate of decomposition, the character of the surrounding mineral soil, the porosity of the tile and the character of the ground water flow, seem to be the controlling factors. In strongly acid soils, with the peat wet throughout the season, ordinary tile appear to break down after one year. At Grand Rapids, Minn., the peat is highly acid but the tile were laid shallow and the ground water, except for short intervals, kept well below them through the greater part of the season. Some of the tile were in fair condition at the end of 11 years. At Weyauwega, Wis., in acid soil in the presence of abundant moisture, the tile were not placed directly in peat but in the sand below it. The system began to give trouble after two years and was very bad in three. In Wood and Juneau Counties, Wis., in acid peat above acid subsoil poor tile break down in a year.

DISINTEGRATION IN HIGH-LIME PEATS

In the high-lime peats the situation is much more complex. Water still is a controlling factor. At St. Peter, Minn., a rather poor grade of tile broke down in a season. This was in a so called running slough in Prairie Country which would therefore be high in lime, but the bottoms of the tile were wet throughout the season. At Coon Creek, during the two and one-half seasons they were in the ground, the tile disintegrated most rapidly where there was a gentle flow or soakage of water regardless of the presence of lime in the form of marl. Where the flow of ground water was stronger, even though marl was not present, disintegration was not so great. Where the tile were well above the ground water level throughout the greater part of the season, disintegration amounted to very little.

On University Marsh the conditions were very unusual. The marsh surface was below the level of Lake Mendota. There is, in addition, heavy seepage from the surrounding hills. This seepage coming through the marl bed was so strong that the peat was prevented from attacking the bottoms of the tile. This is the reverse of what is commonly found. The tops of the tile were, however, above the seepage water and were destroyed after six years. This is the more remarkable in the light of the fact that during the winter the seepage was sufficiently strong to turn the deeper peat strongly alkaline and almost neutralize it to the surface.

In Ozaukee County the least proportional disintegration was found. The marsh in this case was narrow and the main ditch comparatively deep. The tile would be above the water level throughout the greater part of the season. As was to be expected, the greatest disintegration was found where the tile were kept continuously moist. In none of the three observations made in this county was there any serious disintegration after four years.

PRODUCTION OF ORGANIC ACID

One of the most interesting facts brought out by the investigation was the extremely rapid production of organic acids where moist peat was kept at a warm temperature. In the laboratory culture of peats from University Marsh, both the peat from the surface, which was very faintly acid, and the peat from 5 feet deep, which was strongly alkaline, in 10 days became strongly acid. In another 10 days the surface peat contained 5 times as much acid as it did at the end of the first 10 days. The deep peat in the second 10 days not only increased its acidity to 7 times what it was at the end of the first 10 days but actually outstripped the shallow peat. The northern peat which was but faintly acid in the beginning became violently acid at the end of 10 days.

The same thing was done during this past winter with peat from Coon Creek. The peat culture in distilled water required a week to overcome its original alkalinity, after which time the increase in acid was rapid.

It is not assumed that exactly the same sort of accumulation of acid will occur in nature though the process of production may be identical. What will occur will be the production on an enormous scale of the products of decay. Much of these will naturally escape and be carried away by ground water. In the cooler months, particularly in the winter, the escape of the products of decay will probably more than keep pace with

their production. But in the summer months when run-off is light and decay rapid, the products of decay must accumulate to a very great degree. If there is any run-off through the tile lines, these products of decay will find their way to the tiles along with the drain water. The percentage of concentration of the acid in the drain water may be very low, but the actual amount passing during a season would be enormous. With the drain water also carrying carbonic acid, the complex compounds formed with the cement would be immediately carried away, presenting a fresh face to the action of the organic acids.

Though this process is evidently very similar to the leaching of lime from surface soil, a little thought will show that they are not exactly analogous. The lime in the soil is present in a comparatively inert form. It has been demonstrated that the concrete of the tile is chemically active even to the very end of the tile. It is possible that the ordinary soil is better able to retain the complex lime-organic compounds than is the peat. It has been shown that tile near the surface of a well-drained peat are not greatly affected. It is possible that the greatest injury is done by organic compounds that are produced in quantity only where the conditions are suitable. It is possible that these conditions may not exist in a mineral soil. This leads us to the statement that the deductions from this investigation can not be stretched to include the use of concrete tile in mineral soils whether acid or alkaline.

ECONOMIC FACTORS

Though, under certain conditions, the life of a concrete tile in peat soil may be very short, under certain other conditions it may continue a long time. In general, it may be said that the average life of concrete tile, as they have been made, is about six years. The tile may hold on for a considerable time during which the violent alkalinity is being neutralized, after which the final collapse is rapid. In peat soils the collapse of the tile may not be as serious as it would be in a mineral soil, nor may the collapse at once become apparent. If the drain is not closed by the tramping of stock or by farming operations, the underground channel may remain open for years, functioning nearly as well as the original drain.

It will probably be said that the quality and density of concrete tile is improving rapidly and that the tile at Coon Creek, for instance, were not good tile and were condemned before they went into the ground. Even though they were not good tile, however, they were no worse than many hundreds of miles of tile that have been used. They were shipped from the factory while still "green," and the first test was made when they were only partly cured. That is not the point, however. The point is that during the two years in the ground the tile lost in every quality that fits them for the work for which they are intended.

If the average expectation of the life of the tile were 40 or 50 years or upward, there would probably be no question as to the advisability of using concrete tile, but with an expectation of only 6 or 10 years it does not seem that, as now made, their use in peat is sound economics. It would seem to be a question whether or not they would pay for the investment and give a sufficient profit during that time to reimburse the land owners for the trouble and business hazard involved. No doubt improvements in the quality of the tile which are now being generally made will greatly increase the life of the tile, but those improvements are with few

exceptions leading to the improvement of one quality only, and that is density.

But density alone is not sufficient. In the progress of the field work, the greatest possible range of soils was investigated. It is significant that in no place where concrete tile were in peat for two or more years were the tile free from some percentage of disintegration. We can not assume that all of these tile, covering some 30 odd observations in two States, were below the average in quality.

IMPROVEMENT NECESSARY

It would seem that the first requisite for making the concrete permanent in a soil high in organic acids is the elimination of the free alkali. It is generally understood that an excess of lime is added to the materials of which cement is made in order to give it setting qualities and plasticity. If these qualities are necessary for a general purpose cement, it is possible that a special cement might be made for use in drain tile in peat soils which would not have the objectionable features of the standard product.

The second suggested improvement would probably be covered by the first, but if the first were not put into effect it would seem that a very distinct improvement could be effected by finer grinding. This would permit of more complete hydration at the time of setting. The "setting" of the old tile when it was reground would seem to indicate that perhaps the original particles of cement were too large for the process of hydration to be complete even after five years.

The third suggested improvement in the manufacture of concrete tile for use in peat soils is the addition of some substance which would either render the constituents of the cement chemically inert or would so coat the particles of the tile that the entrance of water would be prevented. If this could be done the tile would last as long as the coating remained intact. If such a material were used it must of necessity be of such a character that it itself would not decay.

The fourth method of improvement is the one that is at present being used. It involves greater care in the mechanics of manufacture of the tile, and includes washing, grading, and elimination of soft materials from the aggregate, better mixing and blending and better packing and curing. The result is a stronger, denser tile that is greatly superior to the tile made by a more careless process. Its life in any soil, but particularly in soils which carried inimical solutions, would be very greatly lengthened. It is possible that this method combined with the addition of a preservative substance will produce the desired results. Very great improvement has already been made along that line, and the utmost thought and care should be expended in an effort to produce a concrete that is unquestionably permanent.

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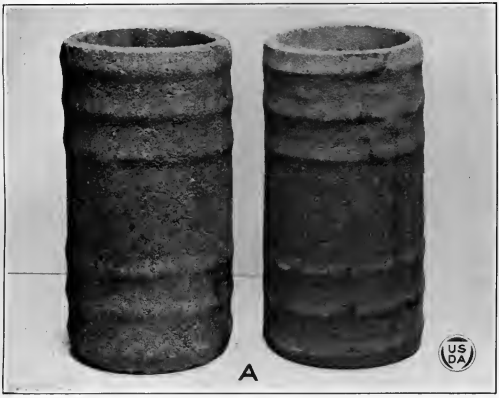
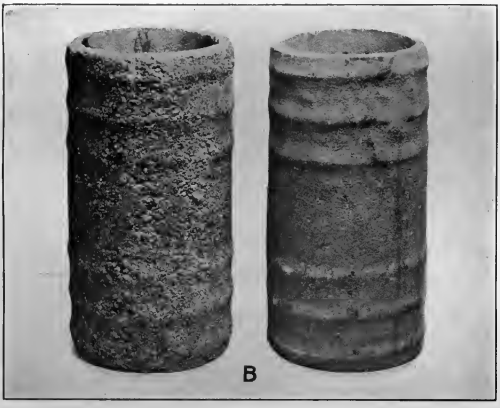
PLATE 1

A.—Concrete tile on left laid in deep peat on University Marsh in 1918. Taken up in 1919. The tile to the right is new.

B.—Concrete tile at left laid in peat 3½ feet deep underlain by marl in University Marsh in 1914. Taken up in 1919. The tile to the right is new.


A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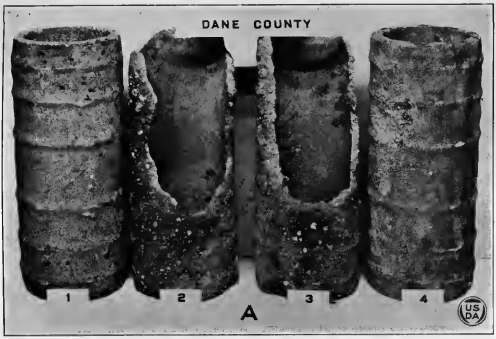

DANE COUNTY
1
2
3
4
A
USDA

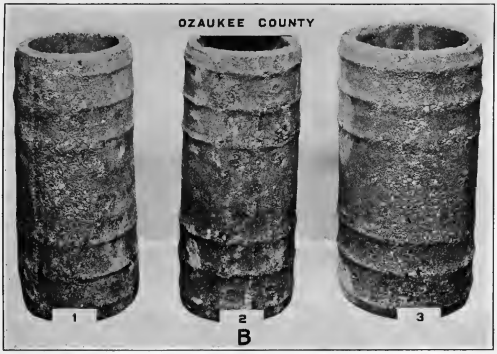

OZAUKEE COUNTY
1
2
3
B

PLATE 2

A.—Concrete tile laid near Madison, Wis., in 1914; taken up in 1920. No. 1 has not been in the ground. Nos. 2 and 3 were laid 3½ feet deep in peat. No. 4 was in silt loam overlain by 2 feet of peat.

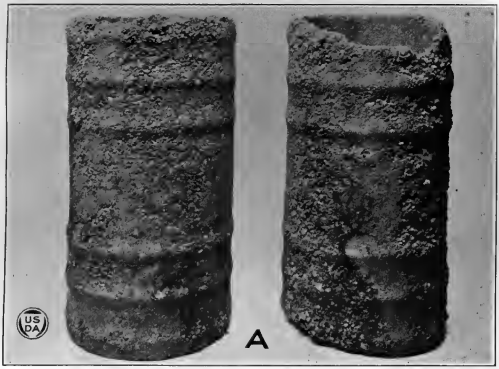
B.—Concrete tile laid in recent soil high in lime in 1916; taken up in 1920. No. 1 was in peat carrying a high percentage of alluvial matter and getting high ground wash. No. 2 was at the outlet of a drain in 4 feet of peat. No. 3 was in 4½ feet of peat overlying sand.

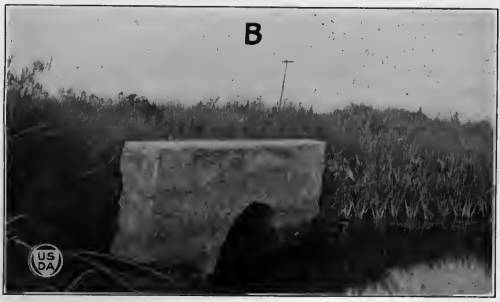
39363—23——5

PLATE 3

A.—Tile from University Marsh. From same line as in Plate 1B, but taken up in fall of 1920. Note progressive disintegration in Plates 1, A, 1, B, and 3, A.

B.—Concrete culvert in Gaynor Road, Grand Rapids, Wis. Built about 1910. Photographed September, 1920. Acid water from the cranberry marshes stands against the concrete at all times.


A


B


WAUPACA COUNTY
1
2
3
4
5
A
USDA


B
X 6205
USDA

PLATE 4

A.—Concrete tile laid in Central Plain of Wisconsin in 1915, taken up in 1919. No. 1 lay on the ground. No. 2 was in sand below 8 inches of black muck. No. 3 was in silt loam with sand at 3 feet 6 inches. No. 4 was in bowlder clay underlying 12 inches of black muck. No. 5 was in sand below 8 inches of black muck.

B.—Concrete tile laid in a sewer in Milwaukee about 1885. Taken up in 1919.

PLATE 5

A.—Tile from Grand Rapids Experimental Farm. Laid 1910. Taken up 1918. Laid in pile until 1921.

B.—Same as Plate 5, A, showing tile disintegrated on inside at bottom.

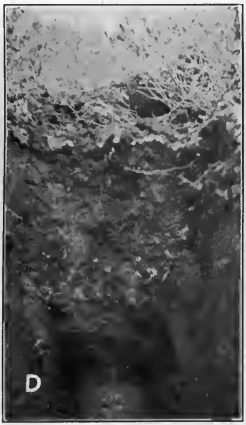
C.—Similar to preceding, showing disintegration at water line.

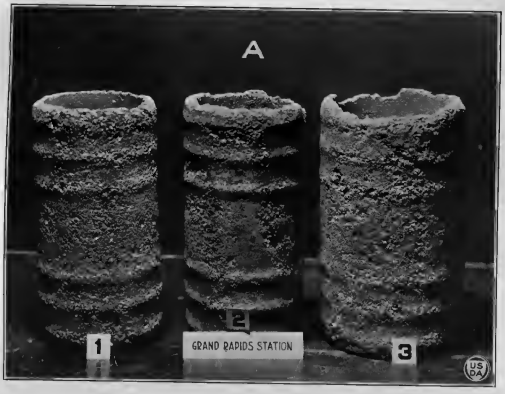
D.—Grand Rapids station, showing sample No. 2 in the ground with the peat arched above it.


GRAND RAPIDS STATION
A
USDA


B
USDA


GRAND RAPIDS STATION
C
USDA


D


A
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GRAND RAPIDS STATION
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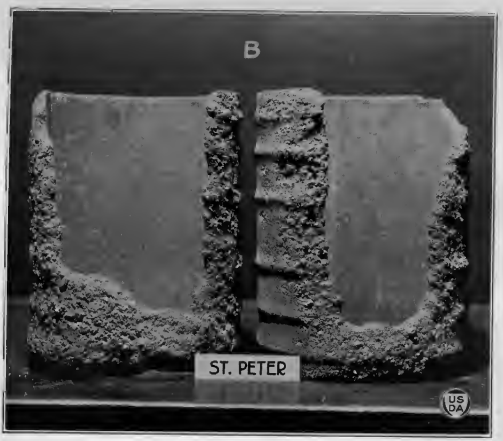
B
ST. PETER
US DA

PLATE 6

A.—Some of same lot laid 1910. Taken up 1918. About one-quarter of tile discarded; remainder relaid. Samples taken up 1921. No. 1, bottom of tile in deep peat. No. 2, top of tile in deep peat. No. 3 taken from 3½ feet deep in mineral soil. Seepage line, but carried peat waters. This portion of line had not been relaid since 1910.

B.—Eight-inch tile from line in peat northwest of St. Peter. Laid in 1919. Taken up after eight months. Second set, relaid in 1920, showed signs of heavy disintegration in September, 1921.

PLATE 7

A.—Upper side of set of tile from Coon Creek, taken by Elliott for testing; 1A, 1B, and 1C taken from pits on lateral 2, peat underlain by marl; 2A and 2B from pits on branch a of lateral 1, 2A peat underlain by marl, 2B peat underlain by sand.

B.—Bottoms of same set as shown in A.


USDA
2B
2A
1C
1B
1A
COON COREK
A


USDA
2B
2A
1C
1B
1A
B

INJURY TO FOLIAGE BY ARSENICAL SPRAY MIXTURES[1]

By D. B. SWINGLE, *Botanist*, H. E. MORRIS, *Assistant Botanist*, and EDMUND BURKE, *Chemist, Montana Agricultural Experiment Station*

INTRODUCTION

Since the first trials of Paris green and of arsenic trioxid for the control of the potato beetle half a century ago there has been a constant increase in the use of arsenical compounds for controlling the ravages of certain species of insects on economic plants.

It is a notable fact that of the many poisonous substances known to physiological chemistry none has been found so well suited to the purpose of poisoning insects that obtain their food by gnawing away portions of the fruit and foliage as certain arsenical compounds. As the control of such insects is a very important field in the science of entomology, much practical and scientific interest centers around this group of compounds. This interest makes imperative the obtaining of more information along certain lines; for it is agreed that an arsenical compound to be a satisfactory insecticide for application to foliage shall approach perfection in these respects: (1) It must promptly kill a large proportion of the insects; (2) it must be relatively inexpensive; (3) it must not seriously injure the plants to which it is applied under the conditions obtaining. Unfortunately, no compound has yet come to notice that perfectly satisfies these requirements, though several do so sufficiently well to be extensively used. It is with the third requirement that this investigation deals, although the others are constantly kept in mind.

The problem of arsenical injury to fruit trees and garden crops as a result of spraying is a troublesome one. Some of the compounds first tried have been abandoned or greatly restricted because of the injury produced and new ones have been proposed to take their places; and these in turn may yet give way to others.

It has long been evident that there are factors influencing arsenical injury that the horticulturist does not understand and others that he is powerless to control. Considerable work has been done to show the nature and relative importance of these factors. All of this work has been fragmentary and most of it has been done under such conditions that it is impossible to judge the relative importance of two or more factors operating at the same time. In most cases the exact composition of the mixture used was not known and in many cases the fact that quite different chemicals appear under the same name evidently was not even suspected. Some of the conclusions drawn are quite contradictory, and others, though perhaps correct, are based on so little evidence that their soundness is questioned. It is probably safe to say that at the time this investigation was undertaken at this station in 1912 enough correct conclusions on this subject had already been drawn to make certain phases of this work largely unnecessary if these correct conclusions had been recognized and isolated from the mass of apparently conflicting data and theories. Such a distinction had, however, been found impossible.

[1] Accepted for publication Sept. 2, 1922.

ARSENICAL COMPOUNDS USED AS INSECTICIDES

As some of the confusion on the subject of arsenical injury can be explained by a study of the processes of manufacture resulting in somewhat different compounds being sold under the same name, a brief review of these processes will be in order. It will also be necessary to discuss briefly the use of the more important arsenical insecticides that the significance of the work that follows may be evident.

ARSENIC TRIOXID

PREPARATION

Arsenic trioxid is obtained in large quantities as a by-product in roasting arsenical ores. This is not pure, but contains some metallic arsenic, arsenic sulphid, dirt, etc., from which it is purified by resublimation. There are two forms of the trioxid known, the amorphous and the crystalline. The amorphous changes slowly into the crystalline form under some conditions. At 25° C. the amorphous form is soluble in about 30 parts of cold water, while the crystalline requires about 100 parts. Both are slowly but completely soluble in about 15 parts of boiling water. The above proportions are approximate, as there seems to be a lack of agreement in the statements of various writers in regard to the solubility of the oxid. The name "arsenious acid," by which it is often referred, is a misnomer, since the oxid is an acid anhydride and has no acid properties until it unites with water, when arsenious acid is formed. In all probability the caustic effect of arsenic trioxid on vegetation is due to its combining with water and forming arsenious acid. Therefore the injury is proportional to the amount of arsenious acid formed and not to the amount of arsenic oxid in suspension.

USE AS AN INSECTICIDE

According to Bourcart (*1, p. 95*)[2] the first trials of using arsenic trioxid for spraying were conducted in America in 1867, when Markham used it to combat the Colorado potato beetle. On account of the serious injury occurring when it was used, other less soluble arsenical compounds were substituted until at the present time the oxid is used almost exclusively in the preparation of poison baits, and as a constituent in the preparation of some of the more usable arsenical compounds.

CALCIUM ARSENITE

Arsenite of lime was probably first recommended as an insecticide by Kilgore (*14*). He recommended that it be made by boiling together for one-half hour, in 2 to 5 gallons of water, white arsenic 1 pound, lime 2 pounds, and diluting the required volume to, say, 100 gallons. It is desirable that the lime should be present in the boiling solution of white arsenic, since it renders the latter insoluble as fast as it goes into solution, thus reducing the volume of water and shortening the time for obtaining the arsenite. Calcium arsenite is not manufactured for an insecticide, but many cases of foliage injury have been reported from its use, showing that it is more or less dangerous to use, and for this reason is not generally recommended.

[2] Reference is made by number (italic) to "Literature cited," pp. 535–537.

COPPER ACETO-ARSENITE (PARIS GREEN)

PREPARATION

Copper aceto-arsenite is a compound of copper, arsenic, and acetic acid, $Cu(C_2H_3O_2)_2.Cu_3As_2O_6$, and theoretically contains 58.65 per cent arsenious oxid (As_2O_3), 31.29 per cent copper oxid (CuO), and 10.06 per cent acetic acid, $C_2H_4O_2$. In the process of manufacture verdigris and arsenic trioxid are the essential materials. Generally verdigris is dissolved in acetic acid and added to a boiling solution of white arsenic and allowed to stand for some time to completely precipitate the copper aceto-arsenite. The light green precipitate is then thoroughly washed with hot water to remove the soluble salts.

Copper aceto-arsenite has several synonyms, the most important of which are Paris green, Schweinfurt green, and emerald green. The compound was discovered in 1814 during the course of experiments, with the object of preparing an improved Scheele's green or arsenite of copper for use as a coloring pigment in the arts. It is supposed to have been first made at Schweinfurt, Germany, but by whom is not recorded. On account of its poisonous nature its use as a pigment was limited, and at present it is scarcely used at all for this purpose, but has become one of the standard insecticide compounds, not only in this country but abroad.

USE AS AN INSECTICIDE

The history of the use of Paris green as an insecticide may be traced back to the period when the Colorado potato beetle became recognized as being of economic importance. The first published account of the destructive propensities of this beetle may be found in the Prairie Farmer, August 29, 1861, (7) and Paris green appeared upon the scene at some time between 1860 and 1870, but who first suggested it and who first used it for the destruction of the potato beetle will perhaps never be told. The use of this material as a standard insecticide undoubtedly began in the West Central States. At first the poison was applied in the powdered form, using flour, plaster, or ashes as a diluent. In 1872 Le Baron (*15, p. 116*), State entomologist of Illinois, in referring to the spring canker-worm, recommended that—

> strong washes, such as Paris-green water, or suds made from whale-oil soap, thrown upon the trees with a garden syringe, will also materially check their depredations.

This is probably the first statement in which the syringing or spraying of apple trees with Paris green is recommended. The first statement referring to the successful control of codling moth by the use of Paris green that attracted attention and which was followed by close investigation, appears to have been made by Edward P. Haynes (Lodeman, *16, 1910*) in 1878. After spraying his orchard with Paris green he said that it not only rid the orchard of cankerworms but the apples on the sprayed part were much less eaten by codling moths. However, the use of this poison for the destruction of foliage-eating insects was for various reasons adopted slowly, and it was not until about 10 years later that Paris green was freely recommended as one of the most valuable insecticides for the destruction of chewing insects.

FERROUS ARSENATE

The use of arsenate of iron as an insecticide in this country is in an experimental stage and this compound has been used to only a limited extent.

PREPARATION

In France, Vermorel and Dantony (*32*), who experimented with iron arsenate for several years, recommended that it should be prepared as follows in order to get the maximum adhesive power: 400 grams ferrous sulphate in 10 liters of water is added to an equal amount of arsenate of soda in 10 liters and stirred constantly. This stock solution is diluted to 100 liters and will contain about 200 gm. ferrous arsenate per hectoliter.

USE AS AN INSECTICIDE

Upon its use as an insecticide Smith (*28*) reports the use of arsenate of iron containing 45 per cent arsenious oxide on elm at the rate of 1¼ pounds to 100 gallons of water without injury, and that it had good insecticidal qualities. Vermorel and Dantony (*32*) used iron arsenate against the codling moth in 1906, 1907, and 1908 and concluded that the action of iron arsenate is equal or superior to lead arsenate, with the advantages of color, presence of iron versus lead, and cheapness. They concluded that the combination of iron arsenate with copper fungicides is not practicable, first, because such combination is not necessary, and, second, because it is very destructive to foliage, and recommend that iron arsenate be used alone and in no case combined with copper. Taylor (*31, p. 15*) reports the use of iron arsenate against the fall webworm in 1909, using 1 to 3 pounds to 50 gallons with promising results and no injury to peach foliage. Melander (*19*) says iron arsenate is a new spray that has the merit of cheapness, but that it is not on the market. He gives brief directions for its preparation, but does not discuss results obtained from its use. Scott and Siegler (*25*) concluded that iron arsenate was a slow-acting poison. In field tests on apple they found that it was not an effective insecticide for the codling moth used at the rate of 1 pound to 100 gallons. When used at greater strengths, however, they thought that it should give fairly satisfactory results, but that it would have no advantage over arsenate of lead.

LEAD ARSENATES

PREPARATION

Arsenate of lead for use as an insecticide is usually prepared by one of two methods, according to Haywood and McDonell (*12*). First, by using lead acetate and disodium arsenate when a precipitate of tri-plumbic lead arsenate, $Pb_3(AsO_4)_2$, is formed, or, second, by using nitrate of lead and disodium arsenate when a precipitate of diplumbic lead arsenate $(PbHAsO_4)_2$ is formed.

COMPOSITION

Theoretically the tri-plumbic lead arsenate contains 74.40 per cent of lead oxid (PbO), 25.60 per cent of arsenic oxid (As_2O_5), and 2.59 per cent water of constitution.

On account of various conditions, the commercial lead arsenate only approximates the percentages given above. This will be obvious for the following reasons: Chemically pure salts are too expensive to use in its manufacture, while various conditions such as temperature, concentration, etc., affect the reaction indicated by theory. Recent work by some investigators tends to show that the lead arsenate commonly on

the market is not either of the above forms, but is a double salt of a complex nature. For the purpose of this paper the lead arsenate will be designated as one of the above forms, or a mixture containing the two forms.

USE AS AN INSECTICIDE

Arsenate of lead was first used as an insecticide during the summer of 1893. In the early work of eradication of the gipsy moth it became evident that the known arsenicals could not be used at the requisite strength for killing gipsy moth caterpillars without serious injury to the foliage. Arsenate of soda was suggested as a substitute, but when used it burned the foliage to a greater extent than the other arsenicals. Mr. F. C. Moulton, who was experimenting upon these insecticides during the winter of 1892–93, proposed acetate of lead to precipitate arsenate of soda and in this way obviate the burning of the foliage caused by the latter; and by this method the first arsenate of lead was produced for insecticidal purposes. Arsenate of lead has the following advantages: (1) On hardy foliage it can be used at almost any desired strength without serious injury, (2) it is visible whenever used, and (3) it adheres well. The principal objection is its rather slow killing properties, probably due to its comparatively low arsenic content and slow solubility.

INJURY TO FOLIAGE

When lead arsenate was first used it was thought to possess all the necessary qualifications for an ideal insecticide. It has proved of great value and is used very extensively on apple and other more hardy foliage, but reports from its use on peach are variable, sometimes no injury being reported and in other cases the injury being so severe as to defoliate the trees. Fernald (*8*, *9*) states that—

> it [arsenate of lead] can be used in large proportions, if necessary, even up to 25 pounds to 150 gallons of water, without injury to the foliage. . . . It does not injure the foliage of the most delicate plants, even when used in as large a proportion as 25 pounds, or even more, to 150 gallons of water.

Smith (*26*, *p. 437*, *27*, *p. 8*) states:

> Its great advantage is its harmlessness to plant life of all kinds . . . it is absolutely harmless to foliage at any strength . . . It is the only effective poison of this character that can be safely applied to peach foliage and on conifers.

Many other statements similar to the foregoing are available to show that many considered lead arsenate as almost an ideal insecticide. However, other investigators conducting careful experiments occasionally reported serious injury. Haywood and McDonnell (*12*) say:

> Rather severe injury was caused to the foliage and fruit of the peach by pure lead arsenate, made either from lead acetate or lead nitrate.

Woodworth (*36*) states, in speaking of spraying in the Pajaro Valley with lead arsenate, that:

> While the codling moth was well controlled, the amount of burning was so large that the progress of spraying was entirely checked. . . . The most significant discovery of the year 1906 was that where a lead arsenate was so compounded that all the arsenic acid present was combined with lead no injury was produced on the most delicate foliage. Such a compound is known as the neutral or ortho-arsenate of lead. At that time no manufacturer was able or willing to produce an arsenate of lead of this description, and to this day, excepting the product manufactured here at Watsonville, there is no strictly neutral lead arsenate on the market.

These statements serve as illustrations that foliage injury is not due directly and entirely to the arsenical but is greatly influenced by other

conditions. Haywood and McDonnell (*12*) point out the great variation in the composition of the different samples analyzed, and this, no doubt, has had very much influence on contradictory results. Lead arsenates which are safe to use under dry arid conditions may cause serious injury under conditions of high humidity. Atmospheric conditions following sprayings have a great influence on the action of the spray mixture of the foliage, and for this reason duplicate experiments, when a short time intervenes between them, may not control. Gillette (*11*), Woodworth and Colby (*37*), and others concluded that leaves kept perfectly dry can hardly be injured by the arsenites, but that under conditions of high humidity the injurious action is greatly increased. However, at the present time lead arsenate is recognized as the standard insecticide, not only for orchard spraying but for field and truck crops as well. Against insects it is known as a rather slow poison, but it is effective. In this respect there is considerable difference in the two forms, viz, the triplumbic and the diplumbic ortho-lead arsenate, due in all probabilities to the difference in their arsenic content, which is the poisonous principle.

LONDON PURPLE

PREPARATION

London purple consists of calcium arsenite, calcium arsenate, and inert ingredients as dye residue, dirt, etc., and is prepared by boiling a purple residue from the dye industry with slaked lime. The analysis on a package recently received at this station was as follows: Total arsenic (As), 21 per cent; active ingredients, calcium arsenate and calcium arsenite, 54 per cent; inert ingredients, dyestuffs, etc., 46 per cent; arsenic (As) in water soluble forms, 7½ per cent. Analysis by our station chemist substantiated the claim as to the total arsenic content. If judged from the arsenic content, London purple has only about one-half the killing strength of Paris green when used as an insecticide.

USE AS AN INSECTICIDE

The name "London purple" was suggested by Dr. C. E. Bessey (private correspondence) in 1878, and he was the first to use it as a substitute for Paris green for the destruction of the potato beetle. Doctor Bessey was one of the three men who first received sample packets of London purple sent by a London firm [3] in a letter dated September 7, 1877.

On October 2, after an exchange of letters, the firm forwarded to him three kegs of the material, which was the first shipment sent to America. The results of the experimental work of Bessey and Budd were favorable to the new poison, and it was soon recommended as a substitute for Paris green, not only for the destruction of the potato beetle but for other insects as well.

The value of this material was recognized with surprising rapidity, probably due to its cheapness and the ease with which it could be applied. The principal objection to its use was its injurious action on the foliage of plants. Its burning tendencies were undoubtedly due to the soluble arsenic it contained, and it was not uniform in composition. The early analysis of London purple shows variations in amounts of arsenious oxid of from 31 per cent to nearly 60 per cent. However, at the present time the manufacturers claim to have perfected a process by which the composition of London purple is as uniform as that of Paris green or others of the standard insecticides.

[3] Hemingway & Co.

From about three years after its introduction, London purple was generally considered to be nearly equal in efficiency to Paris green, and it is so considered to-day.

ZINC ARSENITE

In 1903, an investigation of arsenicals and spray injury was begun by the California Experiment Station. In the course of this investigation, foliage tests were made with zinc arsenite in the summer of 1906. In 1907, about 5 acres of apple trees were sprayed during the blooming season without injury, and the results indicated that this material was a promising insecticide. In 1909, the first commercial material was prepared by the California Spray Chemical Company, Watsonville, Calif.

PREPARATION

The process of manufacture in general consists in boiling together in water, in the presence of ammonia, zinc oxid and arsenious oxid, in approximately the proportion of their combining weights. The boiling is continued until the oxids combine, which is indicated by a marked thickening of the mass, and until the filtrate shows only traces of arsenious oxid.

USE AS AN INSECTICIDE

At the present time zinc arsenite is used extensively in certain localities for the spraying of apple trees just after full bloom, at the rate of 3 pounds of the powder to 100 gallons of water. It is not used for later spraying on account of its tendency to injure foliage; but from recent tests it is possible that it will become an important insecticide for truck crops.

INJURY TO FOLIAGE

Luther (letter of May 20, 1910) advocated the use of zinc arsenite on apple, pear, bean, and potato, but not on delicate foliage like peach. Volck (*33*) pointed out what he believed to be a fallacy in accepting the foliage of any one plant as a reliable index to the injurious action of all arsenicals. He found that either bean or peach foliage was a suitable indicator for testing arsenate of lead that is intended for use on apples. Because of the great ease of obtaining bean foliage, it was adopted as a standard testing medium. Later he says:

> Arsenite of zinc may prove entirely neutral to bean foliage and yet when applied to peaches do marked injury. Samples which injure peach will later prove injurious to apples if applied in sufficient quantities, and bean foliage is not suitable for testing samples.

Luther (*17*) in speaking of investigations in the Pajaro Valley states:

> On apples it [zinc arsenite] has been sprayed as thick as whitewash without the least bit of injury. On small field crops, such as beans, potatoes, etc., it has given no injury, but on the peach, which is supposed to be more hardy than the bean, the injury was severe.

Woodworth (*36*), in speaking of spraying conditions in the Pajaro Valley, says:

> This [zinc arsenite] has proven to be the safest of the arsenicals that can be procured in the form of dry powder. It is not so safe, of course, as the neutral lead arsenates, but has been used without very serious evidence of burning in the orchards where dusting has been adopted instead of spraying, . . . There is no doubt that the zinc arsenite stands foremost at the present time among the available arsenicals with high arsenic content.

Cooley (*3*) reports its use on the foliage of potato and cabbage without injury. Johnston (*13*) reports the use of zinc arsenite in the proportions of 1, 1½, and 2 pounds to 50 gallons of water on potatoes, without foliage injury. Clinton and Britton (*2*) report two applications of zinc arsenite on apple trees at intervals of seven days, using ¾ pound to 50 gallons, with foliage injury so severe that the trees dropped many of their leaves. Volck (*34*) recommends the substitution of zinc arsenite for lead arsenate for the first two sprayings, i. e., full bloom and 10 days later, but in the following combination: Zinc arsenite (dry basis), 6 pounds; iron sulphid, 6 pounds; Black leaf 40, 1 pound; water, 200 gallons; because when iron sulphid is added, the foliage-injuring properties are largely restrained. If used alone, only the first (full bloom) spraying should be applied. For later sprayings arsenate of lead is recommended.

Melander (*19*) says "It [zinc arsenite] is easy to use, adhesive, and has not scorched in our tests [on apple]." Schoene (*24*) found that on apples zinc arsenite alone or in combination with soap, glucose, or lime sulphur caused more or less injury, but that lime or Bordeaux prevented this injury. In other experiments with zinc arsenite, slight injury occurred on the foliage of pear and plums, peach and grape leaves were severely scorched, while potatoes and cabbage were uninjured. It is also suggested in this bulletin that the solvent action of carbonic acid is partly responsible for the damage. Scott and Siegler (*25*) record injury to foliage of apple when zinc arsenite was used alone at the rate of ¾ pound to 50 gallons of water or combined with milk of lime or with lime sulphur; that it caused moderate burning on bean foliage, except where lime was added, in which case no burning resulted; that when added to slaking lime for Bordeaux mixture it caused no foliage injury to either apple or grape; and they suggested its possible use with Bordeaux mixture in certain sections for the control of codling moth, bitter rot, and blotch.

By various workers zinc arsenite is considered an effective insecticide against insects that are rather resistant to the poisoning effect of arsenic, such as larvæ of webworm, tussock moth, etc.

METHODS OF INVESTIGATION

It has been apparent for some time that there are several factors that influence the injury of foliage by arsenical compounds. Some of these are inherent in the plants themselves, some are dependent upon the chemical nature and solubility of the compounds used, while still others are to be classed as environmental conditions. It is obvious that to determine the relative importance of these several factors one of them must be varied while the others are kept as nearly constant as possible, for it is quite impossible to eliminate all but one.

METHODS OF APPLYING CHEMICALS

A large part of the spraying was done in the college orchard at Bozeman, Mont.; but this was quite extensively supplemented by orchard work in other parts of the State, and by spraying plants of various kinds in field plots, on the college campus, and in the botanical greenhouse.

The mixtures were applied with a bucket spray pump under good pressure, using a Bordeaux nozzle. In the case of small plants, such as potatoes, sugar beets, etc., entire plants were sprayed, but on orchard trees this was rendered impossible by the very large number of applications

that were made, reaching into thousands. In orchard spraying, therefore, a single branch 2 or 3 feet long was sprayed with each mixture, and it is probable that as accurate results were secured as though entire trees had been covered. Indeed, it was possible to cover these single limbs more evenly than whole trees could have been covered.

It has been found most convenient to use two liters of spraying mixture for each application, and for this reason the strength of the chemical used is indicated as the number of grams in two liters. This method of expression will be found in most of the tables in this paper.

PRECAUTIONS

Care was taken to avoid spraying on windy days, and if rain followed the application the results were rejected excepting as they could be used for data in relation to precipitation. For the regular work foliage was chosen that was normal in development and free from mechanical or other injuries. Both the upper and the under side of every leaf was drenched thoroughly, and except in special cases care was taken not to shake off the spray mixture before it dried upon the leaves.

As the number of applications exceeded 6,000 in the orchard and plots and 4,000 in the greenhouse, it is evident that the greatest care was necessary to prevent errors in labeling, recording, etc., if the results were to be thoroughly reliable. This was realized from the first, and a complete and yet simple system of checking was adopted and scrupulously followed. From the nature of this system and from the fact that in the checking an error in the original record was very rarely found, we feel assured that the few erratic results that appeared were all due to other causes.

In presenting these data comparisons are not made in the same table between tests run under different conditions, and the reader is warned not to make direct comparisons between injuries which resulted from spraying on different dates, in different places, or under other differing conditions, except for the purpose of studying the effects of these specific conditions, others being practically constant.

DEVELOPMENT AND CHARACTER OF INJURY

When a leaf is materially injured by arsenic, it shows visible symptoms in a day or two which become more and more pronounced until certain portions or, in severe cases, the whole leaf is dead, brown, and more or less shriveled. In severe cases the leaves drop in from one to four weeks from the time of treatment. Most commonly the first visible symptoms appear on the second day after treatment. At this stage there is very little change in color, but the surface of the leaf in the injured portion has lost its normal luster and becomes duller in appearance. The tissues under these duller areas have lost much of their turgidity and become more or less flabby. Very soon, perhaps the second day, a dull brown tinge is apparent, which at first is indefinite in outline and becomes more and more sharply defined. After 10 days of treatment no further change takes place except that the dead portion becomes frayed by the whipping of the leaves in the wind, or, if it is severely injured, the leaf drops off. No exact time can be given for this course of development, as it is hastened by hot, dry weather and retarded by cool, wet weather. Most commonly the first visible injury may be detected on the first or the second day. The condition shown in Plate 1, A may be seen on the

third day; Plate 1, B on the fourth day; Plate 1, C on the fifth day; Plate 1, D at the end of a week; Plate 1, E in about 10 days, and Plate 1, F in 3 or 4 weeks.

If the leaf is not entirely killed, as was usually the case in these experiments, the injury is worse at the margin and in roundish spots of all sizes in the interior. If there are abrasions through the epidermis, these become centers of discolored areas. In most of these spots, however, there is no visible mechanical injury, nor do they correspond to depressions in the leaf where the spray mixture collected in greater abundance.

The degree of injury was measured by the proportions of injured to uninjured surface. In the case of the apple, if more than half of the leaf is killed it usually drops prematurely. The position of the injured area varies this rule somewhat. Injury near the midrib or near or on the petiole is more likely to cause dropping than if on the margin, especially toward the tip. Not all plants are equally inclined to shed their leaves. The bean, for example, will do so much more readily than the apple, while the tomato retains them more tenaciously.

For the purpose of comparing results for tabular data, etc., a condensed method of expressing the degree of injury is highly desirable. For this purpose the terms, very slight, slight, moderate, bad, very bad, partly defoliated, and defoliated were adopted. As applied to individual leaves the following definitions apply to the records in this paper:

Very slight = the least amount of injury that is easily seen.

Slight = a few small spots up to one-eighth of the area of the leaf killed.

Moderate = one-eighth to one-fourth of the area killed.

Bad = one-fourth to one-third of the area killed.

Very bad = one-third to nearly all of the area killed.

As applied to the sprayed plants or branches as a whole the following definitions served as a guide:

Very slight = a few small spots on a small proportion of the leaves; no leaf seriously injured.

Slight = about one-eighth to one-fourth of the leaves showing spots, but few, if any, of them seriously injured.

Moderate = about one-third of the leaves more or less injured. Most of the leaves showing only small spots, but a few of them may be injured to the degree classed as "bad." In the definitions given above for individual leaves we have applied this term to the most serious injury that can be done without commercial injury to most crops.

Bad = approximately half the leaves injured, most of them only slightly, but some in "moderate" degree. This we have considered to be just enough injury to class as of commercial importance to most crops.

Very bad = most of the leaves more or less injured, some of them nearly killed.

Partly defoliated = a portion, but not all of the leaves entirely killed or so badly injured that they dropped off before the notes were taken. The leaves of some kinds of plants when all or nearly all the tissue is killed, will drop off. The leaves of other plants, as tomato, still cling after death, but for uniformity we class as defoliation a killing of the leaves whether they actually drop off or not.

Defoliated = practically all the leaves are killed.

Occasionally there would be some combination of injured leaves other than those listed above, as, for example, a few leaves badly injured and the others practically all sound, and judgment had to be used in designating the degree in such cases, but these definitions will, in general, serve to place the authors and readers on common ground.

CONDITIONS AFFECTING THE AMOUNT OF INJURY

It is a well-recognized fact that arsenic and its compounds tend to injure all forms of life with which they come in contact, but the degree of injury varies with several conditions, some of which are more or less under the control of the entomologist, the horticulturist, or the farmer, who makes use of these compounds for destroying insects upon growing crops. A study of these conditions constitutes the most important part of this investigation.

DIFFERENCE IN SUSCEPTIBILITY OF PLANTS

It has been found that different species of plants vary greatly in their natural resistance to arsenical action. Confining ourselves to the higher plants and especially to those that for economic reasons are likely to receive applications of arsenical insecticides, we find that some will be almost or quite killed by applications that will injure others but little.

DIFFERENCE IN GENERA AND SPECIES

Numerous spraying tests were made on a rather wide range of plants, including some that were only distantly related. Table I, which records the results of a test made on August 2, 1915, is fairly typical of the results obtained with these plants. It should be added, however, that in other tests sugar beet has not usually proven quite as susceptible as here indicated, being about the same as potato. Also, squash has usually proven a little more susceptible, being in about the same class as apple. Cabbage is distinctly the most resistant of the plants we have tested, though in the experiment recorded in this table cucumber was injured but little more.

TABLE I.—*Plants in field plots sprayed with calcium arsenite, 1.2 gm., and soap, 7.2 gm. per liter of water, to show the relative susceptibility of different species*

Name of plant.	Injury.
Apple, Wealthy	Bad.
Bean, White Navy	Very bad.
Cabbage, Late Flat Dutch	Slight.
Cucumber, Improved Long Green	Do.
Potato, Early Ohio	Moderate.
Pea, Senator	Bad.
Rutabaga, Monarch	Moderate.
Squash, Yellow Crookneck	Do.
Sugar beet, Klein Wenzlebener	Bad.
Tomato, Enormous	Moderate.
Turnip, White Globe	Do.

We suggest a table of susceptibility of plants we have sprayed, as shown in Table II. In using this table one must keep in mind that different varieties of the same crop vary somewhat among themselves in this respect. In this table an attempt is made to range the plants from the most resistant to the most sensitive.

TABLE II.—*Approximate order of foliage susceptibility to arsenical injury*

1. Cabbage.	6. Tomato.	11. Pea.
2. Sugar beet.	7. Rutabaga.	12. Squash.
3. Potato.	8. Turnip.	13. Cucumber.
4. Apple.	9. Cherry.	14. Peach.
5. Pear.	10. Plum.	15. Bean.

Something about the condition of plants of the same kind seems to vary at times to make them more or less susceptible. This accounts for some apparent discrepancies between Tables I and II. Repetition for confirmation is recognized as of fundamental importance in all scientific experiments, but is indispensable in studies of arsenical injury.

TABLE III.—*Fruit trees sprayed with copper aceto-arsenite and with calcium arsenite to show relative susceptibility of different species*

Name of tree.	Injury.	
	Copper aceto-arsenite 2.4 gm. to 1 liter of water.	Calcium arsenite 1.2 gm. to 1 liter of water.
Apple, McIntosh	Very slight	Moderate.
Crab apple, Transcendent	None	Do.
Cherry, Montmorency	Moderate	Very bad.
Cherry, Royal Ann	Slight	Nearly defoliated.
Pear, Flemish Beauty	Very slight	Moderate.
Plum, Bradshaw	Very bad	Defoliated.

For the variety used, the order of injury here indicated is plum, cherry, pear, apple, and crab, of which the plum is distinctly most susceptible. From the conflicting reports of other investigators it seems likely that this order will vary according to the variety used. We made no tests on the peach, as no trees were available, but it is quite generally conceded that it is more tender than any of the fruits named in Table III.

Roses were sprayed several times for a comparison with apple. Harrison's yellow rose seemed about like Okabena apple in susceptibility, and the Rugosa a little more tender.

DIFFERENCES IN VARIETIES OF THE SAME SPECIES

It is important to know if arsenical spray injury on any variety that happens to be chosen will be the same on other varieties under similar conditions. Among apples it is quite generally conceded that Ben Davis is especially susceptible, though strictly comparative data are not abundant in the literature.

In our work we have had many opportunities for comparing susceptibility of apples, and we have thoroughly tested Alexander, Ben Davis, Charlottenthaler, Gano, Hybernal, Lieby, McIntosh, McMahon, Okabena, Oldenburg, Rome, Shiawassee, Wagener, Tetofsky, Yellow Transparent, Greenwood, Crab, Hyslop crab, and Transcendent crab. Of these the crab apples have shown themselves distinctly more resistant than the standard apples, especially the Transcendent, which is almost in a class by itself. Among the standard varieties Ben

Davis, Gano, and Tetofsky have been conspicuously susceptible, while the others have shown no very consistent differences. They are so nearly alike that in one test certain ones would be injured a little more than the rest, while in other tests other varieties would be most injured. For practical purposes they may be considered about alike. It seems probable that this varietal susceptibility of apple trees is due to the thickness or character of the cuticle on the leaves rather than to a difference in the living cells within, for we have shown (*30, p. 304*) that when fresh wounds through the bark are treated with arsenical compounds, Ben Davis is not more injured or Transcendent less injured than other varieties.

Of the common garden beans, four varieties were compared. These were the White Navy, Red Kidney, Dwarf Horticultural, and Burpee Stringless. In their susceptibility to calcium arsenite, copper aceto-arsenite, and London purple they were almost identical.

From our experiments and those of Woodworth (*35*) we may conclude that among plants of different species, and different varieties of the same species, few general rules may be laid down, and each species and variety must be actually tested in comparison with others to know its susceptibility. To be sure, some groups, as beans, are especially susceptible (probably all of them) and probably all cabbages are relatively resistant. But among plants of intermediate susceptibility such as apple, cherry, rose, potato, and sugar beet, varietal difference is sufficient to make it unsafe to draw comparisons between these species without stating the varieties.

INDIVIDUAL DIFFERENCES

Do individuals of the same species and variety show differences in susceptibility? If such be the case, general conclusions can not be drawn with safety from single tests, and some repetition is necessary to get the prevailing tendency, the amount of repetition depending upon the degree and frequency of individual variation and the exactness of the method used.

In carrying on this investigation, practically all the herbaceous plants used, whether in the greenhouse or in the field plots, were sprayed in triplicate and in some cases three branches of as many different orchard trees were sprayed in the same way. Opportunity was thereby offered for a very large number of comparisons. The results showed that the tendency to individual variation is quite marked. As this tendency is a matter of degree and we have no standards for expressing it, reference may be made to Table IV, which is quite representative of this condition. (See also Tables IX to XIV.) Doubtless this variation is partly apparent and partly real. With the greatest of care it is difficult to keep a large number of plants under identical conditions after spraying. One may be kept in a little more shaded place than another, or have better air circulation, or be brushed more in working among the plants. This difficulty is even greater in treating branches of orchard trees. Using every precaution to eliminate such factors, individual differences still appeared sufficient in degree to convince one that the triplicate plants at the time of spraying were not always alike in susceptibility. We believe that this is due in part to the environmental conditions under which the plants were grown, though all showing visible abnormalities were rejected before spraying, and there also may be a natural tendency to more or less resistant strains.

Nevertheless, the investigator in this field must give it due recognition and repeat all work until a predominant tendency is clearly established. We have made it a point, therefore, in all cases in which certain results shown in a published table differed from those most commonly observed under the same conditions, to call attention to this fact.

TABLE IV.—*Tomato plants in greenhouse, sprayed in triplicate, showing individual differences in susceptibility*

Chemical.	Amount of chemical in 2 liters of water.	Amount of soap in 2 liters of water.	Injury.		
			Plant 1.	Plant 2.	Plant 3.
	Gm.	*Gm.*			
Lead arsenate, diplumbic, Baker.	9.4	0	None	None	None.
	9.4	7.2	....do	....do	Do.
	9.4	14.4	....do	....do	Do.
	9.4	28.8	Moderate	Moderate	Moderate.
Calcium arsenate, Merck.	2.8	0	Very slight	Very slight	Very slight.
	2.8	7.2	Moderate	Slight	Slight.
	2.8	14.4	Slight	....do	Do.
	2.8	28.8	Moderate	Moderate	Moderate.
Calcium arsenite, Adler.	2.4	0	Moderate	Moderate	Moderate.
	2.4	7.2	Bad	Bad	Bad.
	2.4	14.4	Very bad	Very bad	Very bad.
	2.4	28.8	Partly defoliated.	Partly defoliated.	Partly defoliated.
Calcium arsenite, Baker.	2.4	0	Moderate	Moderate	Moderate.
	2.4	7.2	Very bad	Very bad	Very bad.
	2.4	14.4	....do	....do	Do.
	2.4	28.8	....do	....do	Do.
Calcium arsenite, Merck.	2.4	0	Slight	Slight	Slight.
	2.4	7.2	....do	Moderate	Do.
	2.4	14.4	Very slight	Very slight	Very slight.
	2.4	28.8	....do	Slight	Moderate.
Arsenic trisulphide, Baker.	0.8	0	None	Slight	Very slight.
	.8	7.2	Slight	....do	Moderate.
	.8	14.4	Moderate	Moderate	Slight.
	.8	28.8	Very bad	Very bad	Very bad.

DIFFERENCES IN PARTS OF THE PLANT

In the spraying of trees and other plants all aerial portions are necessarily coated. This may or may not be necessary from the standpoint of controlling insects and diseases, but as it is practically unavoidable it makes the relative susceptibility of different parts of the plant to injury by the spray mixture of much importance in all spraying operations. This is especially true of fruit trees where, for example, the fruit may be russeted by Bordeaux mixture, greatly reducing its market value, while the same amount of burning on the leaves is unimportant.

STEMS, FRUIT, AND FOLIAGE

On all kinds of plants sprayed in this investigation the leaves were the most susceptible portion. Most of the herbaceous plants bore no fruit prior to the time of spraying, but beans, cucumbers, and squashes were, in a few cases, exceptions. On apple trees the leaves proved much more tender than either stems or fruits. This is true to such a marked degree that any arsenical treatment that caused even the slightest direct damage to fruit or bark nearly defoliated the sprayed branch. So far as this fruit is concerned, therefore, and probably in general, arsenical spray injury is a problem of foliage injury almost exclusively. The apple fruit proved slightly more susceptible to very severe treatment than the most tender portion of the bark on the stem.

We have frequently noted that with plants of all kinds, whether herbaceous or woody, the youngest portion of the stem is the most easily injured. With leaves, however, this is not the case. Old leaves nearly ready to fall are damaged most, while young ones just expanding are most resistant.

On the apple the foliage injury takes place almost exclusively through the lower epidermis. Gillette (*10*) sprayed the upper surface and then both surfaces by way of comparison and found that—

> wherever this was done, the damage sustained by the leaves that were wet on both sides was fully double that sustained by those wet only on the upper surface.

Woodworth (*35*) tried painting the leaves with white arsenic, Paris green, and London purple, some on one surface, some on the other, and some on both. The entire surface was coated. He concluded that the lower side is more susceptible than the upper, the difference in his experiments being in the proportion of 47 to 22.

In our own work the arsenical was applied individually to many leaves with a very soft brush, some to the upper side and others to the lower side. It was found that if the painted area extended to the margin it was practically impossible to keep a little of the liquid from extending over onto the opposite surface from that intended. Therefore, after a few preliminary experiments the practice was followed by painting a strip about ¾ by 1 ¼ inches down one side of the midrib. This strip never approached the leaf margin. In Table V each report represents the injury of 10 painted leaves on one shoot. Both sodium arsenite and calcium arsenite were used in this experiment, one being a soluble and the other a nearly insoluble compound. Soap was added in some cases to cause the liquid to spread better and to penetrate the pubescence on the leaf more readily.

The results shown in Table V are quite typical and indicate that the arsenical injury to apple foliage from spraying is brought about largely by absorption from the lower surface. Indeed, in some of our experiments when very toxic solutions were used no trace of injury occurred on leaves treated on the upper surface. We are led to believe that in those cases where injury did occur after treatment of the upper surface, there was some tiny abrasion, not easily visible to the naked eye. In view of our results it is difficult to account for so much injury from treating the upper surface as reported by Woodworth (*35*). The only essential difference in method was that he painted the whole surface instead of the central portion only. When we did the same we could not readily avoid getting some of the liquid over the margin onto the

other side with consequent injury and this may explain the discrepancy. Also, in his experiments the epidermis treated may not have been so free from tiny abrasions.

TABLE V.—*Leaves of apple treated on the upper or the lower surface to determine the relative injury*

Variety.	Chemical.	Strength.	Injury.	
			Upper.	Lower.
Transcendent	Calcium arsenite.	1–1,000	Very slight	Very slight.
Wealthy			None	Very bad.
McIntosh			Very slight	Moderate.
Yellow Transparent			None	Very slight.
Transcendent	Calcium arsenite plus soap.	1–1,000 (14.4 gm. in 2 liters).	None	Very slight.
Wealthy			....do	Do.
McIntosh			....do	Do.
Yellow Transparent			....do	None.
Transcendent	Calcium arsenite.	1–500	Very slight	Slight.
Wealthy			None	Do.
McIntosh			Very slight	Very bad.
Yellow Transparent			None	Slight.
Transcendent	Calcium arsenite plus soap.	1–500 (14.4 gm. in 2 liters).	None	Slight.
Wealthy			....do	Moderate.
McIntosh			....do	Very slight.
Yellow Transparent			....do	Partly defoliated.
Transcendent	Sodium arsenite.	1–1,000	None	Moderate.
Wealthy			Very slight	Very bad.
McIntosh			None	Bad.
Yellow Transparent			Very slight	Do.
Transcendent	Sodium arsenite plus soap.	1–1,000 (14.4 gm. in 2 liters).	None	Slight.
Wealthy			....do	Moderate.
McIntosh			Very slight	Partly defoliated.
Yellow Transparent			None	Defoliated.
Transcendent	Sodium arsenite.	1–500	Slight	Bad.
Wealthy			None	Very bad.
McIntosh			Moderate	Partly defoliated.
Yellow Transparent			Slight	Very bad.
Transcendent	Sodium arsenite plus soap.	1–500 (14.4 gm. in 2 liters).	Very slight	Very bad.
Wealthy			None	Partly defoliated.
McIntosh			Slight	Do.
Yellow Transparent			None	Defoliated.

Whether the under surface of apple leaves is more susceptible to injury because of the greater prevalence of stomata or because of a thinner or more permeable cuticle is yet to be determined. We are inclined to the latter view.

To determine if this greater protection of the upper epidermis is found also in other plants the leaves of a dozen different shrubs and trees on the college campus were painted with sodium arsenite and soap in different strengths. The procedure was the same as described for apple on page 515. These plants are as follows: Alder, barberry (common, green), birch (white), box elder, buckthorn (English), chokecherry, dog-

wood, lilac (purple), lilac (white), maple (Norway), Siberian pea tree, willow (golden). Repeated tests showed that in all species tested the upper epidermis is decidedly more protective against arsenical injury than the lower.

RELATIVE INJURIOUSNESS OF ARSENICAL CHEMICALS

When a number of arsenical compounds are sprayed upon plants the relative injury done by them may be influenced by two factors, (1) their relative solubility and (2) their relative toxicity to the plants treated. It so happens that with reference to solubility, arsenical compounds fall into two fairly well defined groups, those that dissolve readily in a relatively small amount of cold water and those that are only slightly soluble in cold water. The former we will for convenience designate as the soluble arsenical compounds and the latter as the insoluble arsenical compounds, it being fully recognized that a small amount goes into solution while the greater part remains undissolved when used in spraying work.

READILY SOLUBLE ARSENICAL COMPOUNDS

Compounds of this class can not be used for spraying plants to control insect pests owing to the injury done when enough is applied to kill the insects. Their practical significance in this connection lies in their utility as weed killers and in the possibility of their presence as impurities in the so-called insoluble arsenical insecticides. Their toxicity may be compared on either of two bases, (1) using equal parts by weight of the different chemicals or (2) such amounts of them as will contain equal amounts of arsenic. Our experiments included tests on both these bases.

The results shown in Tables VI and VII correspond very well with those that were usually found in the many tests of these compounds. It will be noted that on the basis of toxicity whether equal weights of the chemicals be used per liter or amounts to contain equal weights of arsenic, these compounds may be divided into three groups; cacodylic acid and the cacodylates are most toxic, the arsenites and arsenic acid next, and the arsenates least. Ammonium arsenate constitutes one exception, as it causes injury equal to the arsenites. Ammonium arsenite was used a little, but is unsatisfactory owing to its instability.

TABLE VI.—*Apple sprayed with soluble arsenical compounds using the same number of grams for 2 liters*

Chemical.	Injury.		
	Concentration 1 to 10,000.	Concentration 1 to 5,000.	Concentration 1 to 2,000.
Acid, arsenic	None	Slight	Bad.
Acid, cacodylic	Moderate	Moderate	Very bad.
Ammonium arsenate	None	do	Bad.
Potassium arsenate	do	None	Moderate.
Potassium arsenite	do	Very slight	Bad.
Potassium cacodylate	do	Moderate	Very bad.
Sodium arsenate	do	None	Slight.
Sodium arsenite	do	Very slight	Bad.
Sodium cacodylate	do	Moderate	Very bad.

TABLE VII.—*Apple and tomato sprayed with soluble arsenical compounds in amounts to contain 7.5 gm. of arsenic in 2 liters of solution*

Chemical.	Injury.	
	Apple.	Tomato.
Acid, arsenic	Very bad	Very bad.
Acid, cacodylic	Defoliated	Partly defoliated.
Ammonium arsenate	Partly defoliated	Very bad.
Potassium arsenate	Bad	Bad.
Potassium arsenite	Partly defoliated	Very bad.
Potassium cacodylate	Defoliated	Partly defoliated.
Sodium arsenate	Moderate	Bad.
Sodium arsenite	Partly defoliated	Very bad.
Sodium cacodylate	Defoliated	Nearly defoliated.

Cacodylic acid and the cacodylates probably never occur in insecticides and are too expensive to be used as weed killers, and are therefore of more theoretical than practical interest. The scientific interest which they have in this connection lies chiefly in their very high toxicity to higher plant life and relative low toxicity to higher animal life. Cushny (*6*) states:

> The action being due to the ion and not to the element . . . organic arsenic combinations in which the metallic atom is directly attached to the carbon atom are only feebly poisonous. ..... The earliest of these is sodium cacodylate, $(CH_3)_2AsOONa$.

Merck's Index (*20*) states also:

> The cacodylates (which see) are now largely used instead of the alkali arsenites, as the former are far less toxic.

It has been stated on high authority (*23*) that—

> Arsenious acid is extremely poisonous, whereas many, both of higher and of the lower plants, can withstand large doses of arsenic acid and can accumulate large quantities of arsenic when supplied to them in this form.

This statement, while perhaps true under some conditions, is wholly misleading if applied to spray mixtures.

NEARLY INSOLUBLE ARSENICAL COMPOUNDS

Turning to the arsenical compounds that are but slightly soluble, we find the comparison more difficult. In the first place it is hard to get these chemicals that are pure and of definite composition, and in some instances they are not fully named. For example, a "lead arsenate" may be diplumbic ortho-lead arsenate or triplumbic ortho-lead arsenate or a mixture of the two, and neither the label nor the statement of the manufacturers reveals which of the three it is. Indeed, it is doubtful if an absolutely pure diplumbic or triplumbic lead arsenate is now on the market. Then, too, some of these chemicals are so slightly injurious that they cause no injury except under such conditions as will permit the more injurious ones to nearly or quite defoliate the plants or branches, making fine comparisons difficult. Furthermore, the number of chemicals in this group is so large, especially if we use several brands of the more important ones, that no single series of comparative tests is likely to be reliable throughout, owing to individual differences in the plants used, unless several are sprayed at the same time with each mixture,

making a series so large that the last must be applied much later than the first. Table VIII will, however, give a nearly correct idea of their relative injuriousness. Under the conditions governing these experiments it should be constantly kept in mind that these results might be different if other brands of the chemicals were used or if climatic conditions were different.

If we consider the results shown in this table and also those from many other spraying tests, we come to the conclusion that the order of injuriousness of the chemicals listed in Table VIII when sprayed on to foliage under our conditions is as follows:

1. Calcium arsenite.
2. Arsenic trisulphid.
3. Barium arsenate.
4. Calcium arsenate.
5. Lead arsenite.
6. Copper aceto-arsenite.
7. Arsenic disulphid.
8. Arsenic trioxid.
9. Zinc arsenite.
10. Lead arsenate diplumbic.
11. Lead arsenate triplumbic.
12. Ferrous arsenate.

TABLE VIII.—*Results of spraying different plants with "insoluble" arsenical compounds in such amounts as to give equal weight of arsenic to 2,000 cc. water to show relative toxicity*

Chemical.	Injury to bean, May 3, 1915 (1 gram arsenic in 2 liters of water).	Injury to apple, June 18, 1915 (2 grams arsenic in 2 liters of water).	Injury to tomato, Aug. 14, 1916 (2 grams arsenic in 2 liters of water).
Arsenic trioxid, Baker	Very slight	None	None.
Arsenic disulphid, Merck	do	do	Do.
Arsenic trisulphid, Baker	Bad	Slight	Very bad.
Barium arsenate, Adler	Moderate	Moderate	Bad.
Calcium arsenate, Merck	do	do	Moderate.
Calcium arsenite, Baker	Defoliated	Partly defoliated.	Partly defoliated.
Ferrous arsenate, Merck	Very slight	None	None.
Lead arsenate, diplumbic, Baker	do	do	Do.
Lead arsenite, Merck		do	Very slight.
Lead arsenate, triplumbic, Baker	None	do	None.
Paris green, Baker	Moderate	Moderate	Do.
Zinc arsenite, "Ortho 40"	Very slight	Slight	None.

Probably the most striking fact brought out by this phase of the work is that arsenic trioxid may be applied with so little injury. It is well known that this compound has been kept out of general use by the injury it has caused under field and orchard conditions in other localities. We have sprayed both apple and several herbaceous plants many times and on many dates during a period of several years and have never found it especially injurious if the mixture was applied as soon as prepared. This might not be true in localities with a high humidity. Even the freshly prepared amorphous form which is more soluble than the crystalline form has given less injury than the majority of the slightly soluble arsenical

compounds, and indeed it was scarcely more injurious than the crystalline form.

On the other hand, it seems surprising that calcium arsenite could have remained in use as an insecticide for so long as it did. It has gradually been abandoned, partly because of its burning of the foliage and partly because some time is consumed in its preparation according to the Kedzie (Woodworth and Colby (*37*)) or the Kilgore (*14*) formulas, which are the ones most generally followed. We have tried three different brands, one of them especially prepared for our use, besides the home-made articles prepared after the Kedzie and the Kilgore formulas, and are left no other alternative than to place it at the head of the list of slightly soluble arsenical compounds, considered from the standpoint of injurious properties. Indeed, used in strengths to contain equal amounts of arsenic, the home-made calcium arsenites were even more harmful than the Baker brand on which we based most of our work.

To determine the variability of compounds supposed to be identical, tests were made with different brands of calcium arsenite, lead arsenate, zinc arsenite, and arsenic trioxid, using four brands of calcium arsenite, 4.9 gm. to 2 liters of water, and applying to apple foliage. Of the four, one[4] caused in a typical case moderate injury; the second,[5] very slight; the third,[6] bad; and the fourth,[7] very slight. In this case there was no consistent relation between the arsenic content of the different brands and the amount of injury produced.

More than 30 brands of lead arsenate were tried. Some were supposed to be diplumbic, others triplumbic, and others mixtures of the two, while still others gave no clue without chemical analysis as to their kind. Some were paste and others were dry. Some were prepared for use as insecticides and others as pure chemicals for technical use. It is doubtful if the results of these tests merit publication in tabular form. Marked differences were found, to be sure, but as some factories do not put out a uniform product, and as others have changed their processes of manufacture since the tests were made, and still others will doubtless do so in the near future, such a list could not safely be used as a basis for selecting lead arsenates for orchard spraying. The same could, perhaps, be said also of the calcium arsenites and zinc arsenites discussed above. The important point is that lead arsenates do vary in composition, as indicated by their analyses and by their injurious properties, even though the labels would not indicate the fact.

It is generally believed that diplumbic lead arsenate has a greater tendency to injure foliage than the triplumbic form. This is doubtless true in a general way, but we have had some brands of the diplumbic that were just as safe to use as most of the brands of triplumbic arsenate.

Four lots of arsenic trioxid were compared, a crystalline form and an amorphous form from one manufacturer,[8] a crystalline form from another,[9] and a crystalline form taken direct from the arsenic retainer of a smelter[10] situated at Anaconda, Mont. In addition, an arsenic trioxid paste was made from each of these four by grinding the dry powder in a mortar with a little water. This was allowed to stand a week or more before using. The injury caused by these various lots of arsenic trioxid was practically equal.

[4] Adler's.
[5] California Spray Co.'s "Ortho 40."
[6] Sherwin-William's.
[7] Thomson's.
[8] J. T. Baker.
[9] Merck.
[10] The Washoe.

EFFECTS OF ADDING OTHER INSECTICIDES, LIME, ETC.

In orchard, field, and garden practice it is often desired to add various substances to arsenical spraying mixtures. These substances are of various kinds: Other insecticides, or fungicides for combination sprays, soap, or other colloidal substances for an adhesive or "spreader" on such plants as cabbage and sugar beet to retard the settling of the arsenical suspension, as recommended by Parker (*22*), or various materials to lessen the tendency to burn the foliage.

LIME SULPHUR

The addition of lime sulphur to kill scale insects or to prevent apple scab is often desirable. This substance brings about a chemical reaction with most arsenical insecticides, and it is of interest to know whether the insecticidal and fungicidal properties of either or both are destroyed and whether the tendency to burn the foliage is increased or decreased. The first of these questions has been quite thoroughly discussed by Cordley (*4*) and Melander (*18*) and is hardly within the province of this paper. The effect of lime sulphur on the injury caused by the different arsenicals is shown in Table IX. The tests from which this table was prepared ran through three seasons and were carried on in four different parts of the State. Several brands of the chemicals were used. The arsenicals were used in such strengths as to contain either 1 or 2 grams of arsenic for each 2 liters of water. The lime sulphur (factory boiled) was added at the rate of 77 cc. of 28° Baumé in every 2 liters of mixture. If in any case the lime sulphur (control) alone caused injury, the series was rejected, but this was extremely rare.

TABLE IX.—*Effects of lime sulphur on the burning properties of various arsenical compounds*

Arsenical compounds to which lime sulphur was added.	Number of brands.	Number of applications.	Number of times injury increased.	Number of times injury decreased.	Number of times the same.	Number of times no injury with or without.
Arsenic trioxids	2	3	2	0	1	0
Arsenic disulphid	1	2	2	0	0	0
Arsenic trisulphids	2	4	3	0	1	0
Barium arsenate	1	1	0	0	1	0
Calcium arsenites	3	16	2	9	3	2
Copper aceto-arsenite	2	20	12	2	2	4
Ferrous arsenates	3	8	0	1	0	7
Lead arsenates, triplumbic	8	52	25	0	1	26
Lead arsenates, diplumbic	13	85	36	0	0	49
Lead arsenates, mixtures	10	27	15	0	1	11
Zinc arsenites	6	42	10	3	2	27

From Table IX it may be seen that lime sulphur causes an increase in the injury produced by most arsenical compounds. This is especially true of copper aceto-arsenite, the lead arsenates and zinc arsenite. With calcium arsenite, however, it usually causes a reduction in the amount of injury, though not to such an extent as to make this compound

safe to use. Even with lime sulphur added calcium arsenite does more damage to the foliage than any other arsenical in the list given in Table IX.

We have given some attention to finding an explanation for the reduced injury by calcium arsenite when lime sulphur is added. As a principle of physical chemistry, the lime sulphur containing calcium sulphids, which are more readily soluble than calcium arsenite, would be expected to prevent to some extent the ionization of the less soluble salt. If this were the only consideration involved, other soluble calcium salts would be expected to have the same effect. To test this experimentally we added calcium nitrate to certain lots of calcium arsenite suspension, and calcium chlorid to other lots. These were sprayed upon tomato, bean, and cucumber plants in comparison with calcium arsenite alone and calcium nitrate and calcium chlorid alone. In almost every instance the injury was increased slightly by the addition of the nitrate and chlorid, though these compounds in themselves caused no injury. After repeated tests we decided that other factors than the restraint of ionization must enter in. This question is now receiving further study.

TOBACCO EXTRACT

This insecticide has come into quite general use for the control of plant lice. It will often save the labor of one spraying application to combine it with an arsenical spray mixture. The results of such combination in injury to the foliage are shown in Table X. The conditions under which the tests there recorded were carried on as to dates, localities, arsenical chemicals, etc., are essentially as described on page 521. The tobacco extract is the "Black Leaf 40." It was used at the rate of 2.5 cc. to each 2 liters of water.

It is evident from Table X that the effect of tobacco extract upon the injurious properties of the arsenical compounds is not marked. It is probable that we may safely state that it does not increase the injury with any of the arsenicals tested, to such an extent as to make its use undesirable and on the other hand it does not decrease the injury in any case enough to make this an important consideration.

TABLE X.—*Effects of tobacco extract on the burning properties of various arsenical compounds*

Arsenical compounds to which tobacco extract was added.	Number of brands.	Number of applications.	Number of times injury increased.	Number of times injury decreased.	Number of times injury was the same.	Number of times there was no injury with or without the extract.
Arsenic trioxids	2	2	0	0	1	1
Arsenic disulphid	1	1	1	0	0	0
Arsenic trisulphids	2	4	0	1	1	2
Barium arsenate	1	2	2	0	0	0
Calcium arsenites	3	22	3	7	9	3
Copper aceto arsenites	2	20	2	6	4	8
Ferrous arsenates	3	15	1	0	1	13
Lead arsenates, triplumbic	7	62	3	2	0	57
Lead arsenates, diplumbic	13	97	5	3	2	87
Lead arsenates, mixtures	10	28	2	0	0	26
Zinc arsenites	6	52	8	3	10	31

SOAP

In spraying some plants, for example, cabbage, and to a less extent sugar beet, the spray mixture does not spread on the surface of the leaf and adhere, but rolls off in droplets, leaving most of the surface unprotected. It has been found that the addition of soap will nearly or quite obviate this. Furthermore, Parker (*22*) has shown that soap will very materially retard the settling of lead arsenate from its suspension in water. The natural tendency of soap to increase the solubility of the slightly soluble arsenical compounds is well recognized and the question arises, will this result in an increased tendency to injure the foliage on the part of some or all of the arsenical insecticides?

During this investigation several different kinds of soap were tried and several strengths were used, but in the tests recorded in Tables XI and XII one brand [11] was used exclusively, 14.4 gm. being added to each 2 liters of spray mixture. The arsenical chemicals were the same in kind and strength as recorded on page 521.

TABLE XI.—*Effects of soap on the burning properties of various "insoluble" arsenical compounds*

Arsenical compounds to which soap was added.	Number of brands.	Number of applications.	Number of times injury increased.	Number of times injury decreased.	Number of times injury was the same.	Number of times there was no injury with or without the soap.
Arsenic trioxids	4	105	16	7	11	71
Arsenic disulphids	1	14	2	0	0	12
Arsenic trisulphids	2	17	9	1	3	4
Barium arsenates	1	9	4	1	4	0
Calcium arsenates	1	77	48	6	17	6
Calcium arsenites	3	123	27	28	63	5
Copper aceto arsenites	2	123	8	64	28	23
Ferrous arsenates	3	29	1	0	0	28
Lead arsenates, triplumbic	8	95	13	1	0	81
Lead arsenates, diplumbic	13	140	56	0	1	83
Lead arsenates, mixtures	10	57	19	0	0	38
Lead arsenite	1	5	4	0	0	1
Zinc arsenites	6	90	11	10	17	52

Table XI shows that of the "insoluble" arsenicals tested all but three cause greater injury if soap is added. This increased injury is most conspicuous in the lead arsenates. Of these, 8 brands were triplumbic, 13 diplumbic, and 10 were probably mixtures of both. The increase of injury through the addition of soap is a little more pronounced with the diplumbic than with the triplumbic arsenates, but the difference is not so great as some have supposed.

One of the most surprising observations made in this phase of the investigation is the reduction in injury caused by copper aceto-arsenite when soap is added.

Not all brands of soap give the same results when combined with arsenical spray mixtures. Some appear to retard the injury by copper aceto-arsenite more than others, and they vary in the degree to which they increase the injury by lead arsenates and other compounds. Neither do they show equal power to keep lead arsenate in suspension. While

[11] "Diamond C."

considerable work was done with different soaps, 18 in number, the results were not sufficiently consistent and conclusive to warrant a detailed statement concerning them. It seems probable that the product of some of the soap factories is not uniform, and the number of brands on the market is great and the assortment found in different parts of the country varies so much that it seemed inadvisable to attempt to study them fully. However, it may at least be said that some quite consistently give bad results with the arsenicals and others are relatively harmless, while still others are variable. The brand most used in this investigation[12] was rather consistent and intermediate in its effect upon the arsenicals.

Soap appears to have some effect also upon the injurious properties of the soluble arsenical compounds, as shown in Table XII. This tendency is in general toward reduction when soap is added, but it is not so marked as to have great significance. Perhaps the more even spread on the leaf surface would account for it.

TABLE XII.—*Effects of soap on the burning properties of soluble arsenical compounds*

Soluble arsenical compounds to which soap was added.	Number of applications.	Number of times injury increased.	Number of times injury decreased.	Number of times injury was the same.	Number of times there was no injury with or without soap.
Arsenic acid	9	0	4	3	1
Ammonium arsenate	7	0	2	3	2
Cacodylic acid	3	0	0	3	0
Potassium arsenate	7	0	4	0	3
Potassium arsenite	8	1	3	4	0
Potassium cacodylate	5	2	1	1	1
Sodium arsenate	10	0	3	3	4
Sodium arsenite	9	1	2	4	2
Sodium cacodylate	5	0	1	2	2

LIME-SULPHUR AND TOBACCO EXTRACT

It may be at times desirable to add both lime sulphur and tobacco extract to lead arsenate or other arsenical spray mixtures, provided the efficiency of neither is destroyed and the resulting mixture is not dangerous to foliage.

TABLE XIII.—*Effects of lime sulphur and tobacco extract on the burning properties of various arsenical compounds*

Arsenical compounds to which lime sulphur and tobacco extract were added.	Number of brands.	Number of applications.	Number of times injury increased.	Number of times injury decreased.	Number of times injury was the same.	Number of times there was no injury with or without soap.
Arsenic trioxids	1	2	0	0	0	2
Calcium arsenites	1	10	0	7	2	1
Copper aceto-arsenite	1	16	7	1	4	4
Ferrous arsenites	1	2	1	0	0	1
Lead arsenates, triplumbic	4	38	19	0	0	19
Lead arsenates, diplumbic	9	58	25	1	0	32
Lead arsenates, mixture	6	12	12	0	0	0
Zinc arsenite	2	30	10	4	0	16

[12] "Diamond C."

A comparison of Tables IX and XIII leads to the conclusion that the addition of lime-sulphur and tobacco extract to arsenical spray mixtures has practically the same effect upon the foliage as lime sulphur alone.

Arsenic trisulphid may constitute an exception, but our tests with this compound combined with both tobacco extract and soap are too few to be conclusive. It is doubtful, however, if the trisulphid will ever come into general use as an insecticide.

LIME SULPHUR, TOBACCO EXTRACT, AND SOAP

This combination with arsenical compounds is not a desirable one as it often forms a curdled mass that is difficult to apply to the foliage. It also has a greater tendency to injure the foliage than any other combination we have tried. Calcium arsenite in combination with these three substances is the only exception, as may be seen by Table XIV.

TABLE XIV.—*Effects of lime-sulphur, tobacco extract, and soap upon the burning properties of various arsenical compounds*

Arsenical compounds to which lime-sulphur, tobacco extract, and soap were added.	Number of brands.	Number of applications.	Number of times injury increased.	Number of times injury decreased.	Number of times injury was the same.	Number of times there was no injury with or without soap.
Arsenic trioxids	2	5	3	1	0	1
Arsenic disulphid	1	2	2	0	0	0
Arsenic trisulphids	2	5	5	0	0	0
Barium arsenate	1	1	0	0	1	0
Calcium arsenites	3	17	2	8	4	3
Copper aceto-arsenites	2	20	12	2	3	3
Ferrous arsenates	3	8	2	1	0	5
Lead arsenates, triplumbic	8	51	29	0	0	22
Lead arsenates, diplumbic	13	84	57	0	0	27
Lead arsenates, mixtures	11	30	26	0	0	4
Zinc arsenites	6	40	17	1	1	21

GELATIN, AGAR, AND MILK

While soap is probably the best material known to make spray mixtures spread on leaves of cabbage, sugar beets, etc., where there is a tendency to roll off the smooth or waxy surface, its tendency to increase injury by most arsenical compounds gives an incentive to search for some other spreader. Moore (*21*) has given a very thorough discussion of the principles and practices of spreaders in spraying work, and a full bibliography of the subject. Gelatin, agar, and separated milk, having possibilities along this line, were tested in our work with various arsenical compounds to determine if they would influence the burning.

The gelatin was used in strengths of 0.1 per cent and 0.4 per cent (i. e., 1 and 4 grams to the liter). The agar was used in strengths of 0.01 and 0.04 per cent. The separated milk was used in strengths of 0.1 per cent and 0.4 per cent. Various arsenical spray mixtures, including lead arsenate, Paris green, and calcium arsenite, were tried singly and in combination with these three "spreaders" both on greenhouse plants and in the field, and also in the orchard. Repeated experiments showed no tendency on the part of these materials to increase the arsenical injury, and while they are not so efficient as soap in pro-

moting the adhesion of the mixture, yet they may be regarded as very serviceable. The agar in strengths used increased the spreading power least and would have to be used much stronger, perhaps 0.1 per cent, but gelatin 0.4 per cent and milk 0.4 per cent were quite satisfactory for sugar beets, though only moderately so for cabbage.

LIME

It has been recommended by Kilgore (*14*) that lime be added to Paris green and other arsenicals to restrain the injurious action on the foliage.

On tomatoes and beans in the greenhouse we have tested quite thoroughly unslaked lime with calcium arsenite and, with fewer repetitions, with lead arsenate (Corona) and with Paris green. Quite consistently the use of lime has very materially reduced the burning action of these three arsenical compounds as shown by Table XV.

TABLE XV.—*Effect of lime in combination with arsenical compounds*

Date.	Plant.	Chemical.	Amount in 2 liters.	Lime.	Injury.
			Gms.	*Gms.*	
July 25, 1917	Tomato	Calcium arsenite	1.9	None.	Bad.
Do	do	do	1.9	4.6	Do.
Do	do	do	3.9	None.	Defoliated.
Do	do	do	3.9	4.6	Partly defoliated.
Sept. 3, 1917	do	do	2.0	None.	Moderate.
Do	do	do	2.0	9.0	Slight.
Do	do	do	3.9	None.	Partly defoliated.
Do	do	do	3.9	9.0	Moderate.
Mar. 3, 1918	Bean	Lead arsenate (Corona).	9.7	None.	Very slight.
Do	do	do	9.7	9.7	None.
Do	do	do	19.5	None.	Moderate.
Do	do	do	19.5	19.5	None.
Do	Tomato	do	9.7	None.	Slight.
Do	do	do	9.7	9.7	None.
Do	do	do	19.5	None.	Moderate.
Do	do	do	19.5	19.5	None.
Sept. 3, 1917	do	Paris green	6.9	None.	Bad.
Do	do	do	6.9	6.9	Slight.
Do	do	do	9.2	None.	Very bad.
Do	do	do	9.2	9.0	Slight.

FERROUS SULPHID

Volck (*34*) especially has advocated the use of ferrous sulphid to decrease the burning effect of zinc arsenite. We have used this compound (7.2 gm. to 2 liters) many times in combination with different brands of calcium arsenite and of zinc arsenite and have found no general benefit in restraining the injury to the foliage. Usually the injury with and without the ferrous sulphid was the same, but occasionally it was a little more or a little less if the iron salt was added.

EFFECTS OF LETTING MIXTURES STAND BEFORE APPLYING

In the case of that group of arsenicals that are but slightly soluble in water and are often called "insoluble," one would naturally expect that if the suspension were applied as a spray as soon as mixed the amount

in solution would be less than if allowed to stand for some time and the injury correspondingly less. To what extent this principle will operate can be determined by experiment only.

To this end we have made repeated trials with arsenic trioxide and a few with calcium arsenite, calcium arsenate, lead arsenate, and Paris green.

Quite without exception the burning by arsenic trioxide steadily increased as the mixture was allowed to stand. Table XVI illustrates a typical demonstration of this fact. In this case suspensions of arsenic trioxide[13] (2.6 gm. in 2 liters) were prepared at different intervals so spaced that all would be ready for spraying at the same time, having stood the time periods indicated in the left hand column of the table. These suspensions after mixing were kept in an incubator at blood temperature and were stirred at intervals of about six hours, day and night. At the time of spraying the suspensions were thoroughly shaken and a small sample removed and filtered through Swedish filter paper, and the filtrate analyzed. The remainder was sprayed upon tomato and bean plants in triplicate.

TABLE XVI.—*Effects of letting arsenic trioxid stand after mixing*

Time after mixing.	Arsenic trioxid in solution.	Injury.	
		Bean.	Tomato.
	Per cent.		
5 minutes	0.001392	Slight	None.
1 hour	.002784	Moderate	Do.
2 hours	.006960	Very bad	Slight.
4 hours	.012520	Defoliated	Moderate.
8 hours	.016720	.....do	Do.
12 hours	.024128	.....do	Bad.
24 hours	.032480	Dead	Very bad.

Table XVI shows strikingly the progressive increase in burning by allowing arsenic trioxid to stand in suspension and the corresponding increase in the amount of the chemical that went into solution. Other experiments showed that this increase in injury as a result of standing continued for at least 48 hours and was strongly in evidence whether or not soap was added to the mixture. Kilgore (*14*) has shown that arsenic trioxid suspended in water continues to dissolve for 10 days.

The tendency of calcium arsenite, calcium arsenate, lead arsenate, and Paris green to increase in burning power as a result of standing in suspension is relatively slight.

EFFECTS OF REPEATED SPRAYING

It is often necessary for the control of insect pests to spray plants or trees two or more times at intervals of a few days or weeks, and in such cases it is important to know if the second or later applications are especially dangerous from the standpoint of injury to the foliage. Clinton and Britton (*2*) showed that the second application of zinc arsenite may do very serious damage, even though the first did little or no harm. Our work with two brands of zinc arsenite, Thomson's and "Ortho 40,"

[13] Baker's.

strongly confirms this observation. Quite uniformly the second application did much more damage than the first, and indeed a strength of 3.2 grams per liter, applied twice at intervals of about three weeks, did more harm than 6.4 grams applied once on either date. With calcium arsenite this tendency was noticeable but not nearly so pronounced as with zinc arsenite, while with lead arsenate and with copper aceto-arsenite there was but little more injury after the second spray than after the first.

EFFECTS OF HUMIDITY

That humidity is one of the great contributing factors in arsenical spray injury is one of the most striking facts constantly in evidence throughout this investigation. This observation is by no means new and had already been noted by Gillette (*11*) and others; and certain manufacturers of lead arsenates have come to recognize the necessity of using safer mixtures on the Pacific coast and other humid regions than are required elsewhere.

Even with these facts in mind, however, we were almost astonished at the difference in results on leaves kept in moist and dry air. To demonstrate if possible the effects of humidity, an extensive series of spray mixtures was applied about simultaneously in different parts of the State, including an apple orchard near Flathead Lake (Mont.), where the humidity is generally relatively high. As it happened, however, this section was unusually dry during the course of the experiment and much more so than in the other localities where tests were made. Not a drop of rain fell for weeks and the dew point was not reached on a single night during the experiment. The days were rather hot, and wet and dry bulb thermometer readings made three times daily indicated a very low humidity every day and nearly every night. As a result almost none of the spray mixtures used caused any injury whatsoever. Even calcium arsenite (2 gm. in 2 liters of water) caused only slight injury. In the majority of cases, including all localities and dates, this compound in half this strength caused very serious injury to apple foliage, and sometimes defoliation.

A still more striking demonstration of the effects of humidity in extreme cases was made repeatedly by spraying tomato and other plants in pots and covering certain ones with bell jars and keeping duplicates outside. Under these conditions the moisture transpired by the leaves kept the air under the bell jars saturated.

The plants were all kept in diffused light in a basement laboratory to prevent rise of temperature under the bell jars as would happen if kept in direct sunshine. The plants were kept in this condition for usually one or two days and aerated twice daily. The bell jars were then removed and all plants placed under like conditions in the greenhouse until the notes were taken. Table XVII is representative of the results under these conditions.

Probably nothing in the whole series of experiments on arsenical spraying is more striking than the contrast of injury in dry air and in the saturated atmosphere under the bell jars, especially when soap is added. It might seem at first that the bell jar makes an extreme condition of humidity, but a saturated atmosphere out of doors is by no means unusual, especially near large bodies of water, as on the Pacific coast and in the Great Lakes region.

TABLE XVII.—*Showing the increased injury caused by a high humidity, procured by keeping sprayed tomato plants under bell jars*

Chemical.	Chemical in 2,000 cc.	Soap in 2,000 cc.	Injury.	
			Under bell jars.	Not under bell jars.
	Gm.	*Gm.*		
Arsenic trioxid, Baker	1.3	None.	Slight	None.
Do	2.6	None.	do	Do.
Calcium arsenite, Baker	1.9	None.	Dead	Moderate.
Calcium arsenite, Adler	.6	None.	Very bad	None.
Do	.6	14.4	do	Do.
Lead arsenate, Baker, acid	9.3	None.	None	Do.
Do	9.3	28.8	Moderate	Do.
Lead arsenate, Baker, ortho	12.0	None.	None	Do.
Do	12.0	28.8	Moderate	Do.
Lead arsenate (Corona)	9.3	None.	None	Do.
Do	9.3	14.4	Very bad	Do.
Copper aceto-arsenite, Baker	2.4	None.	Nearly dead	Do.
Do	2.4	14.4	Very bad	Very slight.

To determine if it makes a difference whether the plants were placed in the saturated atmosphere immediately after spraying or after drying a short time, some were put under bell jars for the first three days and then removed while others were not covered at once but inclosed the fourth, fifth, and sixth days; and likewise some were kept under bell jars the first day only, others the second day only, and still others the third day only. Reducing the time, in another experiment some were covered the first hour after spraying, others the third hour, others the first hour of the second day and still others the first hour of the fifth day. The results showed that a saturated atmosphere for even so short a period as one hour considerably increased the injury, though a longer time caused still more damage. Considering the results in all these experiments it appeared to make little difference at what time after spraying the plants were covered, if not more than a few days, provided the time spent in this saturated atmosphere was the same.

It was noted that under the bell jars the plants were dripping with moisture; not only was the air saturated, but the surfaces of the plants were covered with films and drops of water. To learn if an increased amount of humidity is of significance when kept below the point of saturation, sets of tomato plants (three in a pot), were sprayed and dried, then one set was put under a tightly closed bell jar, one was put under a bell jar with the stopper removed and with a tiny crack underneath, and a third was left outside. All were kept in strong diffused light.

Under the jar with the stopper removed were placed wet and dry bulb thermometers and a tiny fan run by a toy motor. This was used to fan the wet bulb for one minute before reading the temperature.

The procedure was to spray 9 tomato plants, three sets of 3 in a pot, with the same mixture, let them dry for a few minutes, then inclose one set tightly under a bell jar 8 by 16 inches on a ground glass plate, inclose another set under a bell jar slightly open at the top and bottom as described above, and keep the third set on the table beside them without covering. After standing under these conditions for three days the plants were all carried to the adjoining greenhouse, where they were left uncovered for about 10 days, after which notes were taken.

Six such experiments were carried out, of which the one shown in Table XVIII is representative. In this case the plants were sprayed with calcium arsenite[14] (1.95 gm., in 2 liters of water; no soap added).

It will be seen that the humidity does not need to reach the saturation point (100 per cent relative humidity) to materially increase the injury over that in a drier atmosphere, although in the saturated atmosphere still greater injury was done. Presumably, other conditions being equal, the degree of injury will be in a certain relation to the relative humidity.

Wetting the leaves at frequent intervals after spraying did not, however, have the same effect as a humid atmosphere.

TABLE XVIII.—*Showing the increased injury caused by humidity produced by transpiration under bell jars*

Conditions.	Time.	Temperatures.			Degree of injury.
		Wet bulb.	Dry bulb.	Relative humidity.	
		F.	*F.*		
Under bell jar closed....	(a)	.....	.....	100	Plant dead.
Under bell jar partly closed.	8 a. m..	58°	62°	79	Partly defoliated.
	1 p. m..	64	66	90	
	5 p. m..	64	66	90	
	8 a. m..	60	62	89	
	1 p. m..	64	66	90	
	5 p. m..	64	66	90	
	8 a. m..	56	58	89	
	1 p. m..	58	60	89	
Not under bell jars.....	8 a. m..	57	64	65	Injury moderate.
	1 p. m..	60	68	63	
	5 p. m..	61	70	61	
	8 a. m..	56	64	60	
	1 p. m..	58	68	54	
	5 p. m..	59	67	62	
	8 a. m..	53	69	32	
	1 p. m..	55	61	68	

[a] Throughout.

Both in the greenhouse and out of doors sprayed foliage was given a light application of water through a fine spray nozzle every few hours throughout the day. The surrounding air was hot and dry and the leaves dried off very soon after each application. Under these conditions, if no soap had been mixed with the arsenical, the injury was not in any case noticeably greater than on the control plants that were sprayed with the same arsenical but not sprayed with water afterwards. In case soap had been added there was a slight tendency to increase the injury by subsequent sprayings with water, but this injury was incomparably less than produced by a humid atmosphere.

EFFECTS OF SOIL MOISTURE

As plants and trees can not always receive a sufficient amount of moisture through the roots it is but natural in spraying operations where foliage burning is liable to occur to raise the question as to whether or not a wilted condition of the leaves would affect the extent of injury.

[14] Baker's.

Greenhouse conditions have been found best for determining the answer, and we have made innumerable trials on tomatoes, beans, and other plants. When slightly or moderately wilted plants were sprayed in comparison with turgid ones, the resulting injury has been invariably practically the same. Any differences that have been noted were within the experimental error. The only cases in which there was a marked difference was when the wilting was so extreme and so long continued that the leaves were practically dying.

EFFECTS OF TEMPERATURE

A factor that might be supposed to affect arsenical injury is the temperature of the leaves and the surrounding air. In an effort to determine the effect of temperature upon the extent of injury to plants sprayed with arsenicals several series were run in a differential thermostat. This consisted of a series of chambers in which were maintained temperatures varying from 5° to 40° C. Light was admitted through glass at the top. Sprayed tomato and cucumber plants were placed in this thermostat for two days and then transferred to an open bench in the greenhouse.

The arsenicals used were diplumbic lead arsenate and Paris green.[15] It was found that between 5° and 15° C. there was little difference in the injury. From 15° to 25° the injury materially increased. Above 25° there was apparently a marked increase in the injury, but the unsprayed controls could not be kept in a healthy condition at these temperatures. Hence the records on the arsenical injury to sprayed plants at corresponding temperatures were of doubtful value.

These experiments show that an elevation of temperature increases the injury, but we wish to emphasize that under field and orchard conditions temperature is of minor importance as compared with humidity.

EFFECTS OF WOUNDING

In connection with studies made on the effects of arsenical compounds on the bark of fruit trees, we have shown (*29*, *30*) that the all-important factor in determining whether or not injury will occur is the integrity of the outer corky layers of the bark, i. e., whether or not this has natural or artificial openings. Are wounds important in determining the extent of injury through the foliage? We have often noticed that there is an excessive injury at the margins of the wounds made by hail stones, whipping by the wind, and other mechanical agencies. In some instances we have purposely made such wounds before spraying. In some of these cases the injury was strictly confined to the margins of such wounds, but this was only where the arsenical treatment was mild. In some instances where the injury was confined to a few scattered spots there was evidence of a tiny puncture in each spot, revealed only by the aid of a lens. Wherever the arsenical treatment was so severe as to injure the foliage badly, there was every evidence of absorption directly through the unbroken epidermis. The injury at the margins of wounds is strictly local, usually making a brown strip about one-eighth of an inch wide and, except where such wounds were unusually extensive, this injury is of little practical importance.

[15] Both Baker's.

EFFECTS OF SHAKING BRANCHES AFTER SPRAYING

Both in the practical orchard and field spraying and in experimental work there is often more or less wind that shakes some of the liquid from the leaves. It is important to know, therefore, the extent to which this will decrease the amount of arsenical injury to the foliage. In all the experimental work of this investigation the spraying was done when there was little or no wind, but this was probably an unnecessary precaution. On several occasions apple limbs were sprayed in duplicate, one being shaken vigorously immediately afterwards and the other hanging quietly until the spray mixture had dried on. Similar experiments were conducted with potted plants in the greenhouse. In no case was there a marked reduction in the amount of burning and usually no difference could be detected. As this shaking removed the excess liquid from the leaves so much more quickly and thoroughly than an ordinary wind, it is fair to conclude that any wind not strong enough to practically prevent spraying operations will have no material effect on the amount of arsenical injury.

EFFECTS OF LIGHT AND DARKNESS

Three experiments were conducted to determine if light increases or decreases the amount of injury caused by arsenical spraying.

Potted tomato plants were placed in a dark room in the evening. Early the next morning two were sprayed with calcium arsenite and put under bell jars in dark boxes wrapped with black cloth; two were sprayed and put under bell jars not in dark boxes, and unsprayed controls were kept under both these conditions. All boxes and bell jars were kept in a strong diffused light for two days and then the plants were removed and placed on a bench in the greenhouse. In two of the experiments the injury to the sprayed plants was equally bad in the dark boxes and in the light. In the third, all the sprayed plants were killed, making comparisons impossible. The unsprayed plants remained in healthy condition.

SIGNIFICANCE OF THE RESULTS

The place of arsenical compounds in the category of insecticides is so important that we can not at present expect their replacement by any other group of chemicals. They have their faults, however, and among these is the marked tendency to injury of the crop they are intended to protect. It is therefore of prime importance to learn the factors that determine this injury and with this knowledge to reduce it to a minimum. Some of the existing beliefs regarding this injury are founded on fact, the difficulty being to know the extent of the tendency. Other beliefs appear to the based on a wrong conception.

It has been found quite difficult to make exact statements concerning the toxicity of the different arsenicals because of the variability of their composition. This is especially true of the lead arsenates. Theoretically one might expect ortho, meta and pyro arsenates each in the form of monoplumbic, diplumbic, and triplumbic salts, a total of 9 possibilities. Actually the number that form is fewer, but, on the other hand, many commercial lead arsenates are not single salts of lead and an arsenic acid but a mixture of different lead arsenates. Furthermore, the product of a company may change from time to time, and even within a short

period different lots from the same factory may vary. It is encouraging that the product of certain companies is becoming stabilized, and it is to be hoped that others will follow their example. At the present time, however, we can not make definite statements about lead arsenate without specifying the brand and lot. These remarks apply in some degree to zinc arsenite and calcium arsenite, while Paris green and arsenic trioxid are much more uniform in composition.

It is rather surprising that calcium arsenate should ever have been used as extensively as it was some 20 years ago when one observes its tendency to burn foliage. To be sure, the competition with other arsenicals, e. g., lead arsenate, did not then exist, and in the less frequent injury by these compounds we find one of the chief causes of its progressive abandonment.

The most striking example of a poorly founded prejudice is the quite general belief that arsenic trioxid (common white arsenic) will burn foliage even when occurring as a very small impurity in other arsenicals. We are not able to explain fully this belief. Probably it lies partly in the fact that chemists expressed their findings of soluble arsenic in insecticides in terms of "arsenious acid," the reasons for which are not always clear to others. Probably also, the letting of the spray mixture stand for hours or days before applying increases the danger from arsenic trioxid more than from other arsenical compounds, and has been a factor. In any event, we feel that we have established quite conclusively that a freshly prepared suspension of arsenic trioxid is not particularly harmful to foliage, and we entertain a hope that this relatively cheap form of arsenic may come into general use for the control of certain insects. Of course its efficacy as an insecticide has yet to be determined.

The hope expressed by Gillette (*11*) that arsenic trisulphid wouid prove less injurious than the arsenical insecticides in common use, seems unlikely of realization, for, while this compound has a low solubility ln pure water, it nevertheless causes considerable burning when sprayed upon foliage.

In the selection of an arsenical insecticide for any crop to be sprayed, the entomologist will do well to consult the table of susceptibility on page 512, for on some plants a mixture may be used that would be quite destructive to others.

The practice of adding to an arsenical spray mixture some other material to act as a supplementary insecticide or fungicide, or as a "sticker" or "spreader," or to decrease the tendency to injury, has been the subject of considerable controversy. Conspicuous among these materials is soap, which, in addition to being a spreader, has been shown by Parker (*22*) to be of considerable value in keeping lead arsenate in suspension. That soap increases the tendency to burning by nearly all "insoluble" arsenicals is clearly established (p. 523), but it is safe to say that the increase in burning tendency with lead arsenate is not sufficient to discourage its use on the more resistant plants such as cabbage, sugar beets, potatoes, and apple. It is indeed a fortunate circumstance that cabbage and sugar beets, on which a spreader is a necessity, are resistant enough to arsenical injury to make the use of soap permissible. It is likewise fortunate that soap has been found to actually decrease the injurious properties of Paris green, for gardeners may now rest assured that the addition of soap to it is not only permissible but desirable. Lime sulphur increases the injury by nearly all the arsenical insecticides in about the same degree that soap does, and must not be used in com-

bination with them on very tender foliage. The fact that lime sulphur decreases the injury caused by calcium arsenite appears to be of only scientific interest, for even with this addition the burning will be so severe that this arsenical is not useable under most circumstances. Tobacco extract gives no increase in the injury by any of the arsenicals.

Of the environmental conditions, only humidity seems to be of large significance, for while the burning is increased in a measure by a rise in temperature, it is doubtful if this tendency is great enough within the range of temperatures encountered in the field to be entitled to practical consideration. The effect of humidity, however, can hardly be overrated and must be given large consideration in all spraying operations. To be sure, this factor is not under the control of the orchardist and farmer, but he can make a fair estimate of the humidity that is likely to prevail following spraying in his locality, and be bold or cautious in the selection of his spraying material.

The varying results observed in this investigation when spraying experiments were repeated under conditions identical, except as to weather, show the significance of atmospheric conditions, but, what is of especial importance to the investigator, these varying results show the great danger in drawing conclusions from single experiments.

SUMMARY

Based on an investigation extending through a period of 10 years, and involving the application of various arsenical spray mixtures to approximately 10,000 separate plants and branches of trees sprayed individually, we submit the following conclusions concerning arsenical injury to foliage:

(1) The name commonly used does not indicate the composition of an arsenical with sufficient exactness. This is especially true of the calcium arsenites, lead arsenates, and zinc arsenites, in which the results obtained by using different lots labeled the same except as to manufacturer may give widely divergent results. The arsenic trioxids and Paris greens are much more uniform in composition.

(2) The arsenical insecticides least injurious to foliage are iron arsenate and certain of the lead arsenates. Possibly new ones will be proposed that will be as safe or safer.

(3) Of the lead arsenates not all pure diplumbic ortho-lead arsenates are identical in burning properties, nor are all triplumbic ortho-lead arsenates identical in this respect. Some diplumbic lead arsenates are as safe to use as some of the triplumbic ones.

(4) Arsenic trioxid is not so dangerous to the foliage as is generally supposed, and indeed this compound is permissible as an insecticide on any but the most delicate foliage, provided it is applied promptly after mixing with water.

(5) Standing after mixing causes a very marked increase in injury by arsenic trioxid and a slight increase in injury by other arsenical insecticides.

(6) Of the readily soluble arsenical compounds cacodylic acid and sodium and potassium cacodylates proved the most injurious. This is quite in contrast to the well recognized fact that these compounds of arsenic are less harmful to the higher animals than most others.

(7) The foliage is more susceptible to arsenical injury than the fruit or the stems.

(8) The injury to leaves is characterized, first, by a lack of luster, then wilting, and a final change to some shade of brown (dependent upon the species of plant) as the affected tissue becomes dead and brittle. The symptoms are not sufficiently distinctive to separate arsenical injury from some others.

(9) The injury to the foliage is practically all through the lower epidermis, regardless of the numbers of stomata in the two surfaces, indicating that it is a result of direct penetration of the thinner cuticle.

(10) Individual plants of the same species and variety vary somewhat in their susceptibility to arsenical injury.

(11) The older leaves of a plant are more susceptible than the younger ones.

(12) Soap added to soluble arsenicals offers a slight protective action.

(13) Soap added to most insoluble arsenicals increases the injury by increasing the solubility to a point more than counteracting its slight protective action.

(14) Soap added to Paris green in suspension distinctly restrains the burning of foliage.

(15) Gelatin, milk, and agar do not increase the arsenical injury to foliage.

(16) Lime sulphur increases the injury caused by most insoluble arsenical compounds.

(17) Lime sulphur distinctly decreases the injury caused by calcium arsenite but not to a sufficient extent to make this a safe insecticide.

(18) Tobacco extract has little influence upon the injurious properties of arsenical insecticides.

(19) Lime restrains, to some extent, the injury by calcium arsenite and Paris green.

(20) We have not been able to decrease the zinc arsenite injury by adding ferrous sulphid.

(21) Repeated spraying with zinc arsenite is liable to result in serious burning.

(22) A slightly wilted condition of the foliage does not result in increased injury.

(23) Light seems not to be an important factor in arsenical injury to foliage.

(24) An increase in atmospheric temperature results in a moderate increase in arsenical injury; but within the ranges of temperature found during the summer in a suitable orchard climate this is of little practical importance if the air is relatively dry.

(25) Humidity is the greatest environmental factor in determining arsenical injury to foliage, and this influence is very marked even before the saturation point is reached.

(26) Using a few experiments as a basis for generalization upon arsenical injury is a treacherous proceeding and may lead to erroneous conclusions. The only safe procedure is to test repeatedly each point under consideration.

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PLATE I

Stages in the development of arsenical spray injury on apple foliage.
A.—Three days after spraying.
B.—Four days after spraying.
C.—Five days after spraying.
D.—Seven days after spraying.
E.—Ten days after spraying.
F.—Twenty-five days after spraying.

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A STATISTICAL STUDY OF THE COMPARATIVE MORPHOLOGY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS[1]

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INTRODUCTION

Eriksson and Henning (*3*)[3] and Stakman and Levine (*14*) have shown that the urediniospores of the various biologic forms of *Puccinia graminis* Pers. differ considerably in size. Stakman and Levine also have shown that the urediniospores of any of the biologic forms become appreciably smaller when the rust is grown on fairly resistant host plants or under other extremely unfavorable environmental conditions.

A mathematical analysis of spore measurements in this connection is of considerable value. Pearl and Surface (*7*) say, "It is certain that not only are quantitative methods needed in biology, but also that a far more serious need is for something of the methodological viewpoint." Rosenbaum (*10*) further emphasizes this need by saying "The biometric methods therefore furnish a means of extending the descriptive method and of expressing quantitatively what investigators have heretofore attempted to express qualitatively." This would seem to have a special application in the case of biologic forms, which have always been distinguished on the basis of their physiologic behavior rather than that of their morphologic structure.

The object of this work was to determine by means of statistical studies the morphologic identity, i. e., the limits of variation and the biometric constants for length and width, of the aeciospores, urediniospores, and teliospores of the different biologic forms of stem rust. The studies described in this paper were limited to *Puccinia graminis tritici* Erikss. and Henn., *P. graminis secalis* Erikss. and Henn., *P. graminis avenae* Erikss. and Henn., *P. graminis phleipratensis* (E. and H.) Stak. and Piem., and *P. graminis agrostis* Erikss. It is intended, however, to extend the study to a number of the more important forms of the wheat stem rust, *P. graminis tritici*, which recently have been discovered.

[1] Accepted for publication Aug. 21, 1922. Data from cooperative investigations between the Bureau of Plant Industry, United States Department of Agriculture, and the Agricultural Experiment Station of the University of Minnesota.

[2] The author takes great pleasure in making acknowledgment to Dr. E. C. Stakman and Dr. H. K. Hayes, plant pathologist and plant breeder, respectively, of the Minnesota station, and agent and collaborator, respectively, of the Office of Cereal Investigations, for many valuable suggestions and helpful criticisms.

[3] Reference is made by number (italic) to "Literature cited," p. 567.

EXPERIMENTAL METHODS

The aecial material used for making spore measurements was obtained from several species of barberry (*Berberis* spp.) artificially inoculated with teliospores obtained from wheat, quack grass, oats, and redtop, respectively. The infected plants were collected in the spring of the year at University Farm, St. Paul, Minn. Young, tender leaves of separate barberry plants were inoculated with the teliospores from each of the hosts. Sets of seedlings of wheat, rye, oats, and redtop were inoculated with the mature aeciospores. The infection results thus obtained verified the identity and purity of each of the biologic forms. The aeciospores used for the determination of the spore size of each biologic form were grown under identically the same cultural conditions and on plants of the same barberry species, namely, *Berberis vulgaris* L. In the study of the effect of the host on the dimensions of aeciospores, the cultural conditions were exactly alike, but the barberry species differed in each case. The aeciospores obtained in this manner were first put away as herbarium material and were not measured until several months after maturity. Fifty aeciospores picked out at random from a considerably larger population were measured for both length and width.

Pure strains of urediniospores of each of the biologic forms of *P. graminis* were obtained from specimens of viable uredinial material on grains and grasses, collected at University Farm, St. Paul, Minn., and cultured continuously in the greenhouse on seedlings of different host plants. The measurements in each case were made of healthy urediniospores from the superficial layer of mature uredinia, care being taken to measure spores from uredinia in the same stage of development. In determining the urediniospore dimensions of the individual biologic forms, 200 spores in each population were measured for length and width, respectively, whereas in the study of environmental and host effects 100 spores were measured in every case. For the latter experiment, however, 200 measurements were made of the controls. In the first instance, that is, in the study of the comparative morphology of the urediniospores, the biologic forms concerned were grown on congenial host plants and under uniform and favorable conditions. In the study of relationship of host to urediniospore dimensions, both susceptible and resistant varieties were used, but the environment was maintained uniform and favorable. In the study of the effect of cultural conditions on the size of urediniospores, only one biologic form, *P. graminis tritici*, and one variety of wheat, Haynes Bluestem, were used, the environmental conditions, such as temperature, light, and soil moisture, being interchangeably altered. Control series were conducted simultaneously with the experimental series.

The telial material used in this study was collected at St. Paul, Minn., and kept in the herbarium for nearly a year before the measurements were made. The rust on the common wheat (*Triticum vulgare* Vill.) was *P. graminis tritici;* on the quack grass (*Agropyron repens* (L.) Beauv.) it was *P. graminis secalis;* on the oat (*Avena sativa* L.) it was *P. graminis avenae;* on the timothy (*Phleum pratense* L.) it was *P. graminis phleipratensis;* and on the redtop (*Agrostis alba* L.) it was *P. graminis agrostis.* One hundred teliospores were measured in each test and the statistical results computed from these.

All of the spore measurements were made with the same microscope, carefully calibrated and standardized. The Zeiss screw micrometer was

used throughout the entire period of the work. The illumination for the microscope was derived from artificial light of uniform intensity. The effect of refraction was eliminated as far as possible by mounting the material in drops of water of approximately equal size. It also was attempted to maintain the quantity of water on the slide nearly constant while the measuring of a given population of spores was in progress.

In order to make the measurements of a given group of spores representative and significant, a sufficient number of measurements must necessarily be made. It was found that in the case of aeciospores a minimum of 50 measurements was required. In the case of urediniospores and teliospores at least 100 measurements were necessary. Greater numbers than these did not seem to add materially to the value of the results obtained in the present investigation, although perhaps they might have been useful in some cases. The longitudinal measurements of teliospores were made from the exterior of the apex to the point of attachment of the pedicel, whereas the transverse measurements were made across the septum separating the two cells.

COMPARATIVE MORPHOLOGY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS

The following figures show the dimensions of spores of *Puccinia graminis* and *P. phleipratensis*[4] as given by several different workers, who made their studies at different times.

TABLE I.—*Spore dimensions of P. graminis and P. phleipratensis as given by various authors*

Authority.	Range of variation for length and width (in microns).				
	P. graminis.			*P. phleipratensis.*	
	Aeciospores.	Urediniospores.	Teliospores.	Urediniospores.	Teliospores.
Eriksson and Henning (*3, p. 25 and 126*).	[a]14–26	[b]17–40×14–22	[b]35–60×11–22	18–27×15–19	38–52×14–16
McAlpine (*5, p. 121*).....	.....	20–36×14–18	35–63×14–25	.....	.....
Plowright (*8, p. 162*).....	15–25	25–38×15–20	35–65×15–20	.....	.....
Saccardo (*11, v. 7, p. 622, and v. 11, p. 204*).	14–26	24–45×14–21	34–60×12–22	18–27×15–19	35–52×14–16
Sydow (*15, p. 695 and 785*).	14–26	22–42×16–22	35–60×12–22	18–30×15–20	38–52×14–16

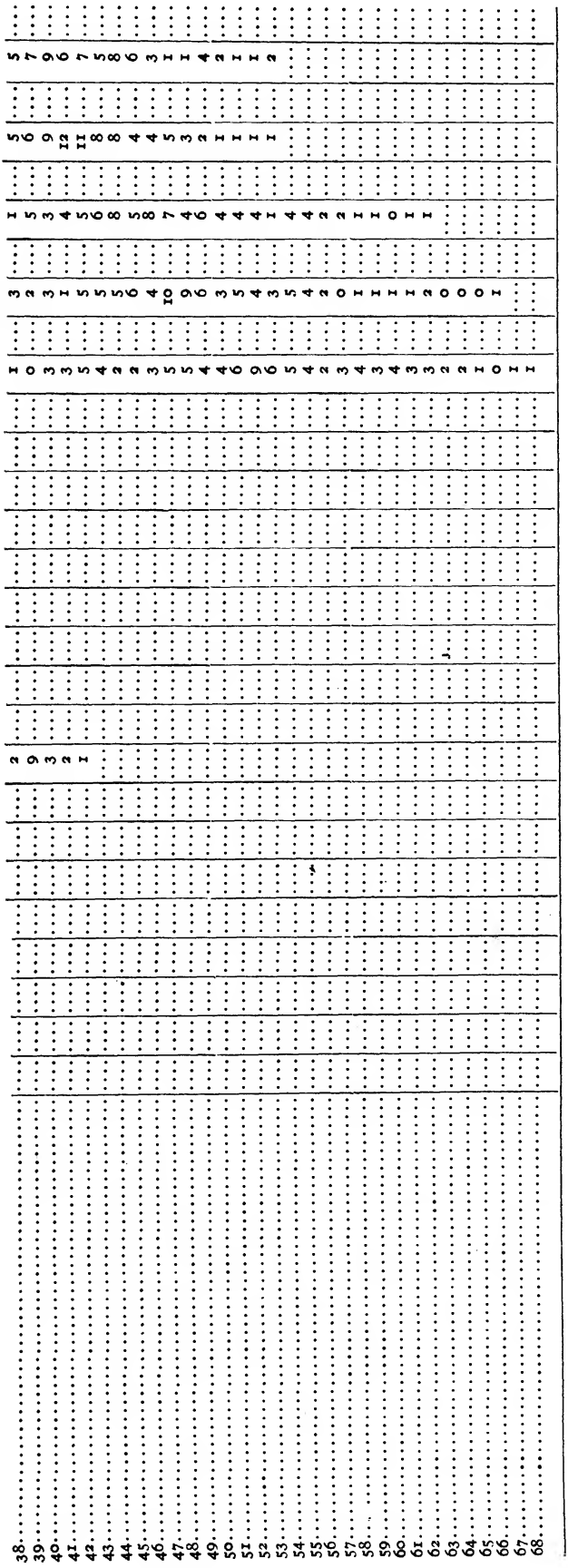
[a] The aeciospore measurements are for range of variation in the diameter of these spores.
[b] These dimensions represent the extremes in range of size of two sets of measurements made by the authors.

[4] *P. graminis phleipratensis* (E. & H.) Stak. and Piem. is cited in this instance as *P. phleipratensis* Eriksss. and Henn. because the measurements quoted were obtained from sources in which this biologic form of stem rust was treated as a separate species.

TABLE II.—*Frequency of different sizes of 50 aeciospores, 200 urediniospores, and 100 teliospores in each of certain biologic forms of Puccinia graminis grown on congenial host plants and under uniform environmental conditions*

[Spores.]

Classes according to length and width.	Aeciospores.								Urediniospores.										Teliospores.									
	P. graminis tritici.		*P. graminis secalis.*		*P. graminis avenae.*		*P. graminis agrostis.*		*P. graminis tritici.*		*P. graminis secalis.*		*P. graminis avenae.*		*P. graminis phleipratensis.*		*P. graminis agrostis.*		*P. graminis tritici.*		*P. graminis secalis.*		*P. graminis avenae.*		*P. graminis-phleipratensis.*		*P. graminis agrostis.*	
	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.	Length.	Width.
Microns.																												
10																				1								1
11				2				3												3								1
12			1	7		1	1	12											1		8		3		2		9	
13		2	1	19		5	2	21								1		4	3		10		6		5		14	
14		4	2	13		13	4	11				3				5		27	6		20		12		13		20	
15	2	14	6	6	1	22	7	3				10				24		52	14		25		22		25		28	
16	2	21	9	3	1	7	14			2		49		3		46	1	72	20		18		25		29		14	
17	2	7	11		6	2	8			6		60		7		58	1	35	27		8		15		17		7	
18	5	2	8		13		7			20		47		17	2	46	8	10	16		4		10		7		4	
19	7		6		18		4			44		25		38	5	16	14		6		3		4		2		2	
20	18		4		9		2			83	2	5	1	70	14	4	23		6				2					
21	7		1		2		0			26	3	1	1	42	16		23		1				1					
22	4		1				1			15	3		3	17	13		38											
23	1								2	4	11		4	6	31		28											
24	1								3		12		9		31		25											
25	1								3		22		14		36		16											
26									6		24		18		27		11											
27									9		38		18		15		8										1	
28									9		31		39		8		3										1	
29									14		24		24		2		1										2	
30									16		11		18										1		1		1	
31									19		8		13										0		0		1	
32									26		3		16										0		2		2	
33									15		3		11								1		0		3		4	
34									17		0		5								1		2		2		6	
35									19		2		4								1		2		2		5	
36									12		3		2								2		2		4		4	
37									13												3		2		5		5	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The differences in the dimensions given by the various workers in the case of *P. graminis* could readily be explained on the basis that more than one biologic form of this species was involved. However, other factors, such as host plant, habitat, personal element, number of measurements made, etc., might have played a part in causing the variation. From Table I, it will be noticed that the size of the urediniospores of timothy rust as given by Sydow differed slightly from those recorded by Eriksson and Henning and by Saccardo. This difference perhaps might be explained as due either to experimental error, variation in the environmental conditions under which the spores were developed, or the number of measurements made. It must be borne in mind, however, that the differences in the dimensions of any of the spores indicated in the table may or may not be really significant. The significance of the difference could not be proved on account of the impossibility of applying a biometric test to the limited data available. In order to determine the value of numerical differences it is necessary to calculate the probable error of these differences and then establish the relationship between the differences and their probable errors. Rietz and Smith (*9*) and Pearl and Miner (*6*) state that a difference may be considered as "certainly significant" only when it exceeds its probable error by more than three times, since a difference which is less than three times its probable error "may reasonably be attributed to random sampling" (*9*), and "that as the ratio, Dev.: P. E., passes 3 the odds against the deviation increase rapidly, reaching a magnitude at 8, which, practically speaking, is beyond any real power of conception" (*6*).

The biometric constants obtained in this study were calculated according to the methods given by Davenport (*2*) and Babcock and Clausen (*1*).

Table II represents a summary of the measurements of all of the biologic forms studied, in their different spore stages, when grown under normal conditions. This table gives the classes according to width and length in microns (each class differing from the next by a single micron), and the number of spores falling into each class both for length and width.

In Tables III and IV, in addition to the variations in the spore measurements, the constants with their probable errors for the different spores of the various biologic forms are given. Table III represents spore lengths while in Table IV spore widths are given. The classes for width differ by one micron (μ) in all cases, whereas the classes for length vary with each spore type. In the measurements of aeciospores the classes differ by 1 μ, in those of urediniospores by 3 μ, and in those of teliospores by 5 μ.

The probable errors of the differences in the statistical results obtained in this study are given in Tables V, VII, and VIII. The bearing they have on the dimensions of the different spore studies is brought out in the discussions of each of the spore types.

In figures 1 to 6, curves are plotted for spore dimensions in microns. These graphs represent the variation in the spore sizes of the biologic forms when the rust was grown on highly susceptible hosts and under very favorable conditions. In each of these graphs the number of spores falling into each class was plotted according to the data contained in Tables III and IV.

TABLE III.—*Variations and constants for length of spores of biologic forms of Puccinia graminis grown on congenial host plants and under uniform environmental conditions*

Spores and biologic forms.	Host plants.	Spore classes according to length.													Total number.	Constants.		
		12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ	23μ	24μ	25μ	Mean.	Standard deviation.	Coefficient of variability.
Aeciospores:																		
P. graminis tritici....	*Berberis vulgaris*....	...	...	...	2	2	2	5	7	18	7	4	1	1	1	50	19.72±0.19	1.98±0.13	10.05±0.68
P. graminis secalis...	.....do.....	1	1	2	6	9	11	8	6	4	1	1	...	...	...	50	17.10± .19	2.00± .13	11.70± .79
P. graminis avenae...	.....do.....	...	...	...	1	1	6	13	18	9	2	...	...	...	...	50	18.62± .11	1.20± .08	6.44± .43
P. graminis agrostis..	.....do.....	1	2	4	7	14	8	7	4	2	0	1	...	...	...	50	16.46± .18	1.93± .13	11.73± .79
		15μ		18μ		21μ	24μ	27μ	30μ	33μ	36μ	39μ	42μ					
Urediniospores:																		
P. graminis tritici...	*Triticum vulgare*....	.....		.....		.....	8	24	49	58	44	14	3			200	32.40±0.19	3.89±0.13	12.00±0.41
P. graminis secalis....	*Secale cereale*.....	.....		.....		8	45	93	43	6	5	....	....			200	27.14± .14	2.91± .10	10.73± .36
P. graminis avenae...	*Avena sativa*.....	.....		.....		5	27	75	55	32	6	....	....			200	28.50± .15	3.24± .11	11.34± .38
P. graminis phleipratensis.	*Phleum pratense*....	.....		7		43	98	50	2	....	....	....	....			200	23.95± .12	2.41± .08	10.01± .34
P. graminis agrostis..	*Agrostis alba*.....	1		23		84	69	22	1	....	....	....	....			200	22.37± .12	2.61± .09	11.67± .39
		25μ		30μ		35μ	40μ	45μ	50μ	55μ	60μ	65μ	70μ					
Teliospores:																		
P. graminis tritici....	*Triticum vulgare*...	.....		.....		.....	12	16	28	20	17	6	1			100	51.80±0.49	7.23±0.34	13.95±0.67
P. graminis secalis...	*Agropyron repens*...	.....		.....		8	14	30	27	14	6	1	....			100	47.35± .45	6.65± .32	14.04± .67
P. graminis avenae...	*Avena sativa*.....	.....		1		8	18	34	22	13	4	....	....			100	46.15± .43	6.46± .31	13.85± .66
P. graminis phleipratensis.	*Phleum pratense*....	.....		3		16	43	29	8	1	....	....	....			100	41.30± .32	4.78± .23	11.56± .55
P. graminis agrostis..	*Agrostis alba*.....	1		7		24	34	23	9	2	....	....	....			100	40.30± .40	5.87± .28	14.56± .70

TABLE IV.—*Variations and constants for width of spores of biologic forms of Puccinia graminis grown on congenial host plants and under uniform environmental conditions*

Spores and biologic forms.	Host plants.	Spore classes according to width.													Total number.	Constants.		
		10μ	11μ	12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ	23μ		Mean.	Standard deviation.	Coefficient of variability.
Aeciospores:																			
P. graminis tritici	*Berberis vulgaris*	...	...	...	2	4	14	21	7	2	...	...	...	...	...	50	15.66±0.10	1.07±0.07	6.83±0.46
P. graminis secalis	do	...	2	7	19	13	6	3	...	...	...	...	...	...	...	50	13.46± .11	1.17± .08	8.69± .58
P. graminis avenae	do	...	...	1	5	13	22	7	2	...	...	...	...	...	...	50	14.70± .10	1.02± .07	6.97± .47
P. graminis agrostis	do	...	3	12	21	11	3	...	...	...	...	...	...	...	...	50	12.98± .09	.97± .07	7.47± .50
Urediniospores:																			
P. graminis tritici	*Triticum vulgare*	...	...	...	...	...	...	2	6	20	44	83	26	15	4	200	19.79± .06	1.27± .04	6.42± .22
P. graminis secalis	*Secale cereale*	...	...	...	...	3	10	49	60	47	25	5	1	...	...	200	17.19± .06	1.26± .04	7.33± .25
P. graminis avenae	*Avena sativa*	...	...	...	...	...	...	3	7	17	38	70	42	17	6	200	19.94± .07	1.36± .05	6.90± .23
P. graminis phleipratensis	*Phleum pratense*	...	...	...	1	5	24	46	58	46	16	4	...	...	...	200	16.88± .06	1.32± .04	7.81± .27
P. graminis agrostis	*Agrostis alba*	...	...	...	4	27	52	72	35	10	...	...	...	...	...	200	15.68± .05	1.12± .04	7.16± .24
Teliospores:																			
P. graminis tritici	*Triticum vulgare*	...	...	1	3	6	14	20	27	16	6	6	1	...	...	100	16.67± .12	1.74± .08	10.42± .46
P. graminis secalis	*Agropyron repens*	1	3	8	10	20	25	18	8	4	3	...	...	...	...	100	14.77± .12	1.83± .09	12.37± .59
P. graminis avenae	*Avena sativa*	...	...	3	6	12	22	25	15	10	4	2	1	...	...	100	15.84± .12	1.78± .09	11.24± .54
P. graminis phleipratensis	*Phleum pratense*	...	...	2	5	13	25	29	17	7	2	...	...	...	...	100	15.63± .10	1.46± .07	9.35± .46
P. graminis agrostis	*Agrostis alba*	1	1	9	14	20	28	14	7	4	2	...	...	...	...	100	14.64± .12	1.72± .08	11.73± .56

AECIOSPORES

No previous record of aeciospore measurements of biologic forms of *P. graminis* could be found. The data available relate to the species as a whole, the assumption evidently having been that there was no difference in the size of aeciospores of the various biologic forms. Figures 1 and 2, however, show at a glance the variation existing between different biologic forms at this juncture in their life cycle. The variation is greater in figure 1, in which the curves for spore length are plotted. Here the class containing the greatest number of individuals in the *P. graminis tritici* curve is greater by 4 μ than the identical class in the agrostis curve,

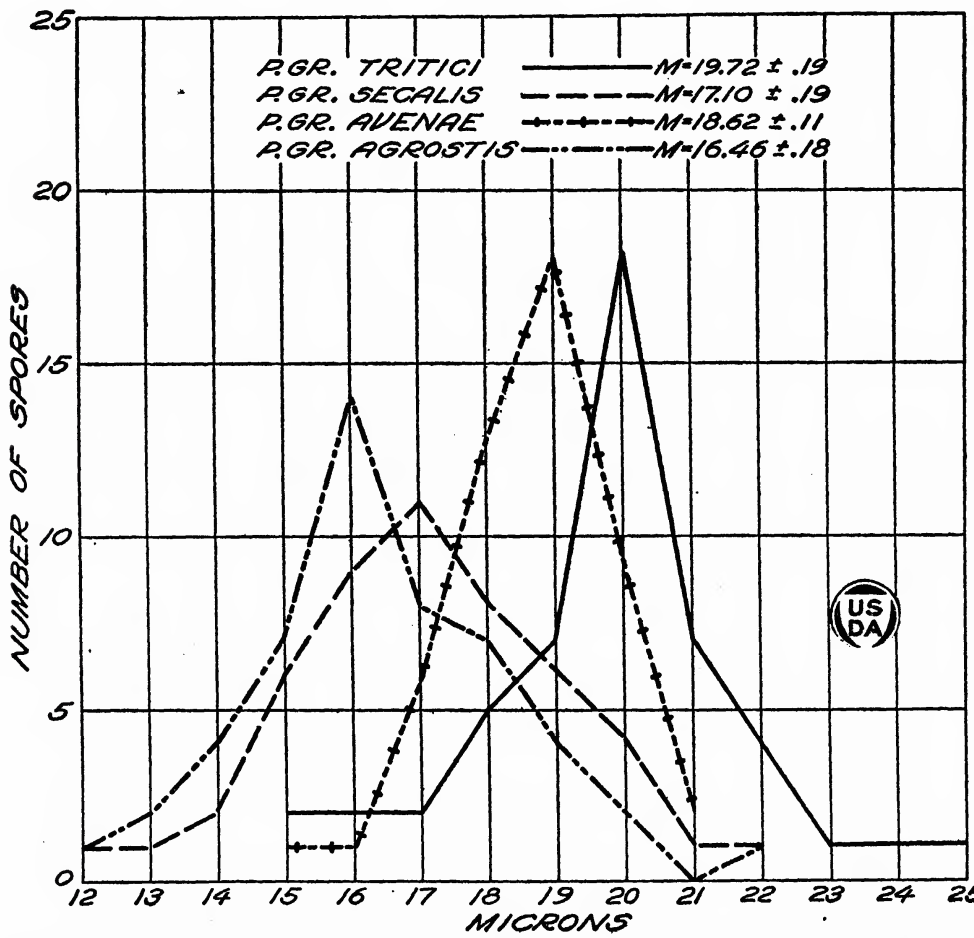

FIG. 1.—Differences in lengths of aeciospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

greater by 3 μ than that in the secalis curve, and greater by only 1 μ than the homologous class in the avenae curve. Figure 2 presents a somewhat different picture. In this graph which represents curves for spore width, the greatest difference between any two classes containing the largest number of individuals is 3 μ, viz, *P. graminis tritici* and *P. graminis agrostis*. In *P. graminis secalis* and *P. graminis agrostis* the modes fall at exactly the same point, i. e., at 13 μ. An examination of the constants in the first section of Tables III and IV and of the differences in the means as presented in Table V will reveal not only the existence of a difference in the spore dimensions but in most cases also the distinct significance of such difference.

P. GRAMINIS TRITICI

The constants, together with their probable errors for the aeciospores of this biologic form, as given in the first section of Tables III and IV, respectively, show this form to have the largest arithmetical mean for both length and width, namely, $19.72 \pm 0.19 \times 15.66 \pm 0.10$ μ.

P. GRAMINIS SECALIS

The aeciospores of this biologic form differ considerably in both dimensions from those of the tritici form, the means being 17.10 ± 0.19 and 13.46 ± 0.11 μ, respectively. An examination of Table V will show that the difference in the means of these two biologic forms is, without doubt, significant for length as well as for width. The difference in the means of length divided by the probable error of this difference is 9.71, while the difference in the means of the width is 14.66 times greater than its probable error. The odds against the occurrence of such differences in random sampling are beyond comprehension.

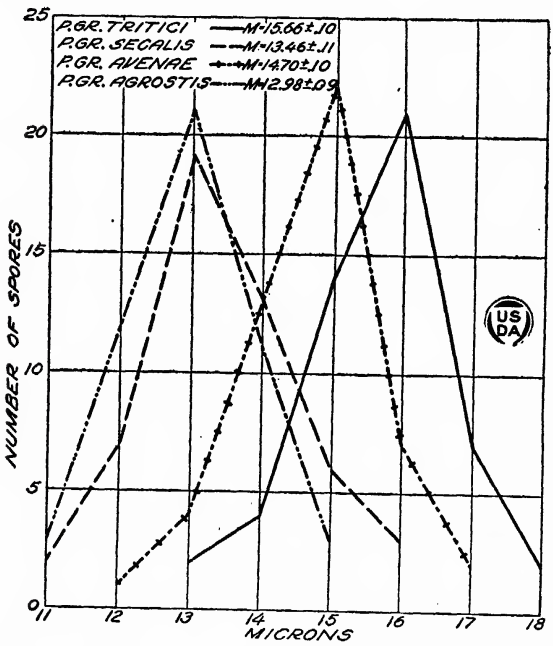

FIG. 2.—Differences in widths of aeciospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

P. GRAMINIS AVENAE

This biologic form differs quite markedly from either of the above, although to a somewhat lesser extent. The differences, nevertheless, are very significant, as can readily be seen from Table V. The means of the aeciospores of this form are $18.62 \pm 0.11 \times 14.70 \pm 0.10$ μ.

P. GRAMINIS PHLEIPRATENSIS

Any attempt to produce aecial infection on different species and varieties of barberry with teliospores of this biologic form has invariably resulted in failure. A study of the aecial stage of this form therefore could not be made.

P. GRAMINIS AGROSTIS

As shown in Tables III and IV, this biologic form has aeciospores which are smaller in size than those of any of the other biologic forms. The means are $16.46 \pm 0.18 \times 12.98 \pm 0.09$ μ. The differences in the means between this form and tritici on the one hand, and avenae on the other hand, as indicated in Table V, are certainly significant. The difference between agrostis and secalis may or may not be due to random sampling, for the difference in the means of spore length is only 2.46 times greater than its probable error and the difference in width does not exceed 3.43 times the probable error. The odds against the normal occurrence of such differences are approximately 9 to 1 in the length differences, and about 45 to 1 in the width differences. It would be difficult therefore

to consider the differences between the agrostis and secalis forms as truly significant, although they might be considered as indicative. A greater number of measurements might possibly throw more light on this question.

The following table gives the differences in the means of the aeciospores of the four biologic forms discussed in the preceding paragraphs, as well as the ratios between these differences and their probable errors.

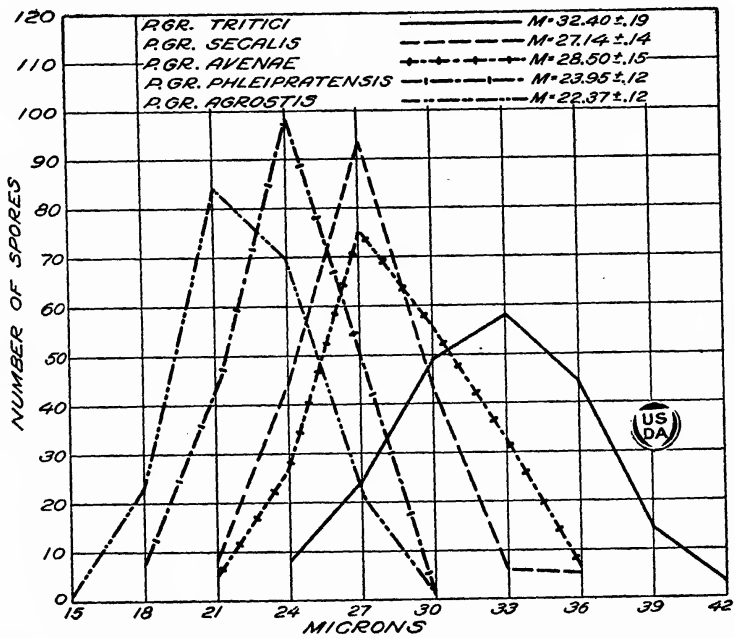

FIG. 3.—Differences in lengths of urediniospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

TABLE V.—*Summary of differences in the means of aeciospores of biologic forms of P. graminis*

Biologic forms.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	Width.	Length.	Width.
P. graminis tritici and *P. graminis secalis*.	2.62±0.27	2.20±0.15	9.71	14.66
P. graminis tritici and *P. graminis avenae*.	1.10± .22	.96± .14	5.00	6.86
P. graminis tritici and *P. graminis agrostis*.	3.26± .26	2.68± .13	12.53	20.61
P. graminis secalis and *P. graminis avenae*.	1.52± .22	1.24± .15	6.91	8.27
P. graminis secalis and *P. graminis agrostis*	.64± .26	.48± .14	2.46	3.43
P. graminis avenae and *P. graminis agrostis*	2.16± .21	1.72± .13	10.28	13.23

UREDINIOSPORES

There is considerably more information found in literature on the relative size of urediniospores of biologic forms of *P. graminis* than on either aeciospore or teliospore dimensions. An interesting phenomenon of spore size relationship becomes apparent when the curves plotted in figures 3 and 4 and the data compiled from previous investigations are examined.

It is that the tritici form possesses the longest urediniospores, the agrostis form the shortest, and the rest group themselves between these two extremes.

TABLE VI.—*Urediniospore dimensions of biologic forms of P. graminis as given by various authors*

Authority.	*P. graminis tritici.*	*P. graminis secalis.*	*P. graminis avenae.*	*P. graminis phleipratensis.*	*P. graminis agrostis.*
Eriksson and Henning (*3, p. 124 and 130*)	29–43×18–21	23–38×15–22	22–40×16–21	18–27×15–19	.....
Freeman and Johnson (*4, p. 27*)[1]	31.33×18.15	.....	.....	.....	.....
Stakman (*12, p. 27*)[1]	37.85×22.76	.....	.....	.....	.....
Stakman and Jensen (*13, p. 215*)[2]	.....	.....	19–35×16–24	17–31×15–23	.....
Stakman and Levine (*14, p. 49*)[2]	23–42×15–25	18–39×13–21	19–37×14–26	16–32×12–21	15–32×12–20

[1] These figures represent averages, size limits not having been given.
[2] Decimal fractions have been converted to the nearest integer.

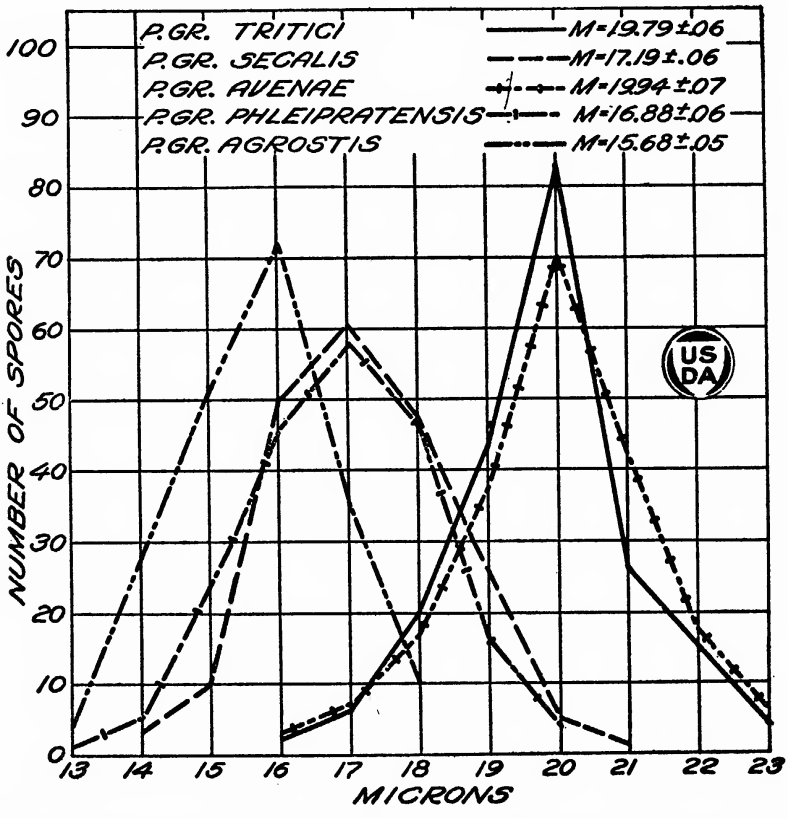

FIG. 4.—Differences in widths of urediniospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

It will also be noted by examining the graphs and the measurements quoted that *P. graminis secalis* and *P. graminis avenae* differ but little in spore length, while the spore width of the latter is much the same as that of *P. graminis tritici.* It will be noted further that according to the graphs as well as the quotations, there is a similarity in the width of the urediniospores of the secalis and phleipratensis forms, but the two diverge noticeably in length. The constants, given in the second section of Tables III and IV, and the differences in the means, presented in

Table VII, bring out not only the variations of the urediniospore dimensions but also the significance of these variations whenever and wherever they are found.

P. GRAMINIS TRITICI

That this biologic form has the longest urediniospores is confirmed by the constants, together with their probable errors, given in the second section of Tables III and IV. In width, the urediniospores are practically the same as those of the oat-rust form. The means for *P. graminis tritici* fall at $32.40 \pm 0.19 \times 19.79 \pm 0.06\mu$.

P. GRAMINIS SECALIS

The rye-rust urediniospores are both shorter and narrower than those of the wheat rust as shown by the means, $27.14 \pm 0.14 \times 17.19 \pm 0.06\mu$, there being a difference of over 5μ in length and more than 3μ in width. There can be no doubt whatever concerning the significance of this difference, as pointed out in Table VII.

P. GRAMINIS AVENAE

Although the urediniospores of this biologic form are only a little more than 1 micron (μ) longer than those of the secalis form, the difference, nevertheless, seems to be quite significant. The means of the urediniospore dimensions of *P. graminis avenae* are $28.50 \pm 0.15 \times 19.94 \pm 0.07\mu$.

P. GRAMINIS PHLEIPRATENSIS

The means of this biologic form are $23.95 \pm 0.12 \times 16.88 \pm 0.06\mu$. The differences between the means for both width and length of this biologic form and those of *P. graminis tritici* and *P. graminis avenae* are quite significant. The difference in the means, of length only, of the timothy rust and the rye rust is also noteworthy. (Table VII).

P. GRAMINIS AGROSTIS

The urediniospores of this biologic form are without any doubt smaller than those of any of the other forms. This is demonstrated by the curves plotted in figures 3 and 4 and confirmed by the constants in Tables III and IV, and also by the differences in the means as shown in Table VII. The means of the redtop-rust urediniospores are $22.37 \pm 0.12 \times 15.68 \pm 0.05\mu$.

The differences in the means of the urediniospores of the biologic forms of *P. graminis* and the ratios between these differences and their probable errors are summarized in Table VII.

TABLE VII.—*Summary of differences in the means of urediniospores of biologic forms of P. graminis*

Biologic forms.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	Width.	Length.	Width.
P. graminis tritici and *P. graminis secalis*.	5.26±0.24	2.60±0.08	21.92	32.50
P. graminis tritici and *P. graminis avenae*.	3.90± .24	.15± .09	16.25	1.67
P. graminis tritici and *P. graminis phleipratensis*	8.45± .22	2.91± .08	38.38	36.38
P. graminis tritici and *P. graminis agrostis*.	10.03± .22	4.11± .08	46.82	51.38
P. graminis secalis and *P. graminis avenae*.	1.36± .21	2.75± .09	6.48	30.56
P. graminis secalis and *P. graminis phleipratensis*	3.19± .18	.31± .08	17.72	3.88
P. graminis secalis and *P. graminis agrostis*	4.77± .18	1.51± .08	26.50	18.88
P. graminis avenae and *P. graminis phleipratensis*	4.55± .19	3.06± .09	23.93	34.00
P. graminis avenae and *P. graminis agrostis*	6.13± .19	4.26± .09	32.25	47.34
P. graminis phleipratensis and *P. graminis agrostis*	1.58± .17	1.20± .08	9.39	15.00

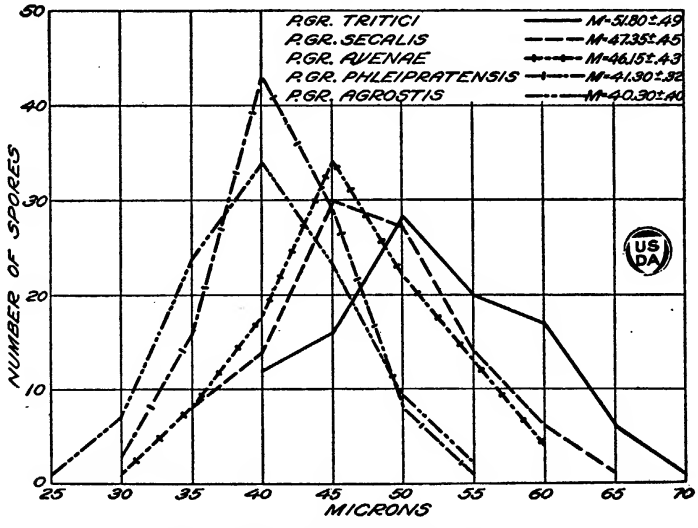

FIG. 5.—Differences in lengths of teliospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

TELIOSPORES

Published data on teliospore dimensions can be found concerning only one biologic form of stem rust and that is timothy rust. The evident reason is because this form had been considered a distinct species. The other data available refer to the species *P. graminis* as a unit. The various authors consulted agree on the range in the size of teliospores of timothy rust. This is given as 38–52 × 14–16μ. Most authors, however, disagree on the size limits of the teliospores of *P. graminis*, perhaps on account of a difference in the biologic forms which had been studied. The curves plotted in figures 5 and 6 point to the existence of a difference in size of the teliospores of the various biologic forms. In general there seems to be a fairly distinct parallellism in the size of the teliospores of the various

forms of *P. graminis* and the size of the urediniospores and aeciospores of the same forms. As in the case of the last two spore types, so in the teliospores, *P. graminis tritici* and *P. graminis agrostis* constitute the extremes with respect to spore size. Figures 5 and 6 show very clearly that the teliospores of the wheat rust are larger in every way than those of any of the other forms. The secalis and avenae forms are practically the same in length but differ noticeably in width. On the other hand, the rusts of rye and of redtop vary insignificantly in spore width. In length the agrostis form approaches that of phleipratensis, but is appreciably different from it in width. The constants given in the third section of Tables III and IV and the differences in the means presented in Table VIII show when these similarities and differences are real and significant and when they are only apparent and inconclusive.

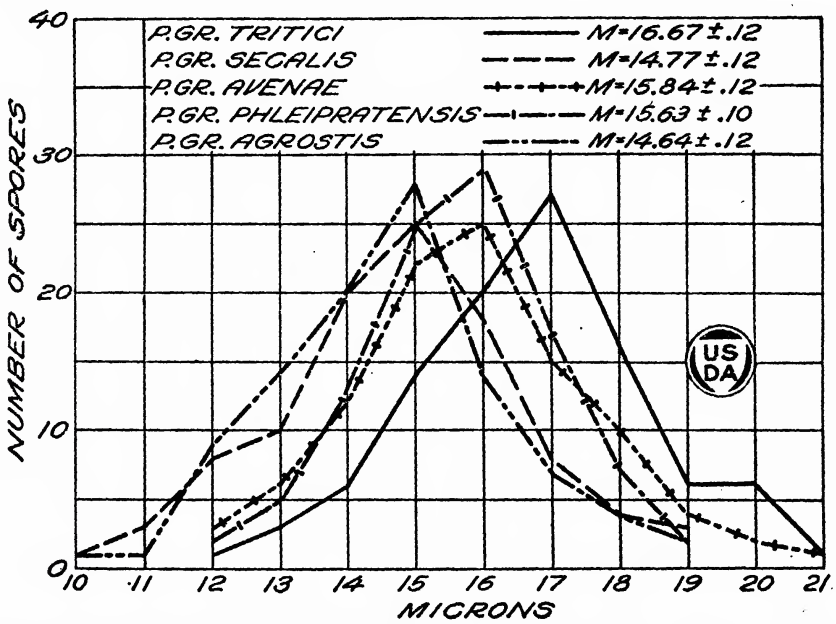

FIG. 6.—Differences in widths of teliospores of biologic forms of *Puccinia graminis* grown on congenial host plants and under uniform cultural conditions.

P. GRAMINIS TRITICI

There is a difference of at least 5 microns between the mode for length of the teliospores of this biologic form and the mode for the longitudinal measurement of any other biologic form. A similar difference is found in the means for length. The greatest difference in the width of the teliospore between this and any other form is about one micron for either mode or means. The means for this biologic form, as given in section 3 of Tables III and IV, are $51.80 \pm 0.49 \times 16.67 \pm 0.12\mu$.

P. GRAMINIS SECALIS

The difference between this biologic form and the one described above can readily be seen by comparing their means, which for the secalis form are $47.35 \pm 0.45 \times 14.77 \pm 0.12\mu$. The difference in the means of the rye-rust and wheat-rust forms is 6.64 times its probable error for length and 11.23 times greater than its probable error for width. Consequently, the difference must be considered as truly significant.

P. GRAMINIS AVENAE

The means for this form were determined as $46.15 \pm 0.43 \times 15.84 \pm 0.12\ \mu$. The difference in means between this biologic form and *P. graminis tritici* is obviously quite significant; not so in the case of the difference in the means of the avenae and the secalis forms, however. Here the difference in the means of the length of the biologic forms is only 1.94 times greater than its probable error and therefore insignificant, because such a difference is likely to occur naturally about once in every five random samples. There is, however, a significant difference in the means of the width of these two forms, it being 6.30 times greater than its probable error.

P. GRAMINIS PHLEIPRATENSIS

A reverse condition prevails in the timothy rust, as seen by examining its constants and differences in means. The means of this biologic form fall at $41.30 \pm 0.32 \times 15.63 \pm 0.10\ \mu$. In other words, the teliospores of this form are considerably shorter than those of the preceding three forms, but of the same or of nearly the same width as the teliospores of the *avenae* form. Table VIII shows that the difference in the means of the width of *P. graminis phleipratensis* and *P. graminis avenae* divided by its probable error is only 1.31, a very insignificant difference. The chances for an occurrence of such a difference are about 38 out of 100.

TABLE VIII.—*Summary of differences in the means of the sizes of teliospores of biologic forms of P. graminis*

Biologic forms.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	Width.	Length.	Width.
P. graminis tritici and *P. graminis secalis*..	4.45±0.67	1.90±0.17	6.64	11.23
P. graminis tritici and *P. graminis avenae*..	5.65± .65	.83± .17	8.69	4.88
P. graminis tritici and *P. graminis phleipratensis*.	10.50± .59	1.04± .16	17.80	6.50
P. graminis tritici and *P. graminis agrostis*.	11.50± .63	2.03± .17	18.25	11.93
P. graminis secalis and *P. graminis avenae*.	1.20± .62	1.07± .17	1.94	6.30
P. graminis secalis and *P. graminis phleipratensis*.....	6.05± .55	.86± .16	11.00	5.37
P. graminis secalis and *P. graminis agrostis*.	7.05± .60	.13± .17	11.74	.77
P. graminis avenae and *P. graminis phleipratensis*.....	4.85± .54	.21± .16	8.98	1.31
P. graminis avenae and *P. graminis agrostis*..	5.85± .59	1.20± .17	9.92	7.06
P. graminis phleipratensis and *P. graminis agrostis*.....	1.00± .51	.99± .16	1.96	6.18

P. GRAMINIS AGROSTIS

The redtop rust has the smallest teliospores of any of the biologic forms, although the difference is not direct or absolute. These spores are much shorter than those of either the wheat, rye, or oat rust, but only a little shorter than those of the timothy rust. On the other hand, they are appreciably narrower than those of the wheat, oat, and timothy forms, but much the same in width as the teliospores of the rye rust.

This characteristic variation is brought out more clearly by an analysis of the differences in the means than by a mere consideration of the constants. The means of this biologic form are $40.30 \pm 0.40 \times 14.64 \pm 0.12 \mu$. The difference in the means between the agrostis and the tritici forms is significant for both dimensions. Between agrostis and secalis, however, the difference in the means is significant for length only. Again, between the agrostis and avenae forms, the difference is significant for both length and width. The difference in the length of teliospore means of the agrostis and phleipratensis forms has no value as it is only 1.96 times greater than its probable error. The difference in the width, however, appears to be very significant because it exceeds its probable error by 6.18 times.

EFFECT OF ENVIRONMENTAL CONDITIONS ON THE MORPHOLOGY OF RUST SPORES

It has been reported (*14*) that resistant host plants and other unfavorable environmental conditions affecting the normal development and vigor of the rust fungus tend to change appreciably the size of its uredinio-spores. However, when normal conditions are reestablished, the uredinio-spores of the following generation are of the original dimensions. This experiment was elaborated and extended to the aeciospores and teliospores. Curves for the distribution of the spores studied were plotted into classes; constants were calculated; and differences in the means of varying conditions established. Tables IX and X represent the spore-size variations for length and width, respectively, as well as the constants together with their probable errors, for the several spore types as affected by different host plants and varying physical factors. The classes in these tables differ in the same order as those in Tables III and IV. The curves in figures 7 to 12 represent the distribution into their respective classes of the spores of the various biologic forms, grown under different conditions. The probable errors of the differences in the means of the spore measurements, together with the ratios obtained from a division of the differences by their probable errors, and the differences in the means themselves are all given in Tables XI and XII.

HOST PLANTS

Species and varieties which are congenial to a certain biologic form of *P. graminis* can not, under favorable cultural conditions, exert any perceptible influence on the spore morphology of that biologic form. However, even under identically the same environmental conditions, the spores of any biologic form will be reduced in size when cultured on resistant varieties. This is substantiated by measurements made of spores grown simultaneously on susceptible and resistant hosts.

SUSCEPTIBLE HOSTS

Aeciospores of *P. graminis secalis* were produced on *Berberis vulgaris* and *B. sieboldii* Miq., two equally susceptible species. The inoculations were made with teliospores of the same origin. The difference in the size of the two sets of aeciospores was practically negligible and highly insignificant (fig. 7 and 8). The means of the spores on *B. vulgaris* were $17.10 \pm 0.19 \times 13.46 \pm 0.11$ μ, as compared with $17.44 \pm 0.15 \times 13.30 \pm 0.09$ μ for the means on *B. sieboldii*.

TABLE IX.—*Variations and constants for length of spores of biologic forms of Puccinia graminis grown on different host plants and under varying environmental conditions*

Experiment No.	Spores and biologic forms.	Host plants or environmental conditions.	Spore classes according to length.												Total number.	Constants.		
			11μ	12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ		Mean.	Standard deviation.	Coefficient of variability.
	Aeciospores:																	
1	*P. graminis secalis*...	*Berberis vulgaris*....	...	1	1	2	6	9	11	8	6	4	1	1	50	17.10±0.19	2.00±0.13	11.70±0.79
2	Do.....	*Berberis sieboldii*....	.....	...	...	1	5	7	15	9	7	5	1	...	50	17.44± .15	1.58± .11	9.25± .61
3	*P. graminis agrostis*..	*Berberis vulgaris*....	...	1	2	4	7	14	8	7	4	2	0	1	50	16.46± .18	1.93± .13	11.73± .79
4	Do.....	*Berberis brevipaniculata.*	2	8	11	15	9	5	...	...	...	...	...	...	50	13.72± .12	1.31± .09	9.56± .65
			15μ	18μ	21μ	24μ	27μ	30μ	33μ	36μ	39μ	42μ						
	Urediniospores:																	
5	*P. graminis avenae*...	*Avena sativa*.....	...	...	5	27	75	55	32	6	....	....			200	28.50± .15	3.24± .11	11.34± .38
6	Do	*Bromus tectorum*...	...	1	32	43	23	1	....	....	....	....			100	23.73± .16	2.37± .11	9.97± .48
7	*P. graminis phleipratensis.*	*Phleum pratense*....	...	7	43	98	50	2	....	....	....	....			200	23.95± .12	2.41± .08	10.01± .34
8	Do.....	*Dactylis glomerata*..	...	1	27	46	26	....	....	....	....	....			100	23.91± .15	2.26± .11	9.48± .46
9	*P. graminis tritici*....	Normal conditions..	.....	.....	...	8	24	49	58	44	14	3			200	32.40± .19	3.89± .13	12.00± .41
10	Do.....	Excessive heat.....	.....	.....	...	10	29	40	18	3	....	....			100	29.25± .20	2.89± .14	9.88± .68
11	Do.....	Deficient light.....	.....	...	1	13	30	37	15	4	....	....			100	28.92± .21	3.13± .15	10.76± .51
12	Do.....	Drought.....	...	...	...	8	19	37	21	14	1	....			100	30.51± .23	3.47± .17	11.38± .54
			35μ	40μ	45μ	50μ	55μ	60μ	65μ	70μ								
	Teliospores:																	
13	*P. graminis tritici*....	*Triticum vulgare*....	.....	12	16	28	20	17	6	1					100	51.80± .49	7.23± .34	13.95± .67
14	Do.....	*Triticum dicoccum*...	3	18	42	27	10	.....	.....	.....					100	46.15± .32	4.79± .23	10.38± .49

TABLE X.—*Variations and constants for width of spores of biologic forms of Puccinia graminis grown on different host plants and under varying environmental conditions*

Experiment No.	Spores and biologic forms.	Host plants or environmental conditions.	Spore classes according to width.															Total number.	Constants.		
			10μ	11μ	12μ	13μ	14μ	15μ	16μ	17μ	18μ	19μ	20μ	21μ	22μ	23μ	24μ		Mean.	Standard deviation.	Coefficient of variability.
	Aeciospores:																				
1	*P. graminis secalis*.	*Berberis vulgaris*	...	2	7	19	13	6	3	...	...	...	...	...	...	...	...	50	13.46±0.11	1.17±0.08	8.69±0.58
2	Do	*Berberis sieboldii*	...	1	7	23	14	5	...	...	...	...	...	...	...	...	...	50	13.30± .09	.90± .06	6.77± .46
3	*P. graminis agrostis*.	*Berberis vulgaris*	...	3	12	21	11	3	...	...	...	...	...	...	...	...	...	50	12.98± .09	.97± .07	7.47± .50
4	Do	*Berberis brevipaniculata*.	2	11	30	6	1	...	...	...	...	...	...	...	...	...	...	50	11.86± .07	.75± .05	6.26± .42
	Urediniospores:																				
5	*P. graminis avenae*.	*Avena sativa*.	...	...	...	...	...	...	3	7	17	38	70	42	17	6	...	200	19.94± .07	1.36± .05	6.90± .23
6	Do	*Bromus tectorum*.	...	...	...	...	...	...	1	10	22	34	29	4	...	...	...	100	18.92± .07	1.08± .05	5.69± .37
7	*P. graminis phleipratensis*.	*Phleum pratense*	...	...	...	1	5	24	46	58	46	16	4	...	...	...	...	200	16.88± .06	1.32± .04	7.81± .27
8	Do	*Dactylis glomerata*.	...	...	...	...	2	8	22	33	22	11	2	...	...	...	...	100	17.06± .08	1.25± .06	7.32± .35
9	*P. graminis tritici*.	Normal conditions.	...	...	...	...	...	...	2	6	20	44	83	26	15	4	...	200	19.79± .06	1.27± .04	6.42± .22
10	Do	Excessive heat.	...	...	...	...	...	...	1	6	14	18	35	17	6	2	1	100	19.71± .10	1.45± .07	.7± .35
11	Do	Deficient light.	...	...	...	...	...	...	...	1	11	20	44	15	8	1	...	100	19.89± .08	1.13± .05	5.69± .27
12	Do	Drought	...	...	...	...	...	...	...	4	18	36	28	13	1	...	...	100	19.31± .07	1.06± .05	5.51± .26
	Teliospores:																				
13	*P. graminis tritici*.	*Triticum vulgare*	...	...	1	3	6	14	20	27	16	6	6	1	...	...	...	100	16.67± .12	1.74± .08	10.42±.46
14	Do	*Triticum dicoccum*.	...	...	1	3	6	16	21	28	13	6	4	2	...	...	...	100	16.57± .12	1.73± .08	10.43±.50

Similar results were obtained with urediniospores of *P. graminis phleipratensis* of identical origin grown simultaneously on timothy, *Phleum pratense*, and orchard grass, *Dactylis glomerata* L. As can readily be seen from experiments 7 and 8 of Tables IX and X, the spores were alike in length and width; the means for the urediniospores developed on the timothy were $23.95 \pm 0.12 \times 16.88 \pm 0.06$ μ, while the means for the urediniospores obtained on the orchard grass were $23.91 \pm 0.15 \times 17.06 \pm 0.08$ μ. Thus the means in both cases differed only slightly.

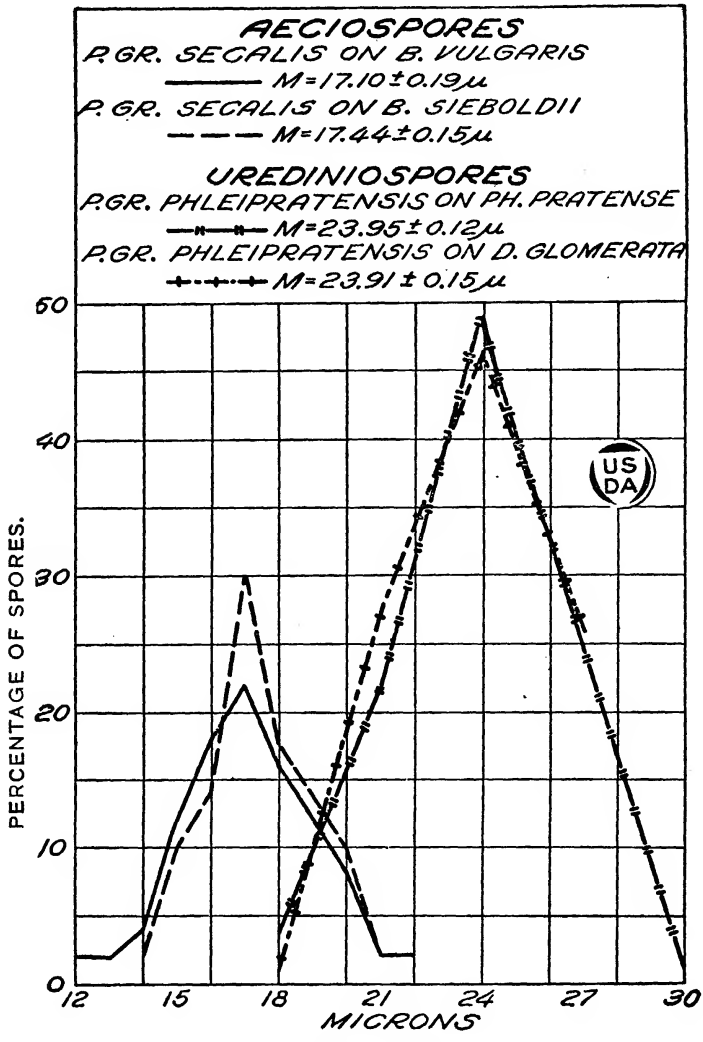

Fig. 7.—Uniformity in length of aeciospores and urediniospores of biologic forms of *Puccinia graminis* grown on different but equally susceptible host plants.

RESISTANT HOSTS

As a rule the width of spores is more or less constant under all circumstances. However, a reduction in both length and width as a result of the effect of resistant hosts was obtained in aeciospores of *P. graminis agrostis* produced on *Berberis brevipaniculata* Schneid. and in urediniospores of *P. graminis avenae* grown on *Bromus tectorum*. A reduction in length only occurred in the teliospores of *P. graminis tritici* found on emmer, *Triticum dicoccum* Schr. Figures 9 and 10 illustrate these variations very distinctly.

The aeciospores of the agrostis form on *B. vulgaris* possessed the following means: $16.46 \pm 0.18 \times 12.98 \pm 0.09$ μ, while the means of these aeciospores on *B. brevipaniculata* were $13.72 \pm 0.12 \times 11.86 \pm 0.07$ μ. The difference between the two conditions, as shown in Table XI, is 12.45 times greater than the probable error for length and 10.18 times greater than that for width, which without any doubt is a very significant difference.

Bromus tectorum L. produced a similar effect on the urediniospores of *P. graminis avenae*. The means of this biologic form on *Avena sativa*

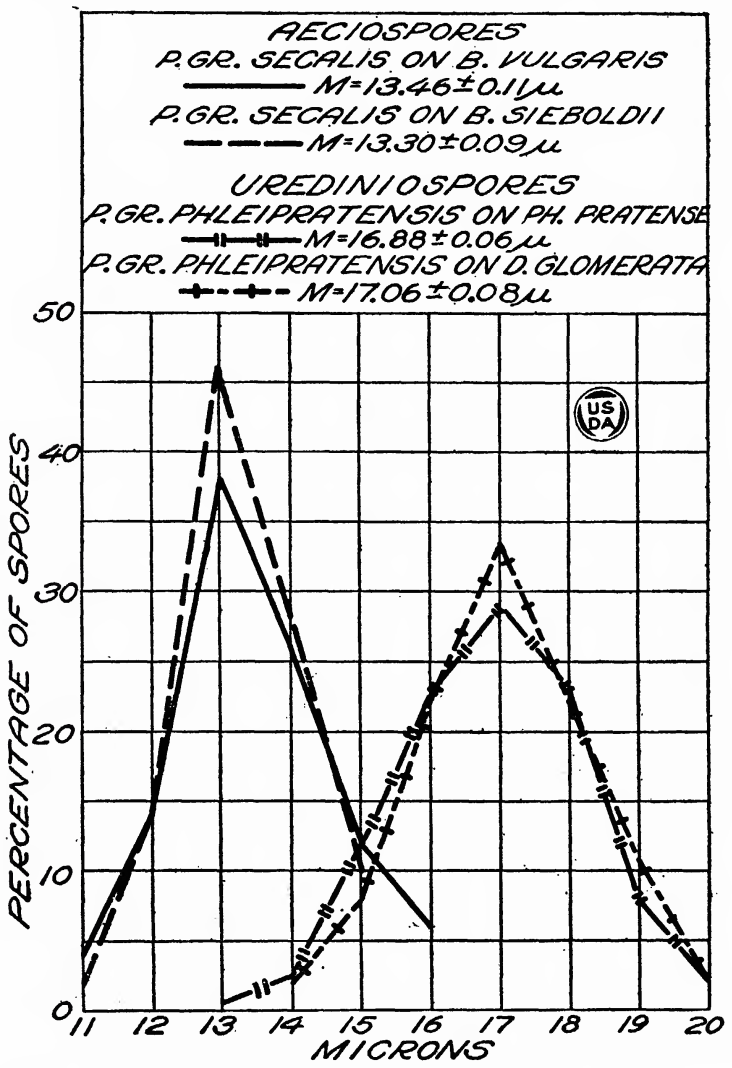

Fig. 8.—Uniformity in widths of aeciospores and urediniospores of biologic forms of *Puccinia graminis* grown on different but equally susceptible host plants.

were $28.50 \pm 0.15 \times 19.94 \pm 0.07$ μ, while on *B. tectorum* they were $23.73 \pm 0.16 \times 18.92 \pm 0.07$ μ. The difference in the means of the urediniospores in the two cases was 21.67 times its probable error for length and 10.20 times for width; both are differences as significant as can be desired.

The teliospores on the common wheat were over 5 μ longer than those on emmer, but practically the same in width. The means for the former were $51.80 \pm 0.49 \times 16.67 \pm 0.12$ μ, while for the latter they were $46.15 \pm 0.32 \times 16.57 \pm 0.12$ μ. The difference in the means of the length only was significant.

Table XI shows the differences in the means of spores of biologic forms of stem rust grown on susceptible and resistant hosts, also the ratios between these differences and their probable errors.

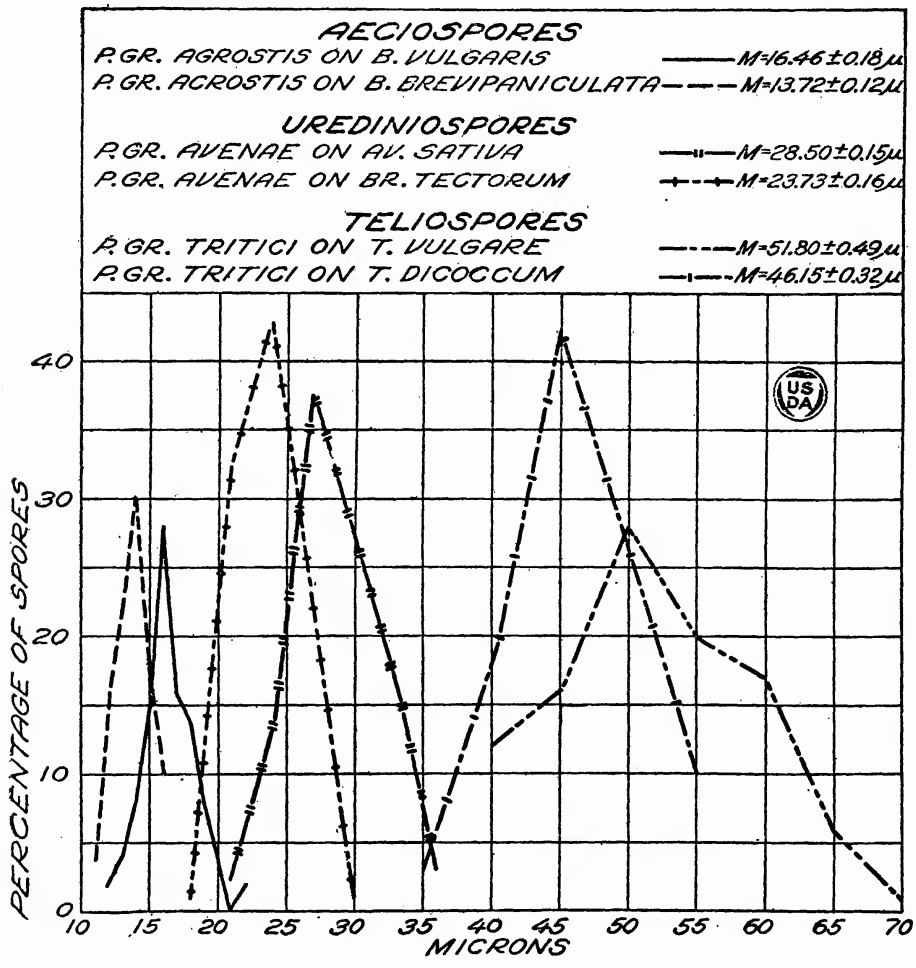

Fig. 9.—Differences in lengths of aeciospores, urediniospores, and teliospores of biologic forms of *Puccinia graminis* grown on susceptible and on resistant host plants.

TABLE XI.—*Summary of differences in the means of spores of biologic forms of P. graminis as affected by various host plants*

Biologic forms.	Host plants.	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
		Length.	Width.	Length.	Width.
P. graminis secalis (aeciospores)	*Berberis vulgaris* and *Berberis sieboldii*	0.34±0.24	0.16±0.14	1.42	1.14
P. graminis phleipratensis (urediniospores)	*Phleum pratense* and *Dactylis glomerata*	.04± .19	.18± .10	.21	1.80
P. graminis agrostis (aeciospores)	*B. vulgaris* and *B. brevipaniculata*	2.74± .22	1.12± .11	12.45	10.18
P. graminis avenae (urediniospores)	*Avena sativa* and *Bromus tectorum*	4.77± .22	1.02± .10	21.67	10.20
P. graminis tritici (teliospores)	*Triticum vulgare* and *T. dicoccum*	5.65± .59	.10± .17	9.75	.59

PHYSICAL FACTORS

Unfavorable cultural conditions affect the virulence and spore size of the rust fungus to an appreciable extent, especially the quantity of rust produced and the length of the spores. The inherent nature of infection and the width of the spores are rarely and only slightly changed, if at all. For obvious reasons the effect of environment on the uredinial stage

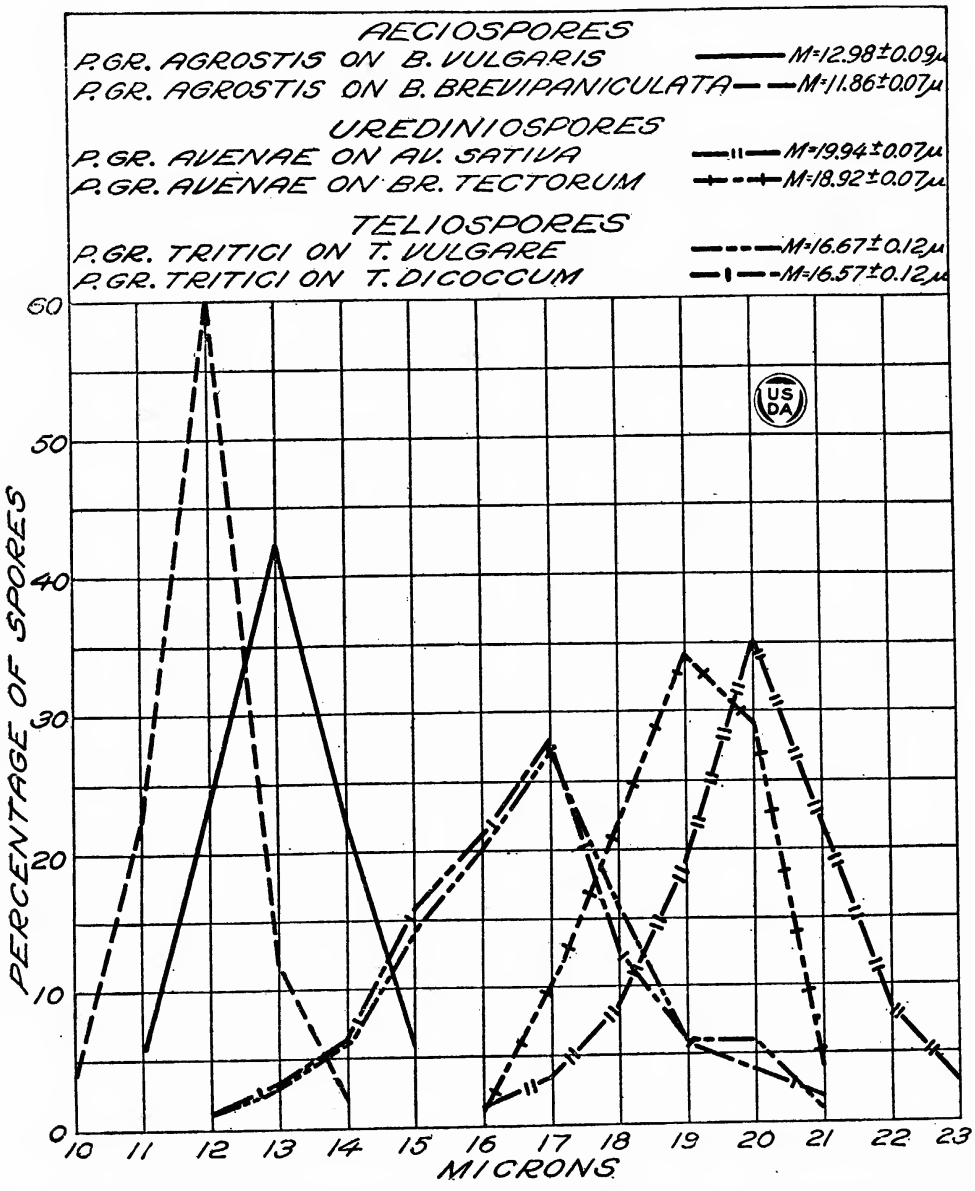

Fig. 10.—Differences in widths of aeciospores, urediniospores, and teliospores of biologic forms of *Puccinia graminis* grown on susceptible and resistant host plants.

alone was studied. Excessive heat more effectively and more conclusively caused the urediniospores of the wheat rust to become shorter than did very low temperature. Deficiency in sunlight and in soil moisture appeared to be equally influential in diminishing the size of the urediniospores. In all of these cases as well as in the control experiment

an extremely susceptible variety of wheat, Haynes Bluestem (Minnesota No. 169), was used as the host plant. Figures 11 and 12 contain curves showing the variations produced by the various conditions.

EXCESSIVE HEAT

The means of the spores in the control experiment were 32.40 ± 0.19 × 19.79 ± 0.06 μ. The means of the urediniospores cultured in high temperature (average for the generation being 81.8° F. with a maximum daily mean of 92.3° and minimum of 76.4°) were 29.25 ± 0.20 × 19.71 ± 0.10 μ. An examination of Table XII shows that the difference in the means of the spore length in the control and temperature experiments was 11.25 times greater than its probable error, whereas the difference in the means of the width was exceeded by its probable error by 1.5 times; a significant difference for length, but none whatever for width.

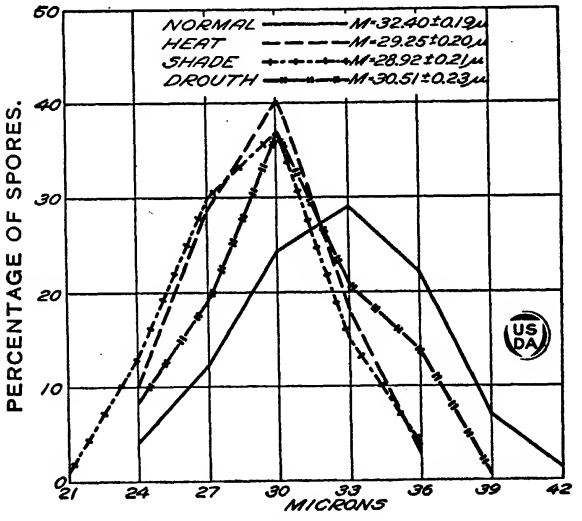

FIG. 11.—Differences in lengths of urediniospores of *Puccinia graminis tritici* cultured under favorable and adverse environmental conditions.

DEFICIENT LIGHT

In this experiment the urediniospores were cultured in a light intensity of a total average of 3.1 per cent with a maximum daily limit of 10 per cent and a minimum of 2 per cent. The light intensity readings were made three times a day with the aid of a Clements photometer. The means of the urediniospores in this test were 28.92 ±0.21 × 19.89 ±0.08 μ, which were practically identical with those which prevailed in the case of excessive heat. Here, too, the difference was significant with respect to spore length only. The difference in the means of the width between the control and shade was exactly the same as its probable error.

DROUGHT

The rusted plants in this test received a total amount of 100 cc. of water from the time they were removed from the incubation chamber until the time when the spore measurements were made, 14 days later. The water content of the soil at this time was determined on the basis of the "oven-dried" method and measured 5.38 per cent. The means of the urediniospores subjected to this condition were 30.51 ±0.23 × 19.31 ±0.07 μ. Comparing these means with those of the control spores, we find a difference in both length and width which appears to be significant.

A summary of the differences and their relations to the probable errors of the differences is given in Table XII.

TABLE XII.—*Summary of differences in the means of urediniospores of P. graminis tritici subjected to various environmental conditions*

Environmental conditions favorable except for—	Difference in means (in microns).		Difference in means divided by probable error of the difference.	
	Length.	*Width.*	*Length.*	*Width.*
Excessive heat (average 81.8° F.).....	3.15±0.28	0.08±0.12	11.25	0.67
Deficient light (average 3.1 per cent)....	3.48± .28	.10± .10	12.42	1.00
Drought (water content 5.38 per cent)....	1.89± .30	.48± .09	6.30	5.34

GENERAL DISCUSSION

No definite statistical information concerning the dimensions of the different types of spores of the biologic forms of stem rust existed prior to this study. Measurements of aeciospores, urediniospores, and teliospores, developed under different sets of conditions and on different host plants, were made with the aim of supplying such information. Biometric methods were employed with a view of ascertaining the significance of the variations discovered. These methods were used merely as a means to ascertain more about the nature and behavior and the identity of the different biologic forms. That results thus obtained are more reliable than a mere mass of individual measurements is now generally conceded.

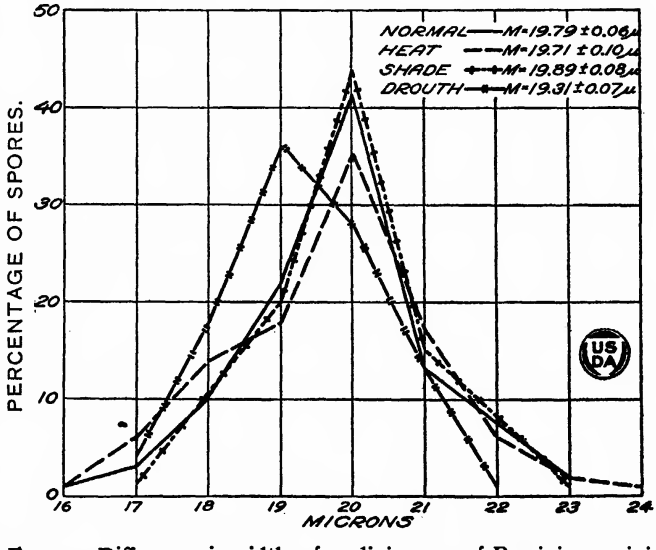

FIG. 12.—Differences in widths of urediniospores of *Puccinia graminis tritici* cultured under favorable and adverse environmental conditions.

The statistical data presented in this paper, in addition to corroborating the empirical results obtained by previous workers concerning the differences in the size of urediniospores, also indicate very strongly the existence of similar differences in the aeciospores and teliospores of the biologic forms of *Puccinia graminis*. The differences in magnitude of the various types of spores seem to be quite marked, although they do not occur in any definite order or logical sequence. Sometimes a significant difference appears in both length and width of spores; on other occasions the difference can be detected in the spore length only, the width being almost identically the same, and vice versa.

A numerical difference alone may or may not be significant in itself. A variation of 1 micron in the means of two measurements, for instance, may in one case be ascribed to experimental error and on another occasion constitute a fundamental difference. The difference in the means of teliospores of *P. graminis phleipratensis* and *P. graminis agrostis* is a

case which can well illustrate this point. The two biologic forms differ by exactly 1.00 ± 0.51 μ in the means of length and by 0.99 ± 0.16 μ in the means of width. The difference in length was probably due to random sampling, as it was only 1.96 times greater than its probable error, whereas the difference in width appeared to be rather significant, because it exceeded its probable error by 6.18 times.

Two numerical differences of unequal magnitude, on the other hand, may sometimes be of identical or nearly identical significance. Thus, for instance, the urediniospores of *P. graminis tritici* exceed those of *P. graminis phleipratensis* by 8.45 ± 0.22 μ in the means of length, but the difference in the means of width is only 2.91 ± 0.08 μ. The difference in the means of length divided by its probable error is 38.38, and that of the width is 36.38. In general, the greater the numerical difference the greater its significance, although in certain cases the situation may be reversed. An excellent illustration of this phenomenon is furnished by the difference in the means of urediniospores of *P. graminis tritici* and *P.*

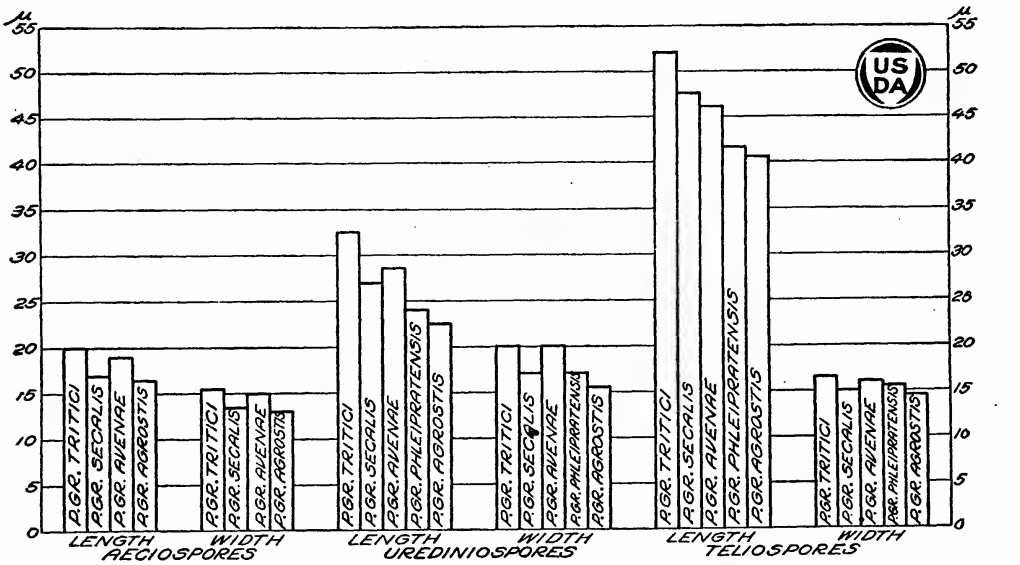

FIG. 13.—Comparisons between the means of lengths and widths of aeciospores, urediniospores, and teliospores, respectively, of biologic forms of *Puccinia graminis*.

graminis secalis. The means of the wheat-rust spores were 5.27 ± 0.24 μ longer and 2.60 ± 0.08 μ wider than those of the urediniospores of the rye rust. But the difference in the means of length exceeded its probable error 21.92 times as compared with 32.50 times for the excess in the case of the difference in width. Thus the important factor in determining the value and significance of differences is not so much the numerical deviation as the probable error of these differences.

The comparative differences in spore dimensions of the various biologic forms are illustrated by photomicrographs and schematic drawings in Plates I and II. Figure 13 represents diagrammatically the apparent correlation between the means of the different forms, while figure 14 shows the rather consistent parallelism in the means of the various spore types.

Although the size of spores is specific for each biologic form, the spores are not rigidly fixed either in size or in shape. Resistant host plants and other adverse environmental conditions, affecting the normal development and vigor of the rust fungus, also affect the size and shape of the

spores. Plate II, F, G, shows the response of the aecial infection of the redtop rust to two species of Berberis, one susceptible, *B. vulgaris* (F), and one resistant, *B. brevipaniculata* (G). The effect of these hosts on the size of the aeciospores is graphically illustrated in figures 9 and 10. Urediniospores and teliospores were also affected in a similar manner. This effect, however, is not permanent; it lasts only as long as the unfavorable circumstances prevail. This is especially true in the case of urediniospores. As soon as the rust is transferred to a congenial host plant and favorable cultural conditions are reestablished it again develops normally and the spores attain their normal dimensions.

The ill effect of excessive heat, insufficient light, and deficient soil moisture was quite definitely proved for urediniospores. This would lead to the assumption that aeciospores and teliospores are probably likewise affected by unfavorable cultural conditions.

Host plants belonging to different species and varieties are not in themselves sufficient to cause a change in the normal development of the rust fungus or in the size and shape of the spores unless they vary mark-

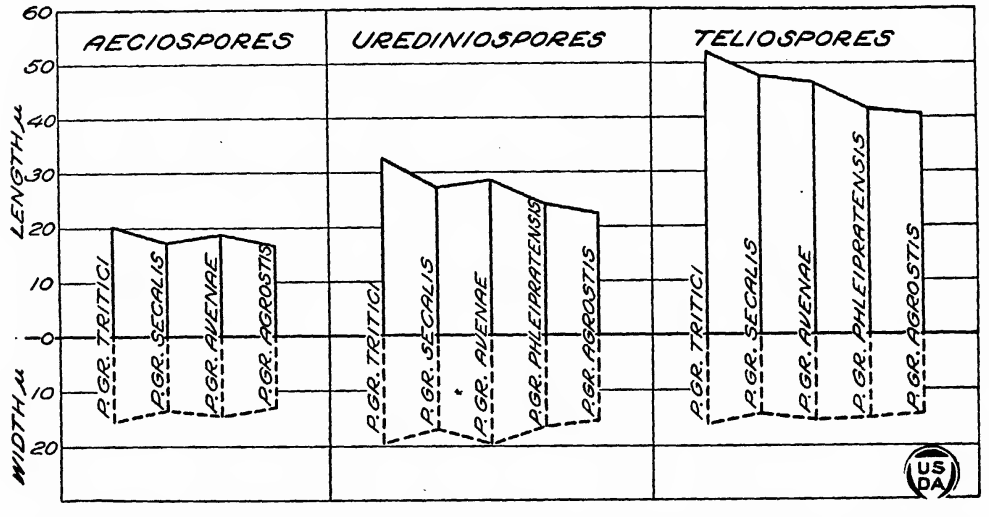

FIG. 14.—Parallelism between the means for lengths and widths of the different spores of biologic forms of *Puccinia graminis*.

edly in the degree of susceptibility to a biologic form. Plate II, D, E, shows an equal amount of aecial infection of *P. graminis secalis* on *B. vulgaris* and *B. sieboldii*. The uniformity in spore size of both cases is illustrated by graphs in figures 7 and 8.

Besides differences in spore size, deviations in the shape of the spores of the various biologic forms were also observed. These were described by Stakman and Levine (*14, p. 48*) as especially pronounced in the urediniospores. Somewhat less pronounced differences in shape were found by the writer in the aeciospores and teliospores, particularly the latter. Unfortunately the necessary statistical data were not available for calculating the ratios of length to width in the different types of spores, and hence constants could not be computed, nor could differences in mean of diameter be established. This is all the more regrettable because of the full realization of the importance of expressing quantitatively the differences occurring not only in the size but also in the shape of spores. A comprehensive method for this purpose has been described by Rosenbaum (*10, p. 250*).

SUMMARY

(1) In studying the nature and behavior of biologic forms careful uniform technic and pure rust strains have been used in order to reduce to a minimum the possibility of experimental error.

(2) Spore measurements can be employed as an additional aid in identifying the biologic forms of *P. graminis*, provided a sufficiently large number of spores are measured for both length and width, and the spores studied are developed on congenial hosts and under favorable conditions. The different measurements should be grouped in classes for length and width, respectively.

(3) On account of the variability in size of the different kinds of spores of each biologic form, a more ready comparison can be made by the use of biometric constants than by a mere mass of individual measurements. By the use of these constants the identity and relationship as well as the nature and behavior of a given biologic form may be further confirmed. Such biometric constants have been calculated and are incorporated in the respective tables.

(4) Numerical differences in spore dimensions may or may not be significant in themselves. The important factor in determining the value of numerical differences consists in the probable error of the differences and in the relation of these differences to their probable errors.

(5) The biologic forms of stem rust differ markedly and significantly in their various kinds of spores when produced under uniform conditions. An interesting parallelism is found to exist between the means of the spore dimensions of the different biologic forms.

(6) In general, the wheat rust, *P. graminis tritici*, has larger spores of each type than any other biologic form. The oat rust, *P. graminis avenae*, occupies the second place; the rye rust, *P. graminis secalis*, the third; and the timothy rust, *P. graminis phleipratensis*, the fourth. The redtop rust, *P. graminis agrostis*, has the smallest spores of all.

(7) The differences in spore sizes do not occur in any consistent direction, nor do they follow a logical sequence. In some cases the spores of two biologic forms may vary in both length and width, in other cases the differences may be in one dimension only.

(8) Congenial hosts do not in any way change the morphology of stem-rust spores. A single host plant, common to several biologic forms, lacks the ability to unify them either in size or shape. Nor can several host plants, which are equally susceptible to a single biologic form, exert any influence on the spore morphology of this form.

(9) Resistant host plants and adverse environmental conditions invariably tend to decrease the size of any type of spores of any biologic form. The reduction in size occurs most frequently in spore length only, but sometimes both length and width are affected. As soon as the unfavorable conditions of host and environment are removed, the spores in question will attain their normal dimensions in a single generation.

(10) It is of interest as well as of importance to establish the quantitative measure of the spore shape of the different biologic forms. For this purpose the ratio of length to width should be determined and grouped into classes, the constants calculated and the significance of the differences ascertained.

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PLATE 1

Median longitudinal diagrammatic figures showing comparative differences in the means of aeciospores, urediniospores, and teliospores of biologic forms of *Puccinia graminis*.

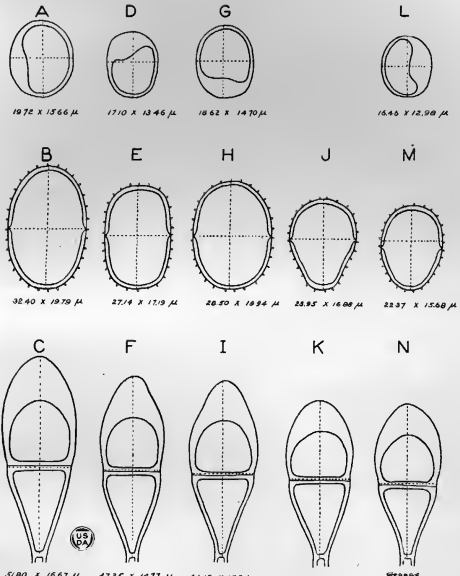
A, B, and C.—Diagrams of aeciospore, urediniospore, and teliospore of *P. graminis tritici*.

D, E, and F.—Similar diagrams of *P. graminis secalis*.

G, H, and I.—Similar diagrams of *P. graminis avenae*.

J and K.—Similar diagrams of urediniospore and teliospore of *P. graminis phleipratensis*.

L, M, and N.—Diagrams of aeciospore, urediniospore, and teliospore of *P. graminis agrostis*.


A
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G
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19.72 X 15.66 μ
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18.62 X 14.70 μ
15.46 X 12.98 μ
B
E
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M
32.40 X 19.79 μ
27.14 X 17.19 μ
28.50 X 18.94 μ
23.95 X 16.08 μ
22.37 X 15.48 μ
C
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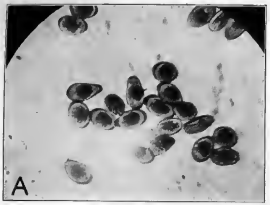
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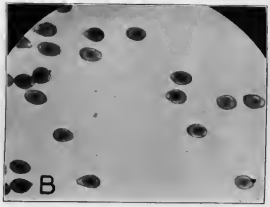
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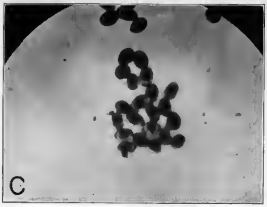
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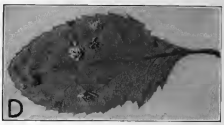
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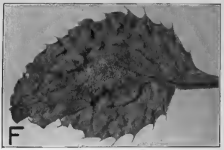
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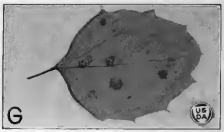
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PLATE 2

Photomicrographs of uniform magnification showing the relative size and shape of the urediniospores of three biologic forms of *Puccinia graminis:*

A.—*P. graminis tritici.*

B.—*P. graminis phleipratensis.*

C.—*P. graminis agrostis.*

Photographs illustrating the effect of susceptible and resistant barberry species on the aecial development of biologic forms of stem rust:

D and E.—Showing similar and normal development of cluster cups of *P. graminis secalis* on two equally susceptible species, *Berberis vulgaris* and *B. sieboldii*, respectively.

F and G.—Showing the difference in the aecial development of a single biologic form, *P. graminis agrostis*, when grown on susceptible and resistant barberry species, viz, *B. vulgaris* and *B. brevipaniculata*, respectively.

RELATION OF CERTAIN SOIL FACTORS TO THE INFECTION OF OATS BY LOOSE SMUT[1]

By LUCILLE K. BARTHOLOMEW, *formerly Assistant Pathologist*, and EDITH SEYMOUR JONES, *formerly Scientific Assistant, Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture*[2]

INTRODUCTION

In order to interpret properly the results obtained by experimenting with a disease, it is desirable to understand the effect of similar conditions upon the host and fungus apart from each other as well as in combination. Accordingly, an attempt has been made in the present paper to analyze the effects of certain soil factors, first upon the fungus, *Ustilago avenae* (Pers.) Jens., and upon the host, *Avena nuda* L., and then upon the two in combination.

The literature relative to the problem in hand has been adequately reviewed in a related publication[3] by the junior author of this paper (in this issue of the Journal of Agricultural Research), so no further detailed consideration of that feature will be attempted in this paper.

CONDITIONS OF THE EXPERIMENTATION

There are certain environmental factors which must be given thorough consideration in any well-balanced series of pathological investigations. Their relative importance may vary with the host and the fungus under consideration, but their standardization and control, in so far as these are possible, are imperative. Probably the four most important of these factors involved in the present investigations are temperature, moisture, composition of the medium, and its acidic or basic reaction.

The temperatures were regulated very satisfactorily by the use of temperature tanks.[4] The soil moistures were computed and maintained in terms of the saturation point of the soil, which was determined by the commonly accepted standard methods. The soil was sifted through wire gauze into standard 1-centimeter cups, which were leveled off by means of a spatula without tamping, and then were set into a dish of water of known temperature and allowed to stand until the soil was thoroughly saturated. The cups then were removed, drained until no more water dripped from the under surface, and weighed. The soil was dried at a temperature of 100 to 110° C. until it reached a constant weight. From these data the water-holding capacity of the soil was calculated. The standardization of soil water on the basis of dry weight, e. g., 10 gm. of

[1] Accepted for publication Aug. 21, 1922.

[2] The research herein recorded was conducted under the direction of Dr. George M. Reed, formerly pathologist in charge of cereal smut investigations, Bureau of Plant Industry, Washington, D. C., but now at the Brooklyn Botanic Gardens. We wish to extend our grateful thanks to him as well as to Dr. L. R. Jones and his associates in the Department of Plant Pathology, University of Wisconsin, for their hearty cooperation at all times.

This paper presents the results of investigations conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, and the Department of Plant Pathology of the Wisconsin Agricultural Experiment Station.

[3] JONES, Edith Seymour. INFLUENCE OF TEMPERATURE, MOISTURE, AND OXYGEN ON SPORE GERMINATION OF USTILAGO AVENAE. *In* Jour. Agr. Research, v. 24, p. 577–591, 3 fig. Literature cited, p. 590–591.

[4] JONES, L. R. SOIL TEMPERATURES AS A FACTOR IN PHYTOPATHOLOGY. *In* Plant World, v. 20, p. 229–237. 1917. Literature cited, p. 236–237.

water to 100 gm. of dry soil, was discarded after trial because of the variation in soil-moisture conditions when soils of different water-holding capacities were used. The chief danger of the soil-saturation method lies in the unequal compacting of the soil but this error may be minimized by using a uniform method for handling all soil samples.

The soil moistures were held constant by the use of galvanized containers, insulated soil surfaces, and daily weighings for the determination of any moisture lost through evaporation. The soil reaction was determined by the Truog[5] method and was found in all cases to be "medium acid." The soil composition offered the greatest difficulties in the matter of uniformity, but these were overcome by securing soil from approximately the same locality for all the series of experiments. It was a sandy loam with a water-holding capacity of 28 to 30 per cent for the first year's experiments and 39 to 41 per cent for those of the second year. Pure-line seed of the previous season was used, and sowings were made at a depth of 1 inch. The soil was thoroughly sifted, poured into the can, and tamped by dropping the can from a given height a uniform number of times, thus obtaining a fairly constant state of compactness.

INFLUENCE OF TEMPERATURE ON GROWTH OF FUNGUS AND HOST

The fungus, *Ustilago avenae* (Pers.) Jens., was obtained from smutted heads of *Avena nuda* L. grown at Columbia, Mo., in the summer of 1918. This material was used for all of the inoculations during the winter and spring of 1918–19. Smutted heads obtained from our own plats of *A. nuda* L. in the summer of 1919 furnished the inoculum for the following year. Pure-line seed of *A. nuda* L., the hulless or naked oat, was chosen as the standard for experimentation because this species shows a high degree of susceptibility to loose smut. Uniform moistures were maintained throughout the infection period and for one week afterwards, following which the containers were removed from the tanks. The plants taken from the tanks during the colder months were allowed to mature in the containers in which they were growing, but those in the series removed from the tanks in the spring months were transplanted at once to open plats and allowed to mature out of doors.

The study of the fungus in culture has been limited, but the results are clear cut. Potato-dextrose agar, with a reaction of +10 on the Fuller scale, was used. Agar sticks were melted, cooled, and heavily inoculated with sporidia from pure cultures of the fungus. These were poured into plates and allowed to incubate until many colonies appeared. Series of plates of the same medium then were inoculated by selecting uniform colonies and transplanting them. The single-spore method of culturing the organism was found impracticable because of the minute size of the spores, their tendency to cling together, and their poor germination, even when incubated under optimum conditions. The results obtained are shown in Table I.

When the cultures were removed from the Altmann incubators at the end of six days and allowed to remain at room temperature, growth occurred in all except those which had been exposed to 36° C. Growth, however, was very slight in cultures taken from 32°, showing that only a small percentage of the sporidia were able to survive exposure to this temperature. This was not due to desiccation of the medium, because sufficiently high humidity was maintained to prevent undesirable evapo-

[5] TRUOG, E. A NEW TEST FOR SOIL ACIDITY. Wis. Agr. Exp. Sta. Bul. 249, 16 p., 3 fig., 1 col. chart. 1915.

ration. Low temperatures appeared to inhibit growth without causing any permanent injury to the fungus. Cultures were kept at the low temperatures in some cases for a period of four weeks or more, but no growth occurred. When these same cultures were transferred to warmer temperatures, growth soon was evident.

TABLE I.—*Effect of temperature on growth of Ustilago avenae in culture*

Average temperature.	Amount of growth.
C.	
5°	None.
8°	Very slight.
14°	Slight.
16°	Fair.
20°	Good.
23°	Fair.
28°	Slight.
32°	Very slight (if any).
36°	None.

EFFECT OF TEMPERATURE ON GERMINATION OF SPORES AND PRODUCTION OF SPORIDIA

The spores of *Ustilago avenae* placed in Van Tieghem cells in beef broth with a reaction of +10, Fuller's scale, germinated between 5° and 34° C., the minimum being between 4° and 5°, the optimum between 15° and 28°, and the maximum between 31° and 34° C. For sporidial production under the same conditions of culture as for spore germination the minimum and optimum are the same as for spore germination, but the maximum, 30°, is lower.

THE EFFECT OF TEMPERATURE ON GERMINATION OF THE HOST, AVENA NUDA L.

It is difficult to estimate the growth of the host as it is not easy to define a standard for growth. The plants in culture, 1 to 2 months old, appeared to be most vigorous where germination and growth of the seedlings had occurred at temperatures of 16° to 24° C. They elongated more rapidly at the higher temperatures but presented an etiolated appearance as compared with the healthy, stalky growth at the lower temperatures. The record is given in Table II.

TABLE II.—*Effect of temperature on germination of Avena nuda L.*

Average temperature.	Observations on growth.
C.	
3°	Germination but no subsequent growth.
5°	Germination but no subsequent growth.
7°	Germination with slight growth.
10°	Germination with slow growth.
15°	Secondary roots produced, plumule 1.3 cm. long.
19°	Optimum growth, plumule 3 cm. long.
21°	Good growth, plumule 2 cm. long.
27–33°	Less vigorous, plumule 1 cm. long.

Table II gives a fair idea of the effect of the range of temperature, 3° to 33° C., upon germination and seedling growth. The optimum lies at an average temperature of 19° C.

If we compare the host and fungus in their temperature relations in the light of the above data, we find a rather close correlation. The fungus shows a tolerance for somewhat lower temperatures, especially as to germination and initial production of sporidia (essential to infection), while the host shows a tolerance for higher temperatures. However, their optima for germination and growth are practically identical.

INFLUENCE OF SOIL TEMPERATURE AND SOIL MOISTURE ON INFECTION

In order to visualize quickly this phase of the problem, it may be well to review briefly the salient points in the life history of the fungus. The spores of *Ustilago avenae*, the loose smut of oats, are for the most part seed-borne. When the oat seed is sown the spores germinate, producing masses of sporidia which continue to bud and multiply much like yeast. As the oat kernel germinates, the sporidia send out germ tubes and infect the young seedling through the coleoptile. By the end of 10 days or 2 weeks the mycelium penetrates through the coleoptile and subsequently establishes itself in the meristematic tissue of the growing point. According to various investigators, infection is limited to the first few days following germination of the oat kernel and none occurs after the growing point emerges from the coleoptile.

As the plant develops, the fungus is carried upward in the growing point of the primary shoot, or of the lateral shoots. Previous to the time of heading, it is impossible to see any difference between smut-free and infected plants, except for a slight lagging in vegetative growth, which is not a safe diagnostic feature. At heading time, however, the fungus appears to gain the ascendency and the mycelium spreads rapidly through the young ovaries, in which are produced the masses of black spores. At times the fungus appears in linear pustules on the leaves, resembling leaf smut of various grasses, and very often the glumes are striated with the black spore masses. The histological features of the fungus have been adequately described by Lutman.[6]

Obviously the problem in hand is concerned with soil infestation and the physical and chemical conditions of the soil at the time of infection of the oat seedling. Subsequent atmospheric conditions may influence the vegetative growth of the host and thus determine the ability of the fungus to maintain its position in a rapidly dividing meristematic tissue. It is interesting to note in this connection, however, that the most vigorously growing plants appear to be the most heavily smutted. Just why two heads on one plant may be entirely smutted and three other heads on the same plant remain free from smut, or conversely, offers an interesting problem of disease escape. Furthermore, it is not uncommon to see a single spikelet of a single head smutted and all the rest of the head smut-free.

As indicated by the three preceding paragraphs, soil temperature and soil moisture may have a marked influence on infection. Consequently, numerous series of experiments were performed in order to determine the influence of these two factors upon infection of the host by the fungus. The data secured are presented in Tables III and IV.

[6] LUTMAN, B. F. SOME CONTRIBUTIONS TO THE LIFE HISTORY AND CYTOLOGY OF THE SMUTS. *In* Trans. Wis. Acad. Sci., v. 16, p. 1191–1244, pl. 88–95. 1910. Literature cited, p. 1225–1228.

A uniform soil moisture of 36 per cent (equivalent to 10 grams of water to 100 grams of dry soil with a soil saturation point of 27.8 per cent) was maintained throughout the experiments from which the data in Table III were obtained.

TABLE III.—*Effect of different soil temperatures on infection of Avena nuda by Ustilago avenae, when soil moisture was maintained at 36 per cent*

Average temperature.	Dry seed.		Soaked seed.	
	Clean plants.	Smutted plants.	Clean plants.	Smutted plants.
C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
8°	33	67	20	80
12°	50	50	10	90
15°	0	100	0	100
18°	0	100	0	100
21°	0	100	0	100
23°	0	100	25	75
26°	33	67	8	92
29°	25	75	89	11

A soil moisture of 67 per cent (equivalent to 19 grams of water to 100 grams of dry soil) with a soil saturation point of 27.8 per cent was maintained throughout the experiment, the results of which are shown in Table IV.

A comparison of Tables III and IV shows soil temperature to be a limiting factor in infection. The extent of its importance, however, varies with the amount of water present in soil or seed.

TABLE IV.—*Effect of different soil temperatures on infection of Avena nuda by Ustilago avenae when soil moisture was maintained at 67 per cent*

Average temperature.	Dry seed.		Soaked seed.	
	Smut-free plants.	Smutted plants.	Smut-free plants.	Smutted plants.
C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
9°	100	0	91	9
12°	47	53	63	37
16°	13	87	72	28
21°	17	83	71	29
26°	39	61	67	33
29°	94	6	100	0

The results given in Table V are based upon the totals secured from a large number of series of experiments and are based on a total of 1,489 plants. The soil moistures are indicated in the table, and the soil-saturation point varies from 39 to 41 per cent. Air-dry seeds were used throughout.

The totals under 80 per cent soil moisture are not based on results obtained from as large a number of plants as in either of the other moisture tests. The high soil moisture was unfavorable for the germination of the grain and many series were reduced or failed utterly as a result, but the percentages given were based on a sufficiently large number to justify inclusion in the results.

TABLE V.—*Effect of soil temperature and soil moisture on infection of Avena nuda by Ustilago avenae*

Average temperature.	Soil moisture.	Average smutted plants.	Soil moisture.	Average smutted plants.	Soil moisture.	Average smutted plants.
C.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
10°–14°	30–35	96.5	60	74.0	80	56.0
14°–16°	30–35	96.5	60	58.5	80	1.0
17°–21°	30–35	98.0	.....	.....	.....	.....
18°–22°	30–35	100.0	60	93.0	(Series failed)	
31°–32°	30–35	24.0	60	0	80	0

When Table V is compared with Tables III and IV, it will be noted that a less pronounced temperature relationship is evidenced, especially at the lower moisture contents. At a temperature of 31° to 32° C. there is a decided falling off in infection at all soil moistures. These results indicate that one would be safe in predicting that high soil temperatures tend to limit infection regardless of soil moistures. When we consider that 32° C. is the maximum temperature for germination and growth of the fungus, we would expect to find this decrease in infection, especially when we consider the rapidity of germination and elongation of the young seedlings at this temperature.

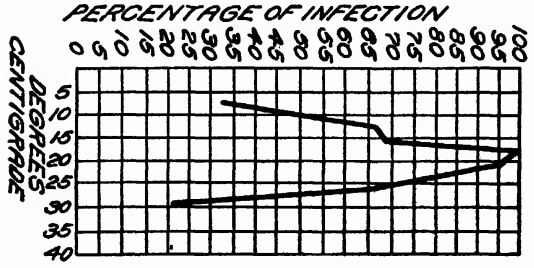

FIG. 1.—Influence of soil temperature upon the infection of *Avena nuda* by *Ustilago avenae* when soil moisture is disregarded.

It will be noted that a temperature of 18° to 22° C. furnishes the optimum for infection in all soil moistures. This coincides directly with the optimum for germination and growth of both host and fungus.

The high percentage of infection at 10° to 14° C. might correlate with the longer time the seed remains in the ground, especially in the case of the lower soil moistures. It may require as long as three weeks under these conditions for the seedling to appear above ground. In this connection, experiments on the relation of soil moisture to the germination of the spores show that the spores germinate as readily in a soil moisture of 35 per cent as in a soil moisture of 65 per cent. If this is correlated with the temperature data already cited, it will be seen that there is no reason why the fungus might not become fairly well established in the soil while the seed remains dormant, or very slowly germinating.

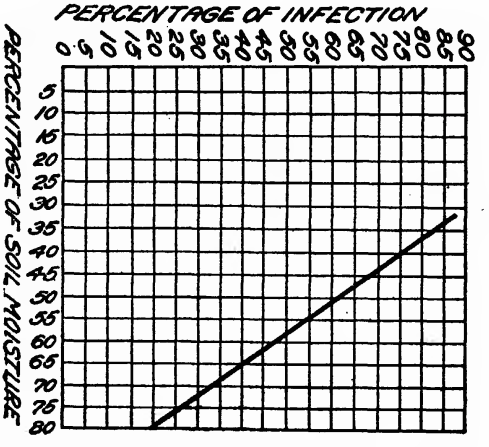

FIG. 2.—The influence of soil moisture upon infection when soil temperature is disregarded.

Such a curve as is shown in figure 1 may help to interpret the common observation that the early sowing of oats in the spring tends to

reduce the amount of infection by loose smut. On the other hand, fall sowing, while the soil temperature is high, would tend also to cut down infection. Such a curve corresponds more nearly to the actual conditions obtaining in nature than a curve derived from series wherein the various factors are considered separately.

The graph in Figure 2 again serves to interpret what is found under field conditions. Conditions of high moisture combined with low temperatures in the spring undoubtedly would tend materially to reduce infection. This conclusion was borne out by some field experiments. Plats containing 28,000 heavily inoculated seeds of *Avena nuda* were prepared in April and May of 1919. The spring was very wet and cold. The average soil temperature was 11.1° C. with a range from 2° to 24° C. during the germination period. The soil moisture averaged from 45 to 50 per cent of the soil saturation point, and ranged as high as 70 per cent at times. The plants matured normally but contained less than one-tenth of 1 per cent of smutted plants. Seed from the same lot treated with the same inoculum, but grown under controlled conditions favorable to maximum infection, produced 100 per cent of smutted plants.

SUMMARY

(1) The temperature range for growth of *Ustilago avenae* when cultured on potato-dextrose agar with a +10 reaction is, minimum 8° C.; optimum 20° C.; maximum 32° C.

(2) The temperature range for germination of the spores in beef broth with a +10 reaction is, minimum 5° C.; optimum 15° to 28° C.; maximum 31° to 34° C.

(3) The temperature range for sporidial production under the same conditions as for spore germination is, minimum 5° C.; optimum 15° to 28° C.; maximum 30° C.

(4) The temperature range for germination of the seeds of *Avena nuda* is, minimum 3° C. with no measurable subsequent growth; optimum 18° to 20° C.; maximum 33° C. plus, not definitely established.

(5) The temperature at which the seed is germinated and the seedlings maintained for two weeks appears to influence the subsequent vigor of the plants. The most vigorous plants were secured from seedlings which had been kept at 16° to 24° C.

(6) High soil temperatures were accompanied by a marked reduction in the percentage of infection under the conditions of our experiments.

(7) Low soil temperatures also were accompanied by a reduction in the percentages of infection, but to a much less marked degree than in the case of high temperatures.

(8) High soil moistures, combined with high soil temperatures, result in complete elimination of the fungus.

(9) Low soil moistures, within a certain temperature range, are accompanied by relatively high percentages of smut infection.

INFLUENCE OF TEMPERATURE, MOISTURE, AND OXYGEN ON SPORE GERMINATION OF USTILAGO AVENAE [1]

By EDITH SEYMOUR JONES, *Formerly Scientific Assistant, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The relation of climatic factors to the development of the smuts has been a matter of speculation during rather more than two decades. An exact determination of the influence of these factors has recently been undertaken by Dr. Lucille K. Bartholomew and the writer (*2*),[2] in order to provide experimental conditions for the determination of the smut resistance of certain pure lines of species of Avena. It has been pointed out in that paper that in order to decide whether any given variety be completely resistant, its performance under conditions resulting in 100 per cent of smut infection in susceptible plants must be ascertained. In determining those factors most favorable to the development of the smut organism, it becomes manifest that these same factors are numerous and closely interwoven, affecting both the host and the fungus. Their effect on both the host and the fungus must be distinguished if a complete analysis of their relation to the development of the disease is to be achieved.

While Doctor Bartholomew and the writer were determining the effect of temperature and moisture (*2*) upon infection by *Ustilago avenae* (Pers.) Jens. (*5*), the writer began to study the influence of temperature upon the germination of the spores of this fungus. When the results of the infection studies began to indicate that soil moisture is a factor controlling infection, the effect of this factor upon the germination of the spores was studied, and later the necessity of studying the relation of oxygen to spore germination became evident. This paper presents the experimental evidence which has been obtained concerning the influence of these three factors upon the germination of the spores of *Ustilago avenae*. Most of these experiments were performed under the guidance and with the help of Doctor Bartholomew, who had carefully formulated the whole problem in its broader aspects.

By way of further introduction to the review of literature and the experiments, it may be well to state briefly the life history of the fungus in its relation to the infection of the host plant. The spores of *Ustilago avenae* overwinter mainly on the seed. The spores germinate simultaneously with the oat seed. They rarely form germ tubes but commonly produce promycelia which, in turn, form either sporidia or thin hyphæ. These sporidia, hyphæ, or germ tubes infect the host through the coleoptile before it is pierced by the first leaf and the fungus establishes itself in the meristematic tissue of the growing point of the main axis and the tillers. Here the mycelium keeps pace with the development of the host, without apparent injury to it, until, at blossoming time, it

[1] Accepted for publication Aug. 21, 1923. This paper presents the results of investigations conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, and the Department of Plant Pathology of the Wisconsin Agricultural Experiment Station.

[2] Reference is by number (italic) to "Literature cited," p. 590–591.

produces its chlamydospores in the young ovaries. Many of these spores, when scattered, come in contact with the seed and the life cycle is repeated.

REVIEW OF LITERATURE

RECOGNITION OF THE RELATION OF CLIMATE TO THE SMUTS

The first recognition of a relation between environment and the development of the smuts of oats, or, in fact, of any closely related smut, was made by Jones (*15*). The observations recorded in that publication indicated, in his opinion, that certain climatic conditions prevailed in Vermont which were especially unfavorable for the development of oat smut. In the following year, however, Jones (*16*) found a higher percentage of smut in Vermont than in other sections of the country. These observations were supplemented by the records, for one season, of the soil temperature during the period of germination and early development of the seedling. From these accumulated data, the writer concludes—

> it is evident that surrounding climatic or soil conditions may at any point in the contest so favor one or the other of the contestants—either the oat plant or the smut fungus—as to decide which shall come out victorious.

ANALYSIS OF THE RELATION OF TEMPERATURE TO THE SMUTS

Following Jones, other workers have recognized soil temperature as a factor of primary importance. Among them Hecke (*12*) distinguishes a threefold effect of temperature, involving particularly (1) the germination of the spores and seeds, (2) the duration of conditions in host plant favorable for infection, and (3) the possibility of the fungus reaching the growing point of the plant. The greater number of the other publications dealing with the relation of temperature to the disease can be classified according to the aspects thus distinguished.

EFFECT OF TEMPERATURE UPON GERMINATION OF SPORES AND SEEDS

Herzberg (*13*) found the cardinal points of temperature for spore germination of *Ustilago avenae* and of four other smuts to be as follows: Minimum between 5° and 11° C., optimum 22° to 30°, and maximum 30° to 35°.

Ravn (*20*) very early noted that the amount of oat smut varies with the time of sowing oats. Later, detailed experiments made possible the definite statement that the time of sowing affects greatly the occurrence of oat smut, that the quantity of smut is small or negligible when oats are sown early, and that it increases in later sowings. A decrease with very late sowing was observed in a few cases. In his discussion of the possible reasons for this climatic influence he presumes that the temperature during germination is the most important factor.

Tubeuf (*22*), experimenting with the smut of oats, found no infection when the temperature was below 7° C. during the seedling stage and 20 to 24 per cent at a temperature of 20° to 21°. In accounting for this difference, he showed that the minimum for the germination of the oat plant is lower than that of the smut, so that when the temperature is sufficiently low the host will escape infection. Thus, he is led to recommend early sowing. In a short paper, Appel and Gassner (*1*) make some general remarks on oat smut, which are essentially in agreement

with the views of Tubeuf (*22*), as given in the first of this paragraph. Also Eriksson (7) considers that damp, warm weather during the sowing favors infection. Heald and Woolman (*10*) have found that a range of temperature between 40° and 65° F. during the germination of wheat is more favorable for bunt infection than either a higher or a lower temperature. It is of interest that this range includes the minimum and the optimum for the germination of bunt spores as given by Hecke (*12*). There are several nontechnical bulletins which mention the importance of temperature. Heald (*9*), in advising the early or late sowing of wheat, shows that during August not only is there a comparatively small number of smut spores in the soil, but that also the soil temperature is generally too high for optimum infection; and by the end of October or the first of November, not only has much of the wind-blown smut germinated, but also there is the frequent occurrence of a soil temperature too low for ready infection. In a similar publication, Heald and Zundel (*11*) state that early seeding of oats results in the minimum amount of smut.

DURATION OF SUSCEPTIBILITY OF HOST PLANT TO INFECTION

Volkart (*23*) was the first to emphasize the fact that low temperature may prolong the infection period. He states that with oats and wheat the slower the initial stages of growth, the more abundant and severe will be the infection. However, extremely low temperatures may retard not only the germination of the seed but also may retard even more the germination of the smut spores and thus remove the danger of infection. This point is more clearly set forth by Hecke (*12*). The latter's experiments dealt with the bunt of wheat which he found occurred most abundantly among plants which had passed their early stages during the low temperatures of late fall and early spring. The temperature of the first five days seemed decisive. That low temperatures at that time caused increased infection he lays in the main to the prolonging of the infection period, although he also discusses what he considers a greater ease with which the fungus may reach the growing point. In comparing oat smut with bunt in this temperature relation, he considers the low temperature not only as a factor retarding the development of the oat seedling, but also as one delaying the germination of the smut spore. The possibility of this being true is substantiated by the cardinal points of temperature. These, according to his citations, are as follows: For oats, minimum 4° to 5° C., optimum 25°, and maximum 30°; for oat smut, minimum 5° to 11° (9°), optimum 22° to 30°, maximum 30° to 35°; for wheat, minimum 3° to 4.5°, optimum 25°, maximum 30° to 32°; and for bunt spores, minimum less than 5°, optimum 16° to 18°; maximum less than 25°.

Munerati (*19*) emphasizes the importance of the duration of the infection period for heavy bunt infection. He states:

> The faster the first phases in the evolution of the life of the seedling are passed through, the more completely is it able to escape attack by Tilletia, and vice versa.

He comes to such conclusions as the result of experiments recorded in two publications (*18*, *19*).

It is obvious that a deeper sowing of seeds might have the effect of prolonging the infection period in a way similar to a low temperature. Jones (*16*) makes note of the factor of the depth of seeding.

Kirchner's (*17*) conception is at variance with that of Volkart (*23*), Hecke (*12*), and Munerati (*19*). Kirchner, also working with bunt, says

there is no connection between the ability of the young plant to push through the ground and the attack of smut. He considers the susceptibility of the host to be dependent upon internal chemical differences, and that the host is not affected by external conditions so as to permit the entrance or bring about exclusion of the smut.

POSSIBLE EFFECT OF TEMPERATURE ON THE ABILITY OF THE FUNGUS TO REACH THE GROWING POINT OF THE HOST AFTER PENETRATION

Hecke's third aspect, the possibility of the fungus reaching the growing point of the host plant, is difficult to distinguish from the second, the prolongation of the infection period. Investigators dealing with the disease do not know without studying the host and the fungus separately which of these is modified by the temperature factor. Hecke classes Brefeld's (*3*) work under the third aspect in which the fungus is considered modified. Using germinated oat smut spores as inoculum, with a lower temperature (one not over 7° C.) he obtained 40 to 46 per cent infection, whereas at a higher temperature (above 15° C.) he obtained 27 to 30 per cent. With the factor of the germination of the smut spores eliminated, Hecke concluded that the fungus reached the growing point of the host more easily at the lower than at the higher temperature. From his results, Brefeld considers that if, in cultivation, the seeding is done in warm weather, followed by cold, more smut will develop than when the temperature remains constantly high. The use of germinated spores as inoculum makes it impossible to compare Brefeld's work with that of other investigators of oat smut.

RELATION OF MOISTURE TO THE SMUTS

The relation of moisture is discussed by Clinton (*4*) and by Hungerford and Wade (*14*). The former notes greater loss from both loose and covered smut of oats in the "hardpan region" of Illinois than in central Illinois. He thinks that the higher moisture content of the soil in this region favors the fungus and causes more infection. The latter observed that in fields sown immediately after rain, a higher percentage of bunt appeared than in those sown under relatively dry conditions. Results obtained from their greenhouse experiments seemed to confirm this observation.

EXPERIMENTAL STUDIES

THE INFLUENCE OF TEMPERATURE ON SPORE GERMINATION

The first problem was to redetermine the cardinal points of temperature for spore germination. The spore material used was grown the spring previous to experimentation and was from 2 to 7 months old when used. To insure its uniformity, all the smutted heads were kept in one envelop, and the spores shaken from the heads were introduced into 30 cc. of medium in approximately the same quantities each time. After trying several different media, a beef broth (4 gm. Liebig beef extract and 10 gm. peptone per 1,000 cc. of distilled water) of +10 Fuller's scale (P_H6.1) was chosen. About two small loopfuls of the spore suspension were mounted in Van Tieghem cells or hollow-ground slides. As Duggar (*6*) has shown the form of the drop and the amount of evaporation to be significant, care was taken that all conditions of mounting

should be as uniform as possible. Duplicate mounts were placed at each temperature, and the experiment was replicated 15 times. Automatically regulated incubators furnished the constant temperatures to which the spores were subjected. At each observation the percentage of germination of 400 spores was recorded for each cell.

The results of a typical experiment are expressed in Table I, and Figures 1 and 2. The results of all the experiments are brought together in Table II.

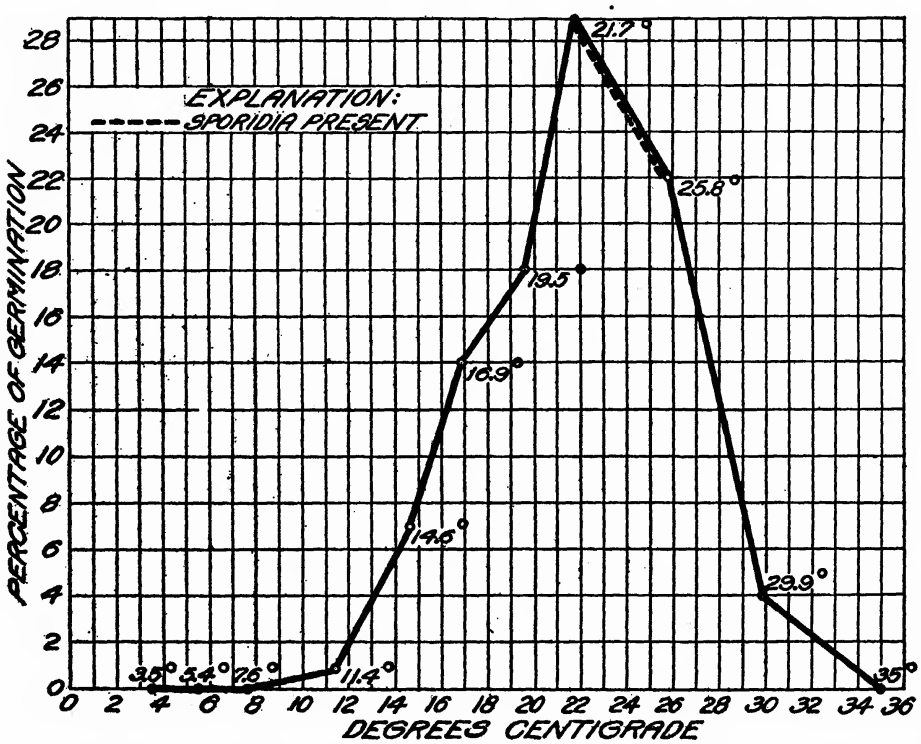

FIG. 1.—Average percentage of germination, and presence of sporidia, of *Ustilago avenae*, in 24 hours, as influenced by temperature of incubation, in experiment 5.

TABLE I.—*Average percentage of germination of chlamydospores of Ustilago avenae produced 5 months earlier, incubated at different temperatures, and recorded at successive intervals*

Temperature average.	Temperature range.	Time.									
		¼	1	2	3	4	5	6	7	8	9
C.	C.	Days.	Days.	Days.	Days.	Days.	Days.	Days.	Days.	Days.	Days.
3.5°	2.6°– 4.8°	0	0	0	0	0	0	0	0	0	0
5.4°	4.4°– 6.0°	0	0	0	0	0	.4	2	3	8	10
7.6°	6.4°– 8.8°	0	0	0	2	14	18.0	....	....	....	....
11.4°	10.0°–12.5°	0	.7	10	27	27	.....	....	....	....	....
14.6°	13.6°–15.9°	0	7.0	22	27	34	.....	....	....	....	....
16.9°	16.1°–18.5°	0	14.0	21	32	....	.....	....	....	....	....
19.5°	17.8°–20.6°	.7	18.0	27	27	....	.....	....	....	....	....
21.7°	19.7°–23.5°	3.0	29.0	27	31	....	.....	....	....	....	....
25.8°	22.5°–28.4°	1.3	22.0	33	....	....	.....	....	....	....	....
29.9°	28.4°–31.4°	.5	4.0	2	5	3	.....	....	....	....	....
35.0°	34.0°–36.0°	0	0	0	0	0	.....	....	....	....	....

In order that Table II be clear to the reader, there are four facts which must be borne in mind. First, in many cases the incubators did not hold the temperatures within satisfactory limits, so that the upper limit of the temperature range was more significant than the average temperature, especially when the spores were incubated for a long time. For this reason the minimum temperature for spore germination rests between 4° and 5° C. In the second place, the age of the spore material influences very markedly the percentage of germination and, to a small degree, extends the range of temperature within which the spores will germinate. This is seen clearly in the germination between 31.7° and 32.7° C. Of six observations made after 6 hours, germination occurred when the spore material was 2 and 4 months old, but not when it was 5 and 6

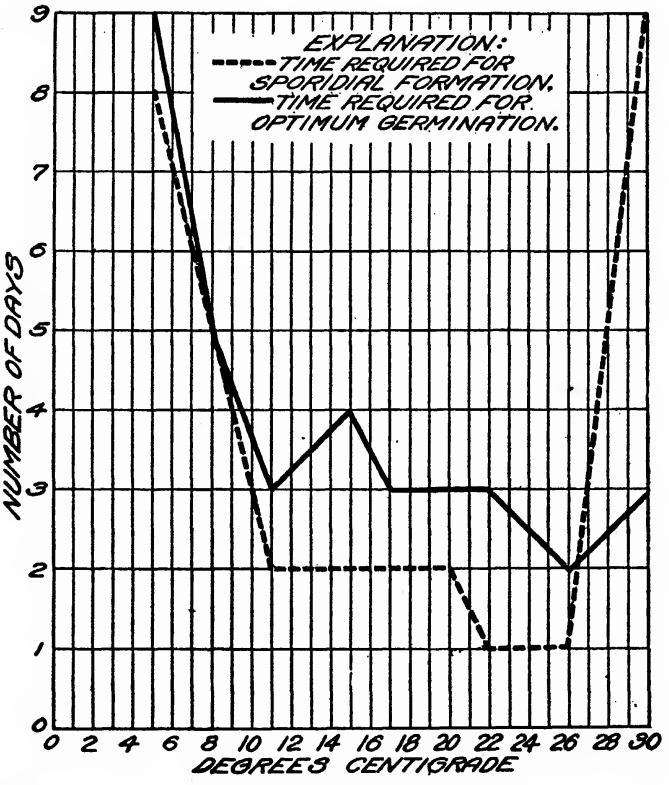

FIG. 2.—Time required for maximum percentage of germination and sporidial formation with *Ustilago avenae*, at temperatures ranging from 4° to 35° C., in experiment 5.

months old. Third, the spore material used for experimentation in the second year showed a higher percentage of germination, and germination at a wider range of temperatures than that used the first year. This accounts for the fact that experiments 11 to 15, inclusive, conducted during the second year, show higher percentages than the earlier experiments. In the fourth place, germination apparently decreased after a certain length of time, as some of the figures in the table show. This, of course, could not be due to an actual decrease, but was due to the fact that the promycelia broke away from some of the spores, leaving them indistinguishable from ungerminated spores. The examinations were discontinued in most cases when lower percentages were obtained. Where the records were made, however, the figures were recorded to show in a more decisive way where the maximum germination was attained.

TABLE II.—*Results of experiments 5 to 10, conducted in 1918, and of experiments 11 to 15, conducted in 1919, showing the average percentage of germination of the chlamydospores of Ustilago avenae of different ages, incubated at different temperatures, and recorded at successive intervals.*

Experiment No.	Age of spores.	Average temperature.	Temperature range.	Time in days.																		
				¼	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	19	20	28
	Months.	*C.*	*C.*																			
8	6	2.9°	2.4°– 3.8°	0	.....	.....	0	0	.....	0	.....	.....	0	.....	.....	.....	.....	.....	.....	.....	.....	0	.....
9	7	2.9°	2.4°– 3.8°	.....	.....	.....	.....	0	.....	.....	.....	0	.....	.....	.....	0	.....	.....	.....	.....	.....	.....	.....
12	2	3.5°	2.8°– 4.0°	0	0	0	0	0	0.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	3.5°	2.6°– 4.8°	0	0	0	0	0	0	0	0	0	0	0	.....	0	.....	.....	.....	.....	.....	.....	.....
7	6	3.5°	2.9°– 4.4°	0	0	0	.....	0	.....	0	.....	0	.....	.....	0.04	.....	.....	.....	.....	.....	.....	.....	.....
6	5	3.6°	3.1°– 4.2°	0	0	0	0	.....	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	3.7°	3.2°– 4.3°	0	0	0	0	.3	2.0	7.0	17.0	11.0	25.0	29.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	3.9°	2.8°– 4.7°	.....	0	0	0	.....	.....	.05	.1	.5	1.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	4.1°	3.2°– 5.0°	.....	.....	.....	.....	.....	.04	.06	.27	5.0	8.0	7.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	5	4.2°	1.8°– 5.4°	.....	.....	.....	0	0	0	.....	0	0	.....	0	0	0	.....	0	0	0	0	.....	.....
15	5	4.2°	3.6°– 5.1°	.....	.....	.....	0	0	0	.....	.03	.3	.....	.....	.4	.....	.....	.....	.6	.....	.9	.....	.....
8	6	4.5°	3.6°– 5.4°	0	.....	.....	0	0	.....	0	.....	.....	.07	.....	.....	.....	.....	.....	.....	.....	.....	.8	3
7	6	4.6°	3.6°– 6.3°	0	0	0	.....	0	.....	0	.....	0	.....	.....	.04	.....	.....	.....	.....	.....	.....	.....	.....
15	5	4.8°	3.7°– 6.0°	.....	.....	.....	.02	2.0	4.0	.....	8.0	8.8	.....	.....	9.4	14.0	.....	.....	.....	.....	.....	.....	.....
9	7	4.8°	4.0°– 5.4°	.....	.....	.....	.....	.....	.....	.....	.....	.5	.....	.....	.....	.2	.....	.....	.....	.....	.....	.....	.....
6	5	5.2°	4.3°– 6.6°	0	0	0	0	.....	.04	.13	.13	.....	.....	.....	.....	.....	.....	1.4	.....	4.0	10.7	.....	.....
5	5	5.4°	4.4°– 6.0°	0	0	0	0	0	.4	2.0	3.0	8.0	10.0	9.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	5.5°	4.2°– 6.4°	0	0	0	.03	.03	.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	5.6°	4.3°– 6.9°	.....	.....	.....	.....	.21	16.0	39.0	.....	44.0	53.0	43.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	5.9°	4.8°– 6.8°	.....	.....	0	5.0	.....	13.0	20.0	20.0	18.0	18.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	6	6.4°	4.4°– 7.4°	0	0	0	.....	2.0	.....	5.0	.....	14.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	7.6	6.4°– 8.8°	0	0	0	2.0	14.0	18.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	7	8.6	7.2°– 8.3°	.....	0	2.0	.....	.....	5.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	7	10.4°	9.5°–11.0°	.....	.13	2.0	.....	.....	15.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	11.4°	10.0°–12.5°	0	.7	10.0	27.0	27.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	12.8°	11.0°–15.6°	0	14.0	27.0	33.0	46.0	36.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	13.9°	12.0°–17.3°	0	19.0	26.0	37.0	38.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	14.6°	13.6°–15.9°	0	7.0	22.0	27.0	34.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	5	16.5°	16.0°–17.3°	0	27.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	16.7°	15.7°–18.8°	.03	58.0	67.0	67.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	16.9°	16.1°–18.5°	0	14.0	21.0	32.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	17.1°	16.2°–18.0°	0	17.0	21.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	18.2°	17.2°–20.0°	2.3	59.0	66.0	66.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	5	18.4°	18.0°–19.1°	.07	21.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	18.6°	17.2°–19.9°	3.1	42.0	40.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	7	19.1°	17.0°–20.2°	.3	18.0	.....	19.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	19.5°	17.8°–20.6°	.7	18.0	27.0	27.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	19.9°	19.2°–20.0°	.05	16.0	19.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	21.6°	20.3°–23.9°	3.8	53.0	57.0	58.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	21.7°	19.7°–23.5°	2.7	29.0	27.0	32.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

TABLE II.—*Results of experiments 5 to 10, conducted in 1918, and of experiments 11 to 15, conducted in 1919, showing the average percentage of germination of the chlamydospores of Ustilago avenae of different ages, incubated at different temperatures, and recorded at successive intervals*—Continued.

Experiment No.	Age of spores.	Average temperature.	Temperature range.	Time in days.																		
				¼	1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	19	20	28
	Months.	*C.*	*C.*																			
12	2	22.3°	21.2°–23.2°	5.6	31.0	30.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	22.5°	21.5°–26.2°	9.3	21.0	24.0	27.0	13.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	22.6°	21.5°–24.0°	8.2	64.0	74.0	59.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	24.2°	24.0°–24.2°	3.8	14.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	24.6°	23.4°–25.6°	14.0	34.0	35.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	24.8°	23.5°–25.8°	16.0	49.0	35.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	25.5°	25.3°–25.8°	5.0	18.0	22.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	25.8°	22.5°–28.4°	1.3	22.0	32.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	26.4°	26.2°–27.0°	4.0	13.0	13.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	26.9°	26.0°–28.0°	11.0	43.0	37.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	27.5°	26.9°–28.3°	9.8	23.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	28.1°	27.3°–28.7°	14.0	54.0	62.0	60.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	28.2°	28.0°–28.3°	5.0	27.0	32.0	34.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	28.5°	27.8°–29.9°	3.7	53.0	48.0	43.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	6	29.1°	27.8°–29.8°	.5	12.0	8.5	.....	9.2	.....	8.5	.....	.....	11.0	.....	10.0	.....	.....	8.2	.....	.....	8.5	.....	.....
6	5	29.7°	29.4°–30.0°	2.2	16.0	18.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	5	29.7°	29.2°–30.3°	6.0	20.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	7	29.8°	29.6°–30.1°	2.0	15.0	17.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	7	29.9°	29.6°–30.6°	.....	.1	.....	.....	4.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5	5	29.9°	28.4°–31.4°	.5	4.0	2.0	5.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	6	30.0°	29.4°–30.8°	0	7.0	8.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	30.1°	30.0°–30.3°	.3	7.8	9.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	30.3°	29.7°–31.7°	.....	.1	.2	.4	.15	.35	1.5	1.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	30.4°	29.9°–31.0°	.9	6.0	2.5	4.7	3.6	.5	.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	31.7°	31.0°–32.0°	.4	5.0	12.0	6.0	6.1	4.5	5.3	3.3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
12	2	31.9°	31.0°–32.2°	.06	.27	.6	.7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	32.2°	31.8°–32.7°	.02	.35	.35	1.7	.5	1.7	.5	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
7	6	32.3°	29.5°–34.2°	0	0	.5	.....	.....	.....	.3	.....	.....	.4	.....	.....	.3	.....	.....	.....	.....	.4	.....
8	6	32.4°	31.7°–33.9°	0	4.0	.....	6.0	.....	.....	.....	.....	.....	.....	4.3	.....	.....	.....	.....	.....	2.1	.....	.....
6	5	32.7°	32.4°–33.8°	0	0	.2	.2	.....	.15	.06	.11	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	7	32.9°	31.7°–33.9°	.....	2.0	.....	.....	3.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
9	7	33.6°	33.2°–35.0°	.....	0	.....	.....	0	.....	.....	.....	0	.....	.....	0	.....	.....	.....	.....	.....	.....	.....
12	2	34.1°	33.5°–35.0°	0	0	.015	.01	.015	.025	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	2	34.3°	34.0°–35.0°	0	0	0	0	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
15	5	34.4°	33.8°–35.2°	.....	.....	.....	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
13	4	34.7°	34.3°–35.2°	0	0	0	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	6	34.7°	34.0°–35.5°	0	0	.....	0	0	.....	0	.....	.....	0	.....	.....	.....	.....	.....	.....	0	.....	0	.....
7	6	34.8°	33.4°–39.5°	0	0	0	.....	0	.....	0	.....	0	.....	.....	0	.....	.....	0	.....	.....	0	.....	.....
5	5	35.0°	34.0°–36.0°	0	0	0	0	0	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	5	35.2°	35.0°–35.6°	.....	0	0	0	.....	0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

With these three considerations in mind, it may be concluded that the spores begin to germinate between 4° and 5° C., and that the maximum temperature for germination lies between 31° and 34°. If the optimum be arbitrarily chosen as that range of temperature over which germination with usual sporidial production will take place under these conditions in 24 hours, it is 15° to 28°. The minimum and optimum for sporidial production are the same as for germination, but the maximum is somewhat lower, ranging from 29° to 30°.

A few interesting observations may be noted here. In the broth solution the spores most commonly germinate into promycelia from which sporidia are cut off, in contrast with the germination on agar placed in the soil, where hyphæ usually are produced in place of the sporidia. The effect of very high temperatures is evident in the type of germ tubes. At the lowest temperatures the tubes appear to be normal, whereas, at temperatures around 29° and 30° C., the sporidia sometimes seem not to be completely formed and the tubes tend to be thin and transparent. At temperatures around 32° the tubes are barely visible and never grow to the usual length.

THE INFLUENCE OF MOISTURE ON SPORE GERMINATION

The object of this phase of the work was to determine the effect of the moisture content of the soil on the spores of *Ustilago avenæ*. Various methods, whereby spores could be placed in the soil under conditions similar to those obtaining in nature and recovered for examination under the microscope, were considered. The following technique finally was adopted. About 30 cc. of 2.5 per cent water agar of known acidity (P_H6.4 or +0.4 Fuller's scale) was poured into open petri dishes and dried thoroughly at a temperature not above 60° C. From the dried agar, smooth pieces 1 cm. square were cut. To distribute the spores evenly over the pieces of agar, a surface of paper was covered as evenly as possible with the spores. The pieces of agar were then laid one by one upon this surface and pressed gently to bring the whole of the surface in contact with the spores. Pieces of filter paper then were clipped above and below the agar with a fine wire. The spores thus prepared were sown under the same conditions as the oat seeds, 1 inch below the surface of the soil.

The soil moistures were made up on the basis of the moisture-holding capacity, as in the experiments of Bartholomew and Jones (*2*), and the acidity determined by Truog's (*21*) method. To insure uniform moisture distribution in the soil, either water was added to the soil 24 hours previous to sowing, or moistened soil was mixed before using, except that the 80 per cent was made originally to 60 per cent and after seeding was brought to 80 per cent. Uniform temperatures were maintained by incubators. The germination of the spores on the agar layer could be counted under the microscope after the removal of the filter paper.

At this point the question will arise as to how closely the conditions to which the spores were subjected in these experiments approximate the actual conditions to which the spores are exposed when naturally distributed in the soil. The availability of water to the spores is probably not the same when the spores are in contact with the agar as when they are in contact with the soil. In order to determine whether the amount of water absorbed by the agar varies directly with the moisture content

of the soil the following experiment was performed. Layers of oven-dried agar were weighed, clipped between filter papers, buried for 24 hours in cans of soil, the moisture content of which was made up to definite percentages, and then removed and weighed. The quantity of distilled water absorbed by 1 gram of oven-dried agar in 24 hours at 22° to 23° C. was found to be 11.8 grams. This maximum quantity of water absorbed under these conditions may be considered the water-holding capacity of agar. In the same time and at the same temperature similar agar placed in samples of a soil containing, respectively, 80 per cent, 50 per cent, and 20 per cent of its moisture-holding capacity, absorbed, respectively, 55 per cent, 30 per cent, and 10 per cent of its water-holding capacity. This shows that agar absorbs water approximately in proportion to the amount in the soil and may be interpreted as indicating that the amount of water available to the fungus fluctuates with the moisture content of the soil. It would seem that, as far as the other factors in the soil are concerned, the conditions to which the spores were subjected very closely approximated those occurring in nature, and it will so be assumed in the remainder of this paper.

In the first moisture experiments, the soil temperature was maintained between 20° and 24° C., and germination was recorded between 24 and 27 hours after sowing. Table III gives a summary of the results of these experiments.

From Table III it will be seen that the production of sporidia, or the small hyphæ which replace the sporidia, seems to be associated with conditions favorable to good germination, and occurs less frequently at 80 per cent soil moisture.

TABLE III.—*Relation of soil moisture to the spore germination of Ustilago avenae incubated at 20° to 24° C. in 24 to 27 hours*

Age of spores (months).	30 per cent water-holding capacity.		60 per cent water-holding capacity.		80 per cent water-holding capacity.	
	Germination.	Type of germination.	Germination.	Type of germination.	Germination.	Type of germination.
	Per ct.		*Per ct.*		*Per ct.*	
9.....	21	Threads, buds,[1] sporidia.	21	Threads.....	4	Short tubes, long tubes, buds.
11.....	19	Threads, sporidia.	19	.....do.....	0	
2.....	35	Threads, buds; sporidia rare.	28	Threads rare; buds abundant; very many short tubes.	.6	Tubes just visible; one long with thread.
2.....	16	Sporidia abundant; threads, buds, long tubes.	11	Long tubes, threads, buds.	5	Threads.
5.....	16	Very long tubes, threads; sporidia abundant.	10	Threads.....	5	Do.
5.....	18	Threads, sporidia very rare.	15	.....do.....	7	Few threads; long and short tubes; buds.
Average.	21	.....	17	.....	4	

[1] Threads are the infection hyphae which may replace sporidia. Buds, a very early state of infection hyphae or sporidia.

It was next desired to ascertain whether the same general relation of spore germination to varying soil moistures obtains at other temperatures. The low temperatures of 10° to 13° C., and the high temperatures of 30° to 33° were chosen for this study. The duration of the different experiments was determined by the length of time required for spore germination at those temperatures. Tables IV and V show an agreement with Table III, and the three tables make clear that at these different temperatures germination is highest at 30 per cent soil moisture, is usually less at 60, and falls off very markedly at 80.

TABLE IV.—*Relation of soil moisture to the spore germination of Ustilago avenae incubated at temperatures of 10° to 13° C. in 48 hours*

Age of spores (months).	30 per cent water-holding capacity.		60 per cent water-holding capacity		80 per cent water-holding capacity.	
	Germination.	Type of germination.	Germination.	Type of germination.	Germination.	Type of germination.
	Per ct.		*Per ct.*		*Per ct.*	
9.....	24	Threads, some short tubes, long tubes, buds.	26	Threads very long; tubes, buds.	8	Threads, short and long tubes.
2.....	9	Sporidia, threads.	3	Long tubes.....	2	Short tubes.
5.....	9	Sporidia rare.....	5	Threads, long and short tubes.	5	Threads.
Average.	14	.....	11	.....	5	

TABLE V.—*Relation of soil moisture to the spore germination of Ustilago avenae incubated at temperatures of 30° to 33° C. in 24 hours*

Age of spores (months).	30 per cent water-holding capacity.		60 per cent water-holding capacity.		80 per cent water-holding capacity.	
	Germination.	Type of germination.	Germination.	Type of germination.	Germination.	Type of germination.
	Per ct.		*Per ct.*		*Per ct.*	
10.....	4	Short tubes, no sporidia, some tubes long, some buds.	3	No sporidia or threads, short tubes mostly just visible.	0.06	Tubes just visible.
2.....	12	Long tubes with few threads.	4	Tubes long and just visible.	.20	Do.
5.....	10	Long tubes, buds.	13	Threads, buds....	3.0	Threads, short and long tubes.
Average.	8	.....	7	.....	1.0	

These results raise the question of the reason for the decrease of spore germination at the higher moisture contents. An increase in water might bring about a change in other factors affecting germination. These factors may be markedly modified when the puddling point is reached. At 80 per cent soil moisture, puddling is easily brought about

by such mechanical agencies as jarring or rapid addition of water. It was observed that when such a point was reached in a few of the preliminary experiments, germination was very much lower than in the later experiments when puddling was avoided by very careful handling of the soil. It is obvious, then, that the condition of the soil containing 80 per cent of its moisture-holding capacity was not far removed from that of puddled soil. Among the several factors that may have been responsible for reduced germination in wet soil, that of absence of sufficient oxygen appears the most probable. For this reason experiments were carried on to determine whether the spores germinate in the absence of oxygen.

THE INFLUENCE OF OXYGEN ON SPORE GERMINATION

In the search for a method whereby oxygen could be excluded from a spore suspension which could be kept under observation, many devices were tried before a satisfactory technique was finally adopted. The apparatus used is shown in Figure 3. Oxygen-free air was forced through an Engelmann cell (fig. 3, C) where spores were suspended in beef broth (P_H 6.4), as in the germination experiments previously described. The beef broth had been freed from oxygen by heating at 100° C. and kept in an air-tight receptacle with a solution of pyrogallic acid. The oxygen was removed from the air in the air-tight bottle (fig. 3, B) by introducing a liter of alkaline pyrogallic acid and shaking it within the bottle for 20 minutes. Normal pressure was restored after shaking by siphoning boiled water from the reservoir (fig. 3, A) into the oxygen-free bottle. The tube from the reservoir to the bottle was then tightly clamped, the reservoir covered with a quarter of an inch of kerosene to exclude oxygen, and the latter raised to the position shown in the figure.

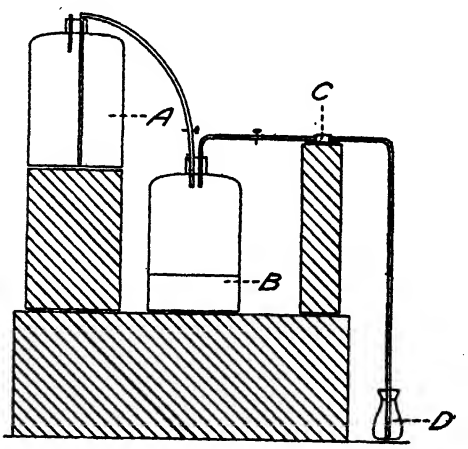

Fig. 3.—Apparatus for excluding oxygen from a spore suspension: A, reservoir of oxygen-free water; B, bottle containing pyrogallic acid; C, Engelmann cell; and D, bottle of water.

The Engelmann cell with its spore suspension was then attached to the outlet tube of the bottle, and a flow of water allowed to pass through the siphon into the bottle of oxygen-free air whose connection with the Engelmann cell was then opened. The oxygen-free air thus forced through the Engelmann cell was allowed to bubble through water in a small bottle placed at a much lower level (fig. 3, D).

By regulating the inflow of water from the reservoir, the oxygen-free air was forced rapidly through the cell for at least 40 minutes, and then more slowly for the remainder of the 24-hour period, except during the time when the air passing through the cell was collected for analysis in a Hempel pipette by the replacement of mercury. During each experiment four or five pipettes of gas were collected and the percentage of oxygen determined over phosphorus.[3] This analysis was made in order

[3] The writer is indebted to Dr. A. S. Loevenhart and the Department of Pharmacology, University of Wisconsin, for the use of the oxgen-analysis apparatus.

that the presence of oxygen entering the apparatus from any source might be detected.

With the apparatus in this final arrangement, three spore suspensions were thus subjected to an atmosphere free from oxygen as determined by the analysis of the samples of air passing over them. In no case did these spores germinate. Control mounts in Van Tieghem cells showed good germination. Before the apparatus was perfected and gas analysis made at the end of each germination, 15 trials had been made in which germination had occurred in only two instances when leaks in the apparatus, which admitted oxygen, were subsequently detected. The data confirm the results of the three final trials cited, and strengthen the evidence that *Ustilago avenae* spores will not germinate in the absence of oxygen. Some of these trials, however, have indicated that the spores will germinate in a low percentage of oxygen. In two experiments, germination of 5.9 per cent was found when the oxygen percentage was 5.85 and 1.95. The controls gave 28 and 35 per cent. In another trial in which germination was 27 per cent, and the control germinated 21 per cent, the gas analysis showed 0.5 per cent of oxygen.

DISCUSSION AND CONCLUSIONS

From the temperature experiments, it may be concluded that within the range from 9° to 28° C. there is no temperature effect upon the fungus which may prevent infection and establishment of the fungus within the host. According to Bartholomew and Jones (*2*) the range of temperature over which the highest percentages of smut occur is from 12° to 28°. This is nearly covered by the optimum range for germination of the fungus, 15° to 28°. Here also, according to Haberlandt (*8*, *p. 43*), is included the optimum for the most favorable and rapid germination of the host; namely, 25° C. At 9°, where the percentage of smut is either zero or very low, it takes about 10 days for the host to appear above ground and from four to five days for a good germination of the fungus and subsequent sporidial production. It would seem that the lack of smut was in no way due to an unfavorable influence of temperature upon the fungus alone. At 29° and below 6° C., however, the temperature seems to be unfavorable to the fungus. At the first and above, the germination is very much reduced and is abnormal, while sporidial production is rare. Here, it seems clear, a temperature effect may be the cause of the low percentage of smut. Below 6° it is possible that the host may develop before the germination of the fungus takes place, as the fungus requires about nine days to produce any considerable germination. These temperature conclusions are essentially in agreement with those of Ravn (*20*), Tubeuf (*22*), Eriksson (*7*), and Heald and Zundel (*11*).

As Bartholomew and Jones (*2*) found a low percentage of smut at the high soil moistures, and as at 80 per cent soil moisture there is a decidedly low fungal germination, with a slight falling off at 60 per cent as compared with 30 per cent, it seems safe to conclude that at the high content of soil moisture the decrease in spore germination may be a direct cause of the low percentage of smut. Analyzing this still further, we can perceive indications that this may be due to a factor involving oxygen supply. Were the oxygen completely eliminated at these high moisture contents, it could be stated conclusively that the absence of oxygen prevented the smut germination, for these experiments have demonstrated for this fungus the commonly assumed hypothesis that the spores

will not germinate in a liquid which is not exposed to oxygen. On the other hand, these experiments also indicate very strongly that with reduced oxygen supply germination still may take place. If it were true that in a soil of high moisture content some smut spores were in contact with oxygen and others were not, the reduction in germination would be clearly explained.

SUMMARY

(1) In order to determine the effect of temperature upon the germination of the spores of *Ustilago avenae*, spores were germinated in Van Tieghem cells or hollow-ground slides at a series of temperatures ranging from 4° to 35° C. in beef broth +10 Fuller's scale (P_H 6.1). The optimum for germination is arbitrarily defined as that range of temperature over which germination with usual sporidial production will take place under these conditions in 24 hours. The minimum temperature for germination was found to be between 4° and 5° C., the optimum from 15° to 28°, and the maximum between 31° and 34°.

(2) Under these conditions, the minimum and optimum for sporidial production are the same as for germination, but the maximum is somewhat lower, ranging from 29° to 30° C.

(3) The relation of moisture to spore germination was studied by placing the spores on layers of agar between filter papers in soil containing 30, 60, and 80 per cent, respectively, of its moisture-holding capacity. Germination was found to be highest at 30 per cent, to fall off slightly at 60 per cent, and very markedly at 80 per cent.

(4) *Ustilago avenae* spores failed to germinate in a suspension exposed to an oxygen-free atmosphere.

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INFLUENCE OF TEMPERATURE ON THE SPORE GERMINATION OF USTILAGO ZEAE [1]

By EDITH SEYMOUR JONES

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Temperature has been regarded by investigators as important among the environmental conditions influencing the infection of corn by *Ustilago zeae* (Beckm.) Unger. Maire (*5*)[2] found that the spores of the corn smut fungus "germinate more quickly if the temperature is a little raised (20° to 25°)" and that the optimum temperature for sporidial and filamentous development is 20° to 25° C. Piemeisel (*7*), in considering the "phenomenon of infection and the optimum conditions for the propagation of the fungus," found the optimum temperature for the budding of sporidia to lie between 20° and 26° C., the maximum at about 40° and the thermal death point near 46°. He also found that incubation at 24° to 38° did not seem to influence the rate or amount of germination of the smut spores.

As the germination of the spores themselves would seem to be at least as significant as the budding of sporidia, and as the above investigators do not give detailed experiments on this stage of fungal activity, a study of the influence of temperature on the spore germination of *Ustilago zeae* was undertaken in order to throw additional light upon the relation of temperature to this early, significant stage in the life history of the fungus. The studies of which the results are presented in this paper have been carried on under the direction of Dr. L. R. Jones.

In searching for suitable media for spore germination it was attempted to duplicate natural soil conditions. Brefeld (*2, p. 67–75*) performed experiments which indicated that the germination of the *Ustilago zeae* spores takes place in the soil. The conception of soil, especially that containing manures, as a usual medium of germination of the spores also has been held even down to the present time. In attempting to find a soil medium like that obtaining in nature, the method of Thompson (*8*) was tested. When a tap-water suspension of spores was poured onto loam, no germination took place, whereas, when a suspension of spores in Pasteur's solution was introduced in the same way, germination followed. It seemed that the conditions for spore germination in such soil were not suitable, whereas they were satisfactory in the Pasteur liquid. In an attempt to find a soil on which the spores would germinate, seven modifications of loam and manure were employed, and the spores of *Tilletia tritici* (Bjerk.), Wint., were used as controls. On one pot which contained cow manure and loam, 1:2, there was considerable germination, and on another pot, one germinating spore was observed. Another test gave slight germination. In all these tests the bunt spores germinated abundantly, but, as a result of all the 27 attempts to germinate the spores of *Ustilago zeae* on soil, no satisfactory method was found.

[1] Accepted for publication Aug. 21, 1922.
The investigations, the results of which are presented in this paper, were conducted cooperatively by the Office of Cereal Investigations, Bureau of Plant Industry, and the Department of Plant Pathology of the Wisconsin Agricultural Experiment Station.

[2] Reference is by number (italic) to "Literature cited," p. 596–597.

An artificial medium was used, therefore, in the study of the effect of temperature on spore germination. Arthur and Stuart (*1*), Brefeld (*2*), Clinton (*3*), Hitchcock and Norton (*4*), Maire (*5*), and Norton (*6*) germinated the spores in many artificial media. Of these, Pasteur's solution was found most favorable for the following experiments.

The spores from a pustule formed in an anther were suspended in a tube of medium, mounted in Van Tieghem cells, and subjected to 13 different temperatures between 8° and 37° C. in thermo-regulated incubators. Duplicate mounts were placed at each temperature and the experiment performed 11 times. To determine the cardinal temperatures for germination, the percentage of germination was counted; the sporidial production, the number of cells in the promycelia, and the cell contents were observed; and the length and width of the germ tubes measured.

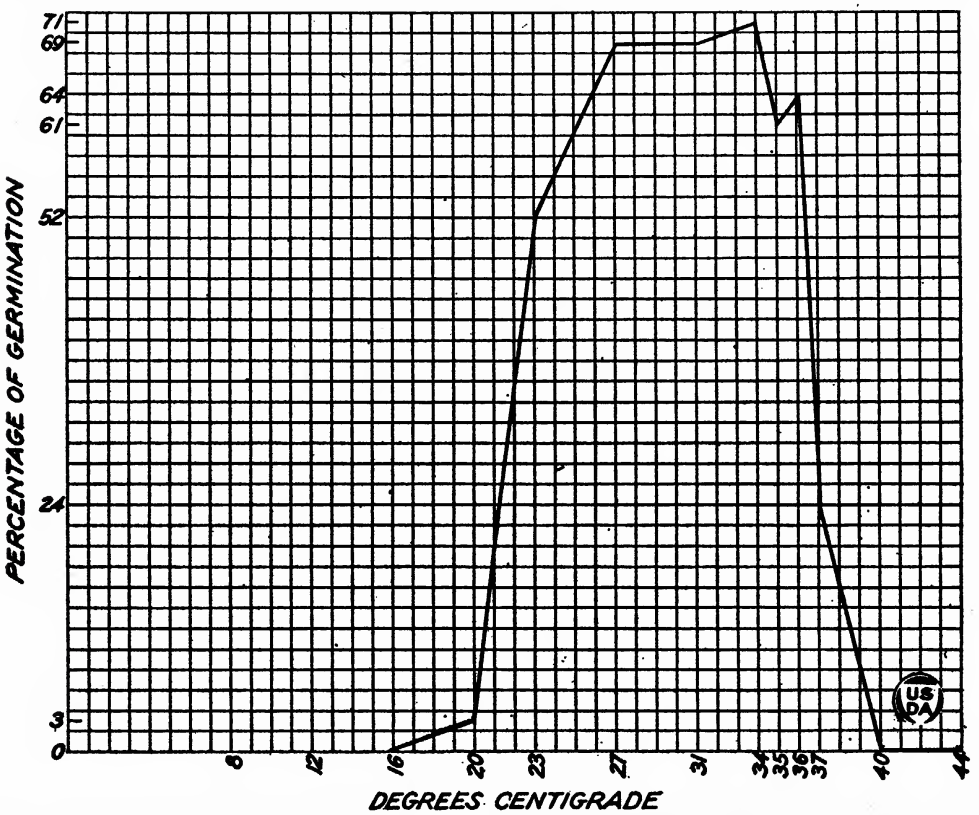

FIG. 1.—Graph showing percentages of germination of chlamydospores of *Ustilago zeae* at different temperatures.

The exact minimum for spore germination of *Ustilago zeae* was not determined. Four experiments showed germination at an average temperature ranging from 7.9° to 8.4° C., the germ tubes appearing on the 12th, 15th, 16th, and 21st days, respectively. The temperature range of the individual tests was greater than desired. That, together with the daily removal of the mounts from the incubators, may have given sufficient opportunity for eventual germination. The optimum temperature for germination is arbitrarily defined as that at which there is the highest percentage of germination within 24 hours together with a production of such germ tubes as behave normally, especially with respect to sporidial production. The highest percentage of germination within 24 hours occurred between 26.5° and 34.7° C. as is shown in Table I and figure 1, which give the average results of all experiments. As

TABLE I.—*Results of experiments on the germination of Ustilago zeae spores, showing range of temperature, time required for sporidial production, length and contents of germ tubes, and percentage of germination at different periods after sowing.*

Number of experiments included.	Average of temperature ranges.	Sporidial production.		Germ tubes.		Germination on given days after sowing.								
		Number.	Time.	Length.	Contents.	¾	1	2	3	4	5	6	7	16
	C.		Days.	μ.		Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.
5	7.9– 8.4	Present	15–21	43	Granular; some vacuolate	.....	.....	.....	.....	.....	.....	.....	.....	4
5	11.7–12.9	None to abundant	3–6	32	.....do	.....	0	0	0.7	3	11	44	57	.....
5	15.3–16.9	Present to abundant	2	36	Granular; granular and vacuolate.	.....	0	33	58	.....	.....	.....	.....	.....
5	19.0–20.9	Few to abundant	1–2	37	Granular; some vacuolate	0.004	3	69	.....	.....	.....	.....	.....	.....
4	23.0–23.9	Present to abundant	¾	31	Granular and vacuolate	13	52	73	.....	.....	.....	.....	.....	.....
5	26.5–28.1	Abundant	¾	33	.....do	54	69	.....	.....	.....	.....	.....	.....	.....
11	30.0–32.9	.....do	¾	45	Granular; granular and vacuolate.	52	69	.....	.....	.....	.....	.....	.....	.....
6	34.0–34.7	Mostly abundant	¾–1	.....	.....	39	71	.....	.....	.....	.....	.....	.....	.....
6	35.0–35.9	None to abundant	¾–1	.....	.....	41	61	.....	.....	.....	.....	.....	.....	.....
4	36.0–36.9	Few to abundant	1–6	59	Homogeneous	24	64	.....	.....	.....	.....	.....	.....	.....
7	37.0–37.8	None to many	¾–2	59	.....do	14	24	.....	.....	.....	.....	.....	.....	.....
9	39.7–40.8	None	.....	.....	.....	0	0	0	0	0	0	0	0	0
3	44.7–44.9	.....do	.....	.....	.....	0	0	0	0	0	0	0	0	0

determined by the number produced on first observation, the optimum temperature for sporidial production lies between 26.5° and 34.7° C. The maximum lies between 36° and 38°. Of 20 observations above 34° only 4 showed abundant production.

At the low temperatures, wherever germination took place sporidia also were produced. The number of cells in the basidia and the width of basidia were alike at the different temperatures and so could not be used as criteria in establishing the optimum temperatures. The lengths of basidia and condition of cellular contents, as shown in Table I, differed at 36.1° to 37.8° C. from those at other temperatures, indicating a deviation from normal at this high temperature. As below 26.5° the percentage of germination is decreased, and, as above 34.7° the sporidial production is decreased and germ tubes are abnormal, the optimum temperature for spore germination may be stated as between about 26° and 34° C. As Table I shows that the maximum temperature rests between 37.1° and 37.8° and as six experiments show no germination at temperatures above 38° after two weeks, the maximum temperature for germination apparently lies between 37° and 38° C.

Attention should be called to the fact that this optimum temperature for germination is much higher for *Ustilago zeae* than for other smuts which have been studied in this laboratory. For example, *Ustilago avenae* germinates best between 15° and 28° C., and, according to Volkart (9), *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn germinate best between 16° and 18° C.

SUMMARY AND CONCLUSIONS

(1) In 27 trials to germinate *Ustilago zeae* spores in soils containing different amounts of manure, there was considerable germination in only one, slight germination in another, and germination barely occurring in a third. The spores germinated readily in many artificial media, from among which Pasteur's solution was chosen for the experiments to determine the relation of temperature to spore germination.

(2) The optimum temperature for the germination of *Ustilago zeae* spores was found to lie between about 26° and 34° C., the maximum between 36° and 38°, and germination was observed to occur at the minimum temperature of 8°. The optimum for sporidial production lies also between about 26° and 34° C., the maximum between 36° and 38°, and sporidia are produced at the lowest temperatures at which germination was observed.

(3) The experiments showing that high temperature is most favorable for spore germination indicate that infection likewise is favored by hot weather whether the germination takes place in the soil or in water held upon the host plant.

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SPORES IN THE UPPER AIR[1]

By ELVIN C. STAKMAN, *Agent, Office of Cereal Investigations, and Plant Pathologist, Agricultural Experiment Station, University of Minnesota,* and ARTHUR W. HENRY, GORDON C. CURRAN, and WARREN N. CHRISTOPHER, *Field Assistants, Office of Cereal Investigations, United States Department of Agriculture*[2]

INTRODUCTION

As a part of the rust-epidemiology investigations which have been made since the spring of 1917 by the Office of Cereal Investigations of the United States Department of Agriculture in cooperation with several State experiment stations, attempts have been made to get as much information as possible on the dissemination of spores by air currents and to correlate the data with the spread of rust on host plants. The usual method in studying the distribution of spores of pathogenic fungi by wind has been to expose spore traps of various kinds near the surface of the earth. The possibility of spores being carried to higher altitudes by convection currents, whirlwinds, and other air movements has been recognized, and spore traps have been exposed on high buildings, on mountain tops, and other elevated points. However, it is desirable to know how many spores there are in suspension several thousand feet above the surface of the earth.

During the spring and summer of 1921 [3] spore traps [4] were exposed on airplanes [5] in the hope of obtaining more information on the dissemination of aeciospores and urediniospores of *Puccinia graminis* and other rust fungi. The general results seem to be worth recording, although the problem of rust epidemiology was not solved.

METHODS

Airplanes were used in preference to balloons, kites, or other similar devices, because long distances could be covered in a short time, spore traps could be exposed easily at different altitudes, and the direction of flight could be changed at will.

Ordinary microscope slides (3 inches by 1 inch), smeared lightly with vaseline on one side, were exposed in different ways. One method was

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[2] The authors wish to acknowledge the enthusiastic cooperation of the commanding officer at Kelly Field, San Antonio, Tex.; Maj. Ira A. Rader, air officer for the Seventh Corps Area of the United States Army, at Fort Crook, Nebr.; Mr. W. I. Votaw, manager of the Aerial Mail Service Station at Omaha, Nebr.: and Mr. W. A. Kidder, of the Curtiss Flying Field (private), St. Paul, Minn. The authors are under deep obligation to these men, and to the pilots working under their direction, for their generosity and courtesy.

[3] The manuscript containing the results obtained in 1921 was submitted for publication before the 1922 results were available. On account of the delay in publication it has seemed desirable to include a few of the most important results obtained in 1922, although details are not given.

[4] The mechanical spore trap was devised by G. D. George, of the Office of Cereal Investigations and the University of Minnesota.

[5] The Air Service of the United States Army furnished the planes and pilots for the flights. The following men assisted in the work for the U. S. Department of Agriculture: E. B. Lambert, E. H. Ostrom, J. J. Christensen, D. L. Bailey, Wallace Butler, and S. P. Harter.

for the operator, seated in the passenger cockpit of the plane, to expose the slide by hand for the desired length of time and then return it to the slide box in which it had been carried. Another method consisted in placing inside of a bottle a microscope slide attached to a wooden paddle, the upper part of which extended through a hole in the cork of the bottle and served as a handle. After the slide had been exposed it was again placed in the bottle. This obviated the necessity of opening the slide box in order to remove or insert a slide, but was rather cumbersome, as it was necessary to carry as many bottles as there were slides to be exposed.

A third method was to expose the slides in a mechanical spore trap. Several types of trap were used, but one devised by Mr. George was the most satisfactory. This was made with six compartments, each containing a slide. It was fastened to the wing struts of the plane and was provided with a wire control which was operated by the observer in the cockpit. One pull on the wire opened the first compartment and exposed slide No. 1; a second pull closed this compartment and terminated the exposure of the slide. The other compartments were opened and closed in the same manner. (Pl. 2.) By this method slides could be exposed, one at a time, for the desired length of time, at various altitudes, and at widely separated points.

Flights were made in southern Texas during the latter part of April and during May, at St. Paul on June 25, and between Omaha, Nebr., and Cheyenne, Wyo., in July, 1921. The exposed slides were sent immediately in tight boxes to St. Paul, where spore counts were made.

RESULTS

Some typical results of exposing slides are summarized in Tables I and II. It is evident that there are many fungous spores at altitudes as high as 11,000 feet above the surface of the earth. In examining the slides special attention was given to rust spores. However, a great many spores of many other kinds of fungi, as well as conidiophores, numerous pollen grains, glumes of grasses, and some small insects also were caught on the slides.

It was practically impossible to identify many of the spores definitely. It was certain, however, that there were many spores of Puccinia, Alternaria, Helminthosporium, Cladosporium, Cephalothecium, Ustilago, Tilletia, and Scolecotrichum. (Pl. 1.) Spores of Alternaria were by far the most numerous, and not infrequently they were found adhering in characteristic chains. Chains of Cladosporium spores also often were found on the slides. On some of the slides there were large clumps of smut spores, there being approximately 60 in one of the largest clumps. Urediniospores were caught more commonly than any of the other spore forms of rust fungi, although teliospores were not uncommon, and, in 1922, aeciospores also were caught. On one slide there was a spore of what appeared to be *Ustilago zeae* with a promycelium and about 20 sporidia still attached. It is possible that this spore may have germinated in the vaseline. This seems quite unlikely, however, as there was no indication whatever that any of the other spores had germinated, and it seemed more probable, therefore, that the spore with its promycelium and attached sporidia was carried up into the air after it had germinated.

TABLE I.—*Results of exposing slides on airplanes*

Slide No.	Location.	Date.	Altitude (above ground).	Length of exposure.	Spores and pollen grains on slides.						
					Stem rust, uredinio-spores.	Leaf rust,[1] uredinio-spores.	Alternaria spores.	Helminthosporium spores.	Miscellaneous spores.	Pollen grains.	Total.
			Feet.	*Minutes.*							
1	San Antonio, Tex.	Apr. 27	1, 000	[2] 1	....	5	1	....	....	28	34
2	.....do	...do	3, 000	5	....	8	5	....	3	28	44
3	.....do	...do	3, 000	5	....	13	....	....	8	47	68
4	.....do	Apr. 28	4, 000	[2] 2	1	29	....	....	6	29	65
5	.....do	...do	Control.	.....	....	....	....	....	....	....	0
6	Waco, Tex., to San Antonio, Tex.	Apr. 29	500 to 1, 500	30	....	355	1	1	3	21	381
7	.....do	...do	1, 500 to 3, 000	30	....	335	3	1	23	38	400
8	.....do	...do	1, 500 to 3, 000	25	....	104	....	....	9	50	163
9	.....do	...do	1, 500 to 3, 000	25	....	70	....	....	8	144	222
10	.....do	Apr. 28	10, 000 to 12, 000	15	....	1	....	....	....	....	1
11	.....do	...do	12, 000 to 14, 000	15	....	....	....	....	1	....	1
12	.....do	...do	14, 000 to 15, 000	10	....	....	....	....	....	....	0
13	.....do	...do	15, 000 to 16, 000	10	....	....	....	....	....	....	0
14	.....do	...do	16, 000 to 16, 500	10	....	2	....	....	....	....	2
15	San Antonio, Tex.	May 2	1, 000	20	....	366	5	2	17	60	450
16	San Antonio, Tex., to Uvalde, Tex.	May 23	Control.	10	....	....	....	....	....	....	0
17	.....do	...do	1, 000	2	3	23	15	2	5	....	48
18	.....do	...do	1, 000 to 2, 000	10	28	33	13	....	14	5	93
19	.....do	...do	1, 000 to 2, 000	30	80	86	36	1	15	....	218
20	.....do	...do	2, 000 to 2, 500	20	16	32	1	1	25	....	75
21	.....do	...do	8, 000 to 9, 000	5	4	36	10	1	9	....	60
22	.....do	...do	9, 000 to 10, 000	5	....	9	19	....	5	....	33
23	.....do	...do	10, 000 to 11, 000	5	....	4	3	....	12	1	20
24	.....do	...do	11, 000 to 12, 000	5	2	7	1	....	....	....	10
25	San Antonio, Tex., to Cuero, Tex.	...do	Control.	10	....	....	....	....	....	....	0
26	.....do	...do	1, 000 to 2, 000	1	4	12	3	....	7	....	26
27	.....do	...do	3. 000	10	40	30	12	....	15	15	112
28	.....do	...do	3, 000	30	80	56	35.	3	25	4	203
29	.....do	...do	3, 000 to 6, 000	10	34	35	3	....	10	....	82
30	Omaha, Nebr.	July 13	2, 000	25	33	14	[3] ab	12	[4] n	15	....
31	Kearney, Nebr., to Gothenburg, Nebr.	...do	6, 000	25	4	3	34	3	14	12	70
32	North Platte, Nebr.	...do	Ground.	Over-night.	2	13	10	2	7	6	40
33	North Platte, Nebr., to Ogalalla, Nebr.	July 14	2, 500	25	19	36	23	2	9	22	111
34	Sidney, Nebr., to Pine Bluff, Wyo.	...do	7, 000	25	3	2	4	....	3	....	12
35	Pine Bluff, Wyo., to Cheyenne, Wyo.	...do	8, 200	30	1	....	5	....	....	....	6
36	Potter, Nebr., to Sunol, Nebr.	July 15	2, 500	20	1	1	19	....	18	1	40
37	Gothenburg, Nebr., to Elm Creek, Nebr.	...do	2, 000	25	126	34	ab	38	ab	68	....
38	Elm Creek, Nebr., to Wood River, Nebr.	...do	3, 500	25	101	28	ab	92	ab	17	....
39	Valparaiso, Nebr., to Omaha, Nebr.	...do	6, 000	35	15	1	n	8	n	1	....
40	Cheyenne, Wyo.	July 19	6, 500	5	32	17	n	4	n	12	....
41	Pine Bluff, Wyo.	...do	8, 000 to 9, 500	15	48	25	n	4	n	18	....

[1] *Puccinia triticina* and *P. coronata*.
[2] Exposed by hand.
[3] ab=Abundant (over 200).
[4] n=Numerous (100 to 200).

TABLE I.—*Results of exposing slides on airplanes*—Continued

Slide No.	Location.	Date.	Altitude (above ground.)	Length of exposure.	Spores and pollen grains on slides.						
					Stem rust, uredinio-spores.	Leaf rust,[1] uredinio-spores.	Alternaria spores.	Helminthosporium spores.	Miscellaneous spores.	Pollen grains.	Total.
			Feet.	*Minutes.*							
42	West of Pine Bluff, Wyo.	July 19	10, 000	5	44	20	[4] n	8	n	10	....
43	Grand Island, Nebr.	...do....	2, 000	10	[3] ab	n	ab	n	n	n	....
44	Big Springs, Nebr., to Sidney, Nebr.	July 22	3, 500	25	198	27	ab	....	ab	24	....
45	Sidney, Nebr., to Bushnell, Nebr.	...do....	5, 000	30	57	20	n	4	n	2	....
46	Lodgepole, Nebr., to Brule, Nebr.	July 23	4, 500 to 5, 000	30	7	11	n	2	n	....	....
47	Brule, Nebr., to North Platte, Nebr.	...do....	5, 000 to 7, 500	35	5	6	57	1	50	25	144
48	North Platte, Nebr.	...do....	Control.	.....	....	....	....	....	....	....	0
49	Curtiss Field, St. Paul, Minn.	June 25	500	2	2	2	....	....	....	....	4
50	.....do.....	...do....	1, 000	2	2	5	....	....	27	....	34
51	.....do.....	...do....	1, 500	2	3	8	....	....	1	....	12
52	.....do.....	...do....	2, 000	2	3	5	....	....	1	....	9

[1] *Puccinia triticina* and *P. coronata*. [3] ab=Abundant (over 200). [4] n=Numerous (100 to 200).

TABLE II.—*Results of exposing slides in a spore trap on the wing of an airplane and by hand from the cockpit for five minutes, at Fort Crook, Nebr., July 22, 1921*

Altitude.	Manner of exposure.	Spores and pollen grains on slides.					
		Stem rust, uredinio-spores.	Leaf rust, uredinio-spores.	Alternaria spores.	Helminthosporium spores.	Miscellaneous spores.	Pollen grains.
Feet.							
10, 500	In spore trap	0	13	21	0	5	2
10, 500	By hand	12	18	147	1	46	0
8, 000	In spore trap	15	2	90	5	65	7
8, 000	By hand	56	13	735	21	[1] ab	158
7, 000	In spore trap	14	1	65	5	48	1
7, 000	By hand	18	17	435	15	ab	36
6, 000	In spore trap	7	1	60	1	11	10
6, 000	By hand	6	15	ab	11	ab	50
3, 500	In spore trap	14	0	84	2	22	2
3, 500	By hand	14	0	ab	19	ab	11
2, 000	In spore trap	4	6	95	3	41	3
2, 000	By hand	32	35	ab	63	ab	17

[1] ab=Abundant (over 200).

TABLE III.—*Results of exposing slides, coated with vaseline or glycerine jelly, simultaneously by hand for 3 minutes on June 14, and vaseline-coated slides by hand for 10 minutes on July 5, from airplanes in the vicinity of a 40-acre tract containing large numbers of escaped common barberry bushes, near Waukegan, Ill.*

Slide No.	Date.	Altitude above ground.	Location.	Number of spores of *Puccinia graminis* on slides.			
				Aeciospores.		Urediniospores.	
				Vaseline.	Glycerine jelly.	Vaseline.	Glycerine jelly.
	1922.	*Feet.*					
1	June 14	100	Over bushes	4	6	0	0
3	...do.....	1,000	.....do	5	15	2	1
4	...do.....	2,000	.....do	6	6	1	2
5	...do.....	5,000	.....do	1	7	0	0
6	...do.....	7,000	.....do	3	0	0	2
7	...do.....	10,000	.....do	0	4	0	0
8	...do.....	12,000	.....do	0	1	0	0
10	...do.....	2,000	10 miles away	4	5	1	2
11	...do.....	2,000	15 miles away	2	2	0	1
12	...do.....	2,000	25 miles away	1	0	0	0
13	July 5	100	Over bushes	14	.....	5	.....
14	...do.....	100	.....do	8	.....	2	.....
15	...do.....	1,000	.....do	7	.....	4	.....
16	...do.....	1,000	.....do	10	.....	0	.....
17	...do.....	2,000	.....do	11	.....	4	.....
18	...do.....	2,000	.....do	9	.....	3	.....
19	...do.....	6,000	.....do	4	.....	3	.....
20	...do.....	6,000	.....do	7	.....	3	.....
21	...do.....	2,000	Over Fort Sheridan	0	.....	2	.....
22	...do.....	2,000	.....do	1	.....	1	.....

On a single one of the slides, exposed for five minutes at an altitude of 10,500 feet (about 2 miles), near Fort Crook, Nebr., 224 spores were caught. On a similar slide exposed for the same length of time at an altitude of 8,000 feet, 827 spores of known identity were caught, and in addition there were about 200 spores of unidentified forms, making the total about 1,000.

The highest altitude at which slides were exposed was 16,500 feet, or more than 3 miles above the surface of the earth. This slide was exposed between Waco and San Antonio, Tex., on April 28, 1921, and two urediniospores of what appeared to be *Puccinia triticina* were caught. It would not be safe, however, to conclude that spores normally occur at this height, as only a few flights were made at such extreme elevations. Spores are very numerous up to about 11,000 feet, but at higher altitudes they apparently are relatively scarce.

The number of spores caught per slide varied according to the method and place of exposure. Fewer spores were caught in the mechanical spore trap than on the slides which were exposed by hand. This probably was due to the fact that the spore trap was attached to the struts just above the upper surface of the lower wing, and it is quite probable that the air currents were deflected in such a manner as to miss the slides to some extent. By referring to Table II it will be seen that more spores were caught on slides which were exposed by hand from the cockpit than on those in the spore trap on the lower wing. In future work it probably would be well, therefore, to expose the slides on the fuselage or under the lower wing.

The real question, however, is not whether a great many spores are in the upper air, but whether they still are viable when they reach the surface of the earth. The difficulty of determining this conclusively is quite apparent. Germination tests were made, but some of them were inconclusive. The spores were embedded in vaseline, and when they were transferred to water they apparently did not imbibe much of it on account of the presence of the vaseline on the exospore. However, Alternaria spores which had been caught at 3,000, 4,000, 8,000, and 10,500 feet, respectively, germinated quite readily.

None of the urediniospores of *Puccinia graminis* germinated in 1921, but many of the tests were made too long after the spores were caught. In 1922, many more tests were made, and it was found that urediniospores germinated readily when caught at altitudes up to 7,000 feet. No tests were made on spores which had been caught at higher elevations. In one test, 64 per cent of the urediniospores germinated on a slide which had been exposed at 2,000 feet. The altitude at which the spores were caught apparently had little or no effect upon their viability. It seems quite likely, therefore, that urediniospores might be carried for long distances in the air and still retain their power to germinate. Germination tests were made of the spores on 24 different slides, and an average of 11.2 per cent of the urediniospores germinated. Considering the fact that the spores often were partially embedded in vaseline or glycerine jelly, a surprisingly large percentage germinated. A few æciospores which had been caught at an elevation of 1,000 feet also germinated. Many of the other kinds of spores also germinated very readily.

GENERAL DISCUSSION

The results of these preliminary experiments indicate that large numbers of spores and pollen grains are carried several thousand feet above the surface of the earth during the growing season. Probably they are carried long distances by the upper air currents, the direction and velocity of which are quite different from those near the surface. If the spores retain their viability, as some of them quite probably do, it is conceivable that a local epidemic might occur in one locality as a result of the blowing in of spores from an infection center in another distant locality. It would be particularly desirable to know more about the dissemination of the spores of those pathogens which often seem to be disseminated over wide areas in some almost miraculous manner, such for example, as *Phytophthora infestans* and various rust fungi.

Attempts were made in 1922 to get data regarding the spread of black stem rust. Slides were exposed on airplanes in Nebraska, Kansas, and Oklahoma, before stem rust had developed in those States but when it was already present in Texas. No spores were caught, however, until rust began to develop near the area in which flights were made. Flights were made from Fort Sill, Okla., toward Denison, Tex., when there was a considerable amount of rust near Denison but none in Oklahoma. It was quite clear that the number of spores in the air decreased rather rapidly as the distance from the rusted area increased. The distance to which spores may be carried undoubtedly depends on many factors, and no final conclusions can be drawn from a limited number of observations. Certainly aeciospores and urediniospores are carried up as high as 10,000 feet, and more, above the surface of the earth. Unless they are brought down by rain or some other agency,

they probably are blown long distances. Just how far they are blown and just how important they are in starting rust far from the place where they were produced can not be stated definitely. More observations will be made.

The airplane is a great aid in studying the distribution of spores of pathogenic fungi. It is likely to be very useful in epidemiology studies and it also may be useful in determining the value of establishing quarantine lines.

SUMMARY

(1) In the spring and summer of 1921 spore traps were exposed on airplanes at various altitudes and at several places in the Mississippi Valley.

(2) Many spores of several different genera of pathogenic fungi, conidiophores, pollen grains, glumes of grasses, and small insects were caught on the slides.

(3) Spores and pollen grains were relatively abundant at altitudes up to 11,000 feet. They were relatively scarce at higher altitudes, but two spores of what appeared to be *Puccinia triticina* were caught as high as 16,500 feet.

(4) Alternaria spores which were caught at altitudes of 10,500 feet and less were viable; urediniospores and aeciospores of *Puccinia graminis*, caught at elevations of 7,000 feet and 1,000 feet, respectively, also germinated.

(5) Airplanes probably will be useful in studying the dissemination of spores of many pathogenic fungi, and probably will aid in the solution of problems connected with the development of epidemics of plant diseases.

PLATE 1 [a]

Photomicrographs of spores caught on slides at various altitudes.

A.—Cluster of Ustilago chlamydospores (3,000 feet, near Wahoo, Nebr., July 16).

B.—Teliospore, *Tilletia tritici* spore, three rust urediniospores, and an Alternaria spore (1,500 feet, near Wahoo, Nebr., July 19).

C.—Helminthosporium spore (10,000 feet, Pine Bluff, Wyo., July 19).

D.—Chain of two Alternaria spores (1,000 feet, between North Platte and Gothenburg, Nebr., July 15).

E.—Alternaria spore, leaf rust urediniospore, and a stem rust urediniospore (2,000 feet, near Fort Crook, Nebr., July 22).

F.—Helminthosporium spore (1,500 feet, near Wahoo, Nebr., July 19).

G.—Urediniospore of stem rust, two Alternaria spores, and a Cladosporium spore (10,500 feet, near Fort Crook, Nebr., July 22).

H.—Cluster of rust urediniospores and one Alternaria spore (4,000 feet, between David City and Grand Island, Nebr., July 13).

I.—Alternaria spore and stem rust urediniospore (10,500 feet, near Fort Crook, Nebr., July 22).

J.—Two Alternaria spores and a Cladosporium spore (10,000 feet, near Pine Bluffs, Wyo., July 19).

K.—Scolecotrichum (?) spore (3,000 feet, near Wahoo, Nebr., July 16).

L.—Fusarium spore (10,500 feet, near Fort Crook, Nebr., July 22).

M.—Budding spores or sporidia, exact identity unknown (3,000 feet, between Meade and David City, Nebr., July 13).

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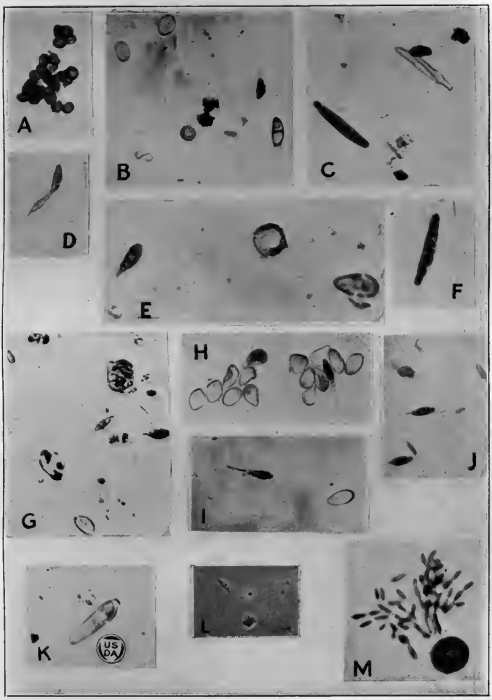
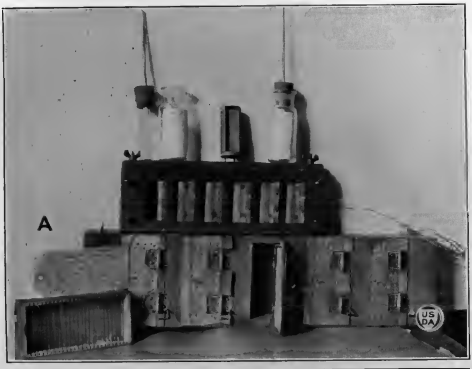
[a] Taken with the following combinations: A, K, and M, with Zeiss microscope No. 47303, 18 oc. and 8 mm. objective; E, with Leitz microscope No. 161086, 7.5 oc. and 6 mm. objective; remaining figures with Zeiss microscope No. 47303, 10 oc. and 8 mm. objective.

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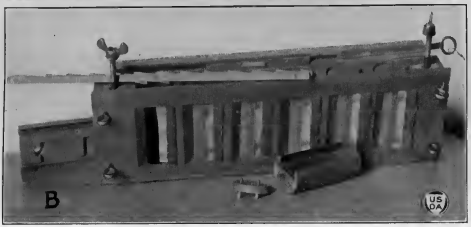
B


C

PLATE 2

A.—Types of spore traps used.
B.—Construction of George spore trap.
C.—View of George spore trap attached to plane.

STUDIES ON THE LIFE HISTORY OF STRIPE RUST, PUCCINIA GLUMARUM (SCHM.) ERIKSS. & HENN.[1]

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INTRODUCTION

Extensive study has been made in European countries on the seasonal occurrence of the so-called yellow rust, caused by *Puccinia glumarum* (Schm.) Erikss. & Henn. It has been held by a few investigators in Europe that the disease may be transmitted from year to year by means of infected grain and grass seeds. Very little research has been carried on to determine if this is possible. The possibility of transmission of this rust through infected seed has an especial significance in the United States, due to its limited geographic distribution. It has been found that stripe rust in the United States is limited in its distribution to the States of the Pacific Coast and Rocky Mountain regions. If the disease can be carried on the seed, grain shipped from the Western States into the Mississippi Valley might become a means of spreading the rust into the Spring Wheat Belt.

It has been demonstrated by various workers that this rust can overwinter in the uredinial stage in central and northern Europe. It seemed important to know if this was true in the United States. Accordingly an investigation was undertaken, in cooperation with the Department of Plant Pathology of the Oregon Agricultural Experiment Station, in 1917, to work out the life history of this fungus under western conditions. The investigation was transferred, in 1920, to Moscow, Idaho, in cooperation with the Department of Plant Pathology of the Idaho Agricultural Experiment Station. The conclusions herein reported are the results of these investigations.

LIFE HISTORY STUDIES

OVERWINTERING UREDINIA

The aecial stage of this rust is unknown. Eriksson and Henning (*3*) were unable to find an aecial host for the rust, although they carried on rather extensive cultural experiments. It has not been possible so far to work upon this phase of the problem in connection with the present investigations. There have been no indications noted in the field which would suggest that there is an aecial host for the rust in this country.

It has been generally concluded by various authors in other countries that *Puccinia glumarum* is able to overwinter in the uredinial stage. Biffen (*1*)[3] states:

> The uredospore stage seems sufficient to enable the fungus to tide itself over the winter, for it is possible to find pustules of rust on the foliage of selfsown wheat or some-

[1] Accepted for publication Aug. 21, 1922. These investigations were carried on in cooperation with the Oregon Agricultural Experiment Station and later the Idaho Agricultural Experiment Station under the direction of the Office of Cereal Investigations, United States Department of Agriculture.

[2] The author wishes to thank Prof. C. E. Owens for his hearty cooperation during the progress of this work, and also to acknowledge his indebtedness to Dr. H. B. Humphrey, Dr. A. G. Johnson, and Prof. H. P. Barss for helpful suggestions on the research and in the preparation of the manuscript.

[3] Reference is made by number (italic) to "Literature cited," p. 620.

times on the ordinary autumn-sown crop even in the depths of winter. The twisted leaves lying on the soil form a series of sheltered moist chambers on the inner surface of which the rust pustules are occasionally present in great numbers. These may develop with great rapidity in the early spring and at times as early as March the whole of the plant's foliage may be yellow with the rust. The winter's cold does not appear to injure these spores for they germinate readily when brought into the laboratory.

Hecke (*4*) concludes that *P. glumarum* can overwinter in Austria. He has observed the disease breaking out in the spring upon the old spots on the leaves which had been infected in the preceding autumn. In about two weeks this would be followed by a second generation appearing on the new growth of the plants. He concludes that the conditions necessary for an epidemic of yellow rust in any given year are a sufficient number of overwintering plants carrying mycelia and meteorological conditions favorable for its development in the spring.

Eriksson and Henning (*3*) conclude that *P. glumarum* may overwinter as mycelium in grain seedlings, and that this method is especially significant in years when there is a continuous covering of snow on the ground throughout the winter. They were doubtful if this means of overwintering is sufficient to carry the rust over from year to year. Henning (*5*) in a recent paper has reviewed the literature upon overwintering of this rust in Europe. He finds that the reports from various countries in central and northern Europe show that this rust can overwinter, at least when the host plants are protected, in the shape of uredinial mycelium. Such overwintering has been observed in Sweden by Eriksson, Henning, and Klebahn; in Denmark by Rostrup; in Austria by Hecke; in Bohemia by Baudys; in Russia by Treboux; and in Germany by Kirchner.

According to the author's observation there does not appear to be any doubt that the uredinial stage of stripe rust in either its urediniospore or mycelial condition can survive the winter in the mild climate of the coast region of the Pacific Northwest. When the winters are mild, it is possible to find fresh uredinia developing in any month from September to July in this region.

In the autumn of 1917 numerous varieties of wheat were sown at Corvallis, Oreg., in order to test them for resistance to stripe rust. A guard row of Chul wheat, which is very susceptible to stripe rust, was sown around the entire nursery in which these varieties were being grown. This entire border row was inoculated on November 21 and December 3 with urediniospores of *P. glumarum*. Infection spread continuously throughout the winter until by January 30 infection was quite general on all susceptible varieties of wheat adjacent to the inoculated plants. By March 1, all the susceptible varieties were heavily infected.

During the winter of 1917–18 a number of pots of the club wheat known as Dale (Dale Gloria), which is a susceptible variety, were grown in the greenhouse and inoculated with urediniospores of stripe rust until they were heavily infected. They were then placed out of doors in an exposed place for five weeks of the coldest weather during the winter. Snow fell three times during this period, and the plants, which were 6 to 8 inches tall, were killed down to half their length by the frost. In six days after they were again taken into the greenhouse they were covered with urediniospores on the portions of the leaves which had not been killed.

Observations also show that the fungus can overwinter readily on various wild grass hosts. In the fall of 1917 and also in 1918, observa-

tion stations were established near Corvallis, Oreg., with a number of wild grasses which were naturally infected with stripe rust. Plants of *Hordeum nodosum* L., *Bromus marginatus* Nees, and *Elymus glaucus* Buckl. were chosen for these observations. At no time during either winter was it impossible to collect viable urediniospores at any of these observation points. Viable urediniospores were collected upon *Elymus glaucus* in 1917–18 in every month from September, 1917, to July, 1918, inclusive.

There is some evidence also which indicates that the fungus may overwinter in the uredinial stage in the intermountain regions, between the Cascade and Rocky Mountain Ranges. Willis (*11*) reports that the rust may overwinter at Moscow, Idaho, on *Hordeum jubatum*. The author also obtained evidence from observations made in the spring of 1920, and again in 1921, that the uredinial stage of the fungus may survive the winter in northern Idaho. Plants of *Hordeum jubatum* and *Bromus marginatus*, which were heavily infected in the fall, developed fresh uredinia in both 1920 and 1921, soon after the snow melted in the spring. In the fall of 1921, various infected hosts in the grass nursery at Moscow, Idaho, and also other hosts located at various places near Moscow, were marked and were examined as soon as the snow melted in the spring. Snow covered these plants continuously from the last week in December until the middle of March. On April 3, 1922, several uredinial sori were found on an old leaf of a seedling of *Hordeum nodosum* growing beside a mature plant of the same species which was heavily infected in the fall. None of these sori had broken through the epidermis of the leaf. The spores were perfectly normal although immature. The appearance of the leaf seemed to indicate that infection had taken place in the fall and after remaining dormant through the winter had started to develop thus early in the spring. By April 15 a number of seedlings of this grass were found infected and producing urediniospores in abundance.

OVERSUMMERING UREDINIA

Observations made during the last five years indicate that along the Pacific coast of Washington and Oregon it is much more difficult for the rust to survive the dry summer months of July and August than to pass through the winter. The summers of 1917 and 1918 were exceedingly dry in the Willamette Valley of Oregon where observations were made during those two years. In 1917 there was no rainfall from June 8 until September 10, and in 1918 from June 5 until September 12. Needless to say, practically all of the wild grasses were completely dried up and very little rust infection of any kind was to be found by September.

Observations were made at Corvallis, Oreg., throughout the summer of 1917 upon *Elymus glaucus*, *Bromus marginatus*, and *Hordeum nodosum*, all of which hosts were heavily infected with stripe rust during the month of May. The *Elymus glaucus* plants were located on the banks of a small creek near the college farm. Urediniospores were produced in abundance upon these plants during May and the first half of June. Infection became gradually less until about July 20, after which no new urediniospores were produced until after the fall rains began. During this period, from July 20 to September 8, the *Elymus glaucus* plants made very little new growth, but a few leaves retained their green color. The old lesions or stripes showed plainly upon these half-dried leaves, but

no new spores were produced thereon. Soon after the fall rains began, however, uredinia began to appear at the ends of the old stripes where the mycelium had survived. The rust developed and spread rapidly until by October 15 an abundance of uredinial infection could be found upon the plants of this species in this locality. This same method of surviving the dry summer months was noted in the same place during the summer of 1918 (Pl. I), and upon *Hordeum nodosum*, *Bromus marginatus*, *Elymus glaucus*, *Elymus canadensis* L., and *Hordeum jubatum* in the rust nursery.

Waterhouse (*10*) has reported that in the hot dry summers of certain parts of Australia, *Puccinia triticina* Erikss. survives the dry period on volunteer wheat.

It seems evident, then, that one of the determining factors for an epidemic of stripe rust in any given year must be the weather conditions during the summer and fall of the previous year. If the precipitation during these months is insufficient to keep the grass hosts of *P. glumarum* and volunteer wheat more or less green in order to tide the fungus over this rather critical period, very little infection material will be present to start an epidemic upon winter wheat. It seems to the writer that the months from July to November in each year may well be considered the critical period for the fungus in regions where the winters are more or less mild. The meteorological conditions in the spring would need to be such as to favor the spread of the fungus which had survived the winter months. However, if fall-sown wheat becomes well infected in the fall, the early spring conditions are usually more or less favorable for subsequent spread. This, of course, would apply only to winter wheat. The weather conditions in the spring would undoubtedly be the determining factor in the infection of spring wheat.

RESISTANCE OF UREDINIOSPORES TO DRYING

It has been shown above that uredinial mycelium of stripe rust may survive the dry summer months in the tissues of the host. It seemed also important to learn the length of time in which urediniospores might remain viable under various conditions. Eriksson and Henning (*3*) found that the urediniospores of this rust were difficult to germinate unless they had been chilled to the freezing point or lower. The writer has had no difficulty in securing good germination of fresh urediniospores at any time. A number of trials were made to ascertain how long urediniospores would remain viable when kept at room temperature in herbarium packets. Table I gives the results of several of these trials.

TABLE I.—*Percentage of initial and final germination of urediniospores of stripe rust, collected from different hosts, kept in paper packets at room temperature*

Host.	Date collected.	Initial germination.	Date of final count.	Final germination.	Total storage.
		Per cent.		*Per cent.*	*Days.*
Wheat	Dec. 1, 1917	80	Jan. 12, 1918	1	43
Do	Nov. 27, 1917	90	Jan. 19, 1918	Trace.	56
Agropyron dasystachyum	Aug. 28, 1916	95	Oct. 24, 1916	1	58
Elymus condensatus	Sept. 5, 1916	85	.....do.....	Trace.	49

An experiment also was conducted to compare the relative resistance to desiccation of urediniospores of several rusts. *Puccinia graminis avenae* Erikss. & Henn. on oats, *Puccinia triticina* Erikss. on wheat, *Puccinia holcini* Erikss. on *Holcus lanatus* L. and *Puccinia glumarum tritici* Erikss. and Henn. on wheat, were chosen. Leaves of the several hosts, heavily infected with the uredinial stage of the rusts, were collected and several portions of the infected leaves were placed in each of a number of small glass vials which were then corked. Other infected leaves of each host were placed in three series of small paper herbarium packets.

The three series of specimens in the paper packets were exposed to different sets of conditions. The first series was placed on a shelf in the open laboratory; the second, out of doors on a window ledge on the north side of the laboratory, and the third on a shelf in a culture room. The temperature and moisture conditions in this culture room were a little more constant, and both temperature and relative humidity were a little higher, than in the open laboratory.

The glass vials containing infected leaves were divided into four groups. The first was placed on a shelf in the open laboratory, the second out of doors on a window ledge, the third in the culture room mentioned above, and the fourth in a small desiccator, the bottom of which was filled with water. This desiccator was kept in the culture room with the other collections. The vials in the desiccator were not corked. Table II gives the results of both these experiments.

TABLE II.—*Resistance of urediniospores of various rusts to desiccation when stored in paper packets and in glass vials, under different sets of conditions*

Kind of rust.	Where exposed.	How kept.	Date collected.	Date of final count.	Final germination.	Storage period.
					Per cent.	*Days.*
Puccinia graminis avenae.	Laboratory	Packets	Nov. 13, 1918	Feb. 4, 1919	0	82
Do	Window ledge	do	do	do	12	82
Do	Culture room	do	do	do	0	82
Do	Laboratory	Vials	do	do	0	82
Do	Window ledge	do	do	do	Trace.	82
Do	Culture room	do	do	do	0	82
Do	Desiccator	do	do	do	0	82
Puccinia glumarum tritici.	Laboratory	Packets	do	do	[1] 0	82
Do	Window ledge	do	do	do	[1] 0	82
Do	Culture room	do	do	do	[1] 0	82
Do	Laboratory	Vials	do	do	[1] 0	82
Do	Window ledge	do	do	do	[1] 0	82
Do	Culture room	do	do	do	[1] 0	82
Do	Desiccator	do	Dec. 3, 1918	do	Trace.	63
Puccinia triticina	Laboratory	Packets	Nov. 14, 1918	do	0	81
Do	Window ledge	do	do	do	5	81
Do	Culture room	do	do	do	0	81
Do	Laboratory	Vials	do	do	3	81
Do	Window ledge	do	do	do	0	81
Do	Culture room	do	do	do	0	81
Do	Desiccator	do	do	do	0	81
Puccinia holcini	Laboratory	Packets	do	do	3	81
Do	Window ledge	do	do	do	5	81
Do	Culture room	do	do	do	Trace.	81
Do	Laboratory	Vials	do	do	Trace.	81
Do	Window ledge	do	do	do	Trace.	81
Do	Culture room	do	do	do	Trace.	81

[1] Trace of germination in 20 days

On January 8, 1919, urediniospores of *P. glumarum tritici* were taken from wheat leaves and placed on open slides and left under a bell jar in a culture room in the laboratory. The bell jar was raised slightly in

order to allow circulation of air. The spores were tested at the beginning and at intervals until no germination occurred. The germination at the beginning was found to be 76 per cent, at the end of 3 days it was 65 per cent, in 5 days 50 per cent, in 10 days 35 per cent, in 13 days 15 per cent, in 16 days 10 per cent, in 20 days 1 per cent, in 23 days only a trace, while in 25 days no more germination could be secured.

No extensive work has been done to ascertain how long urediniospores of *P. glumarum tritici* will remain viable in the field during the winter months. It is possible, as indicated above, to find viable urediniospores at almost any time during an open winter on the Pacific slope. It has not yet been possible, however, to learn if this is the case in the intermountain regions, where the winters are much more severe. Mr. A. F. Thiel states in a personal letter that he found, in connection with his studies of overwintering of stem rust in Montana, that urediniospores of *P. glumarum* were less resistant to the weather conditions than those of *P. graminis tritici, P. graminis avenae, P. graminis phleipratensis* or *P. triticina.*

TIME OF NATURAL INFECTION

Stripe rust is very commonly found upon young seedlings and much has been made of this fact by the advocates of the theory of seed transmission of the disease. Experiments were arranged during the winter of 1917–18 to ascertain how early in its development a seedling might become infected. Seedling plants of Chul wheat were inoculated at various stages in their development, beginning as soon as the plumule emerged and continuing with different plants at intervals until the first leaf was fully expanded. No infection was secured in any case where the inoculation was made before the primary leaf had begun to expand. Twenty seedlings grown from seeds germinated in Petri dishes lined with filter paper were used in each case. When the primary leaf was well expanded, these were transferred to soil in pots.

The details of the experiment are as follows:

No. 1.—Urediniospores placed upon the plumule just as it began to appear. No infection resulted.

No. 2.—When the plumule was one-half inch long, no infection resulted.

No. 3.—When the plumule was 1 inch long, no infection.

No. 4.—When the primary leaf was just beginning to unfold from the sheath, but not well opened, 10 per cent infection.

No. 5.—When the primary leaf was well opened, 100 per cent infection.

Abundant infection has been noted upon the primary leaf of seedlings of *Bromus marginatus, Hordeum nodosum, H. murinum, H. jubatum* and *Sitanion jubatum* which came up in the rust nursery at Corvallis, Oreg., around old infected plants of the same species. (Pl. II, A.)

Uredinia appeared upon young seedlings of Chul wheat 21 days after it was sown on June 10, 1917, alongside of some winter wheat which was heavily infected with stripe rust. (Pl. II, B.) Some of the same lot of seed was sown some distance from any known infection. No stripe rust appeared on the plants from this seed until two weeks after it had appeared upon the seedlings mentioned above. This indicates that infection may occur under field conditions as soon as the primary leaf unfolds. It is very evident from observations made during the winter and early spring, that seedlings infected in the autumn may be a very

common means of carrying the rust over winter, at least on the Pacific Coast.

PERIOD OF INCUBATION

The length of the incubation period of the uredinial stage of various rusts does not appear to be the same. Parker (*8*) found that the incubation period of *Puccinia graminis avenae* Erikss. and Henn. and of *Puccinia lolii avenae* McAlpine, on oats, was about the same. He states, however, that generally, though not always, the uredinia of the stem rust appeared first. He took notes on the appearance of flecks on the seventh to the ninth day, and notes on the formation of uredinia in 12 days. He calls attention to the effect of temperature and light upon uredinia formation. Low temperatures were found to lengthen the period. Durrell and Parker (*2*) state that the incubation period for crown rust upon seedlings of a susceptible variety of oats averaged 7.8 days. On older plants of the same variety the incubation period averaged 9.5 days. Biffen (*1*) found that the incubation period of uredinia of *P. glumarum* on wheat was about 10 days. Marryat (7) states that pale yellow areas appear 6 days after inoculation of susceptible varieties of wheat with urediniospores of *P. glumarum*. Pustules begin to appear on the eleventh and are all open by the thirteenth day.

The author's observations in the greenhouse in connection with inoculation with urediniospores of *P. glumarum tritici* on wheat indicate that with optimum conditions for infection, namely, a susceptible host, fairly high temperature after the first 48 hours, good light, and vigorously growing seedling plants, infection begins to appear on the eighth or ninth day and is evidenced by lighter-colored areas on the inoculated portions of the leaves. Spores appear in abundance on the twelfth or thirteenth day. As observed by Parker (*8*), low temperature and lack of sunlight may materially lengthen this period. It was observed in 1918 in connection with inoculations which were made in the field at Corvallis, Oreg., late in the fall and early winter, that the incubation period at that time of year might be as long as 30 days. It seems possible that uredinial hyphae, after penetrating the host and becoming established, may lie dormant for a much longer time under conditions unfavorable to the development of the host and then again continue growth and the production of spores when growth of the host is resumed.

HEAD AND KERNEL INFECTION

A number of writers have called attention to the fact that both uredinial and telial infection of a number of rusts may be found upon the caryopses of various grains and grasses. The author (*6*) has summarized the literature regarding the occurrence of this phenomena and has called attention to the fact that kernels of wheat infected with *P. graminis tritici* are rather commonly found in wheat from badly rusted plants. The percentage of rusted kernels was not large, however, in any sample examined.

The development of both uredinia and telia of *P. glumarum tritici* upon all parts of the head and kernel of certain varieties of wheat has been found to be very common during severe rust epidemics. In many cases of severe infection it is impossible to find a place on the entire plant from the surface of the ground to the tips of the awns that is not

covered with rust sori. Head infection, which usually indicates that the kernels are more or less infected, is common only in certain varieties of wheat. Chul (C. I. No. 2406), Dale (Dale Gloria, C. I. No. 4231), Talimka (C. I. No. 2495), Baart (Early Baart, C. I. No. 1697), Little Club (C. I. No. 4219), Jones Fife (C. I. No. 3452), and a number of others, are very commonly infected in the head even when the rest of the plant may not be heavily infected. Many times, the inside of the glumes and the kernels of plants of the above-named varieties may be heavily infected without the development of open sori on the outer surface of the glumes. Except in cases of very severe infection, only a few of the spikelets on each head are infected.

If infection develops on the heads soon after they emerge from the boot, sterility of a large number of spikelets may result. (Pl. III, A.) The kernels which develop in such heads usually are very badly shriveled. (Pl. III, B, and Pl. IV, A.) It is easily possible to pick out infected heads even though there be no open sori on the surface of the glumes and awns. Such heads are much lighter in color, being a yellowish green, while the rest of the plant is a normal green. Plate IV, A, shows a number of detached glumes from heads of Chul wheat collected at threshing time. The inside of these glumes shows an abundance of stripe-rust telia.

Kernel infection by stripe rust is much more common in certain varieties of wheat than the author (*6*) found to be the case in wheat infected with stem rust. As high as 60 per cent of the kernels of certain varieties were found to be infected when grown in a rust nursery at Corvallis, Oreg., where a heavy infection of stripe rust was secured by artificial inoculation. Over 35 per cent of infected kernels were found in several samples of wheat grown under field conditions. It seems to the author that the amount of head infection, and consequently the amount of kernel infection, depends largely upon the climatic conditions at heading time, as well as upon the variety of wheat.

EFFECT OF KERNEL INFECTION UPON GERMINATION

The author has reported (*6*) that kernel infection by *P. graminis tritici* does not affect the germinating power of wheat to any appreciable extent. This has not been found true of wheat kernels infected with *P. glumarum tritici*. Numerous germination tests were made with several varieties of wheat which were badly infected with this rust. Parallel series of unrusted kernels from the same samples of seed also were germinated at the same time. The germination of the rusted seed averaged about 50 per cent of that of the unrusted seed. In some cases the germination was less than 25 per cent of that of the unrusted seed. By reference to Plate IV, B, it will be noted that infected kernels are very much shrunken. In many cases a cross section of an infected kernel revealed dozens of sori under the pericarp over practically the entire surface of the kernel. In the case of wheat kernels infected with *P. graminis tritici* the sori usually were confined to the hilar region and were present only in limited numbers.

SEED TRANSMISSION OF STRIPE-RUST INFECTION

HISTORICAL DISCUSSION

The author (*6*) has reviewed the principal literature upon the subject of rust transmission by seed grain and discussed the three theories put forth by various writers to explain the means by which such transmission may take place. These are the mycoplasm theory, the dormant mycelium theory, and the seed-borne-spore theory. The results of experiments carried on by various workers along this line have also been tabulated in the author's previous work (*6*) on this question. This review will not be repeated here. In this paper carefully controlled field and greenhouse experiments with wheat infected with *P. graminis tritici* were also conducted. The results of these studies led to the conclusion that stem rust is not transmitted from one wheat crop to the next by means of infected seed grain. Waterhouse (*9*), more recently, has carried on similar experiments with wheat kernels infected with stem rust which also gave negative results.

Certain European investigators have secured positive results in experiments carried on to determine if *P. glumarum* can be carried from year to year on seed grain. Because of the economic importance of this phase of the question in the United States in connection with the spread of the rust to regions where it is now unknown, experiments were undertaken with stripe rust both in the field and under carefully controlled greenhouse conditions.

EXPERIMENTAL DATA

A number of field observations have been made which at first seem to indicate very strongly that *P. glumarum* may be transmitted by means of seed. In the summer of 1917, among the increase plots of the Farm Crops Department at the Oregon Agricultural College, a plot of Jones (Winter) Fife was found to be heavily infected with stripe rust while none of the plots around it was infected to any extent. It was learned upon inquiry that the seed from which this plot was sown came from McMinnville, Oreg., and it was also learned that seed from the same lot sown at McMinnville produced plants that were not rusted. Upon close examination in the vicinity of the infected plots, numerous volunteer plants of Chul wheat were found which were heavily rusted. A plot of Chul wheat which was grown near this field the year before was known to have been infected with stripe rust. It seemed reasonable to suppose that the rust wintered over on these volunteer plants and infected the Jones Fife. No other very susceptible varieties were grown near this section of the field.

Another case in point which is not so easily explained was noted by Dr. A. G. Johnson, of the Office of Cereal Investigations, United States Department of Agriculture. Doctor Johnson found stripe rust on barley varieties in the nursery on the Belle Fourche Experiment Farm, at Newell, S. Dak., in August, 1917. These barleys were quite uniformly infected, and no other stripe rust was noted at Newell. The writer visited Newell in 1918, but was able to locate no stripe rust on or near the experiment station grounds. Some of the same seed from which these infected varieties had been grown was secured from Mr. John H. Martin, then of the Belle Fourche Farm, and sown in September, 1918. The plants from this seed were grown in the greenhouse at

Corvallis, Oreg., until they were headed but no rust appeared upon them. No rust infection could be found on this seed. Neither has the author been able at any time to locate any barley seed infected with *P. glumarum*. The work which is herein reported and the conclusions drawn relate to wheat only.

The investigations were along two lines: (1) Greenhouse experiments in which large numbers of rusted wheat kernels were sown under isolated conditions and the resulting plants watched for infection; (2) field experiments in which rusted wheat kernels were sown in the field and the resulting plants kept under observation to learn if infection occurred upon them sooner than upon plants grown from clean seed.

FIELD EXPERIMENTS

Rusted wheat seed has been sown for two years in comparison with clean seed and the resulting plants studied every few days for stripe-rust infection. The details and results of these experiments are given in tabular form, for each year separately.

In the autumn of 1917, rusted seed and chaff from rusted heads of several varieties of wheat were saved and sown in an isolated place on the college farm, near Corvallis, Oreg. The details of the experiment and the results secured are given in Table III.

These plots were examined carefully at frequent intervals in the fall and no stripe rust could be found on any of them at any time. In the spring the rust was slow to develop and did not spread rapidly, due to the extremely dry weather.

In 1919, a similar experiment was conducted under the same conditions. The details of the experiment and the results secured are given in Table IV.

As an additional precaution in securing seed which was known to be as free as possible from rust infection, all clean seed used was 1 year old.

The infection which appeared in the fall may have originated from some infected *Elymus glaucus* plants which were later found not far from these plots.

TABLE III.—*Development of rust on plants grown in the field at Corvallis, Oreg., in 1918, from rusted and unrusted seed sown on Oct. 10, 1917*

No.	Variety.	Condition of seed.	Number of rod rows.	Date rust first appeared.
1	Chul	Badly rusted seed and chaff	28	June 25, 1918
2	.....do	Portions of infected heads	3	June 20, 1918
3	.....do	Treated with modified hot-water treatment.	8	Do.
4	Talimka	Clean seed	2	June 22, 1918
5	Tigharia	Rusted kernels and chaff	3	June 25, 1918
6	Hansia Brooch	Rusted kernels	3	June 15, 1918
7	Popatia Nadiad	.....do	2	May 31, 1918
8	Jones Winter Fife	Clean seed	5	June 10, 1918
9	Chul	.....do	5	Do.

TABLE IV.—*Development of rust on plants grown in the field at Corvallis, Oreg., in 1919, from rusted and unrusted seed sown on Oct. 20, 1918*

No.	Variety.	Condition of seed	Number of rod rows.	First rust appeared.
1	Salt Lake Club	Slight infection	10	None until spring.
2	Chul	Heavy infection	10	Do.
3	Dale (Dale Gloria)	Clean seed	10	Do.
4	Baart (Early Baart)	do	10	Do.
5	Talimka	do	10	Do.
6	Dale	Medium infection	10	Do.
7	Federation	Clean seed	10	Do.
8	Chul	Heavy infection	10	Jan. 29, 1919.
9	Baart	Slight infection	10	Dec. 15, 1918.
10	Hansia Brooch	Heavy infection	10	Feb. 11, 1919.
11	Popatia Nadiad	do	10	None until spring.

GREENHOUSE EXPERIMENTS

In addition to the field experiments given above, greenhouse experiments have been carried on under controlled conditions. In order to grow a large number of wheat plants from seed infected with stripe rust, a portion of one of the greenhouses at the Oregon Agricultural College was equipped in the following manner: A partition was built to isolate completely one corner of the greenhouse; double doors were arranged in such a manner that anyone entering the isolated room could pass into a vestibule and close the first door before the second one was opened; the vestibule between the two doors always was sprayed with water before entering. A system of forced circulation of washed air was installed, as shown in figure 1.

The author (*6*) has reported upon this same system of air washing as it was used in connection with similar studies with wheat kernels infected with *P. graminis tritici.* Every precaution was taken to guard against any possible infection from outside. The room was made tight by using roofing cement to seal all cracks and openings in the sides and roof.

The first experiment was started on December 24, 1918. The seed used was for the most part from varieties grown in the rust nursery at Corvallis. It consisted of hand-picked kernels, all of which showed unmistakable evidence of stripe-rust infection. The varieties used were Hansia Brooch (C. I. No. 4690), Popatia Nadiad (C. I. No. 4696), Talimka (C. I. No. 2495), Baart (C. I. No. 1697), and Chul (C. I. No. 2406). In this, as in all other experiments of this series, temperature and humidity records were kept and it was found possible to maintain the temperature and relative humidity within the normal limits of plant growth. Flats filled with about 5 inches of sandy-loam soil were used for growing the plants. In all, 1,465 wheat plants were grown to maturity in this experiment, and no rust appeared on any of these plants at any time.

The second experiment was started on November 20, 1919. Wheat kernels were picked in the same manner as for the first experiment and the same methods were followed. Chul wheat (C. I. No. 2406) was used for the entire lot of plants in this experiment. The total number of plants grown was 2,470. No rust appeared at any time on any of the plants grown from this rust-infected seed and the experiment was discontinued when the wheat was mature.

In addition to the two experiments outlined above, 786 plants were grown from rusted seed in one of the rooms in the greenhouse without any special attempt to isolate the room to keep outside infection from the plants. These were grown to maturity during the winter months without the development of rust on any of them.

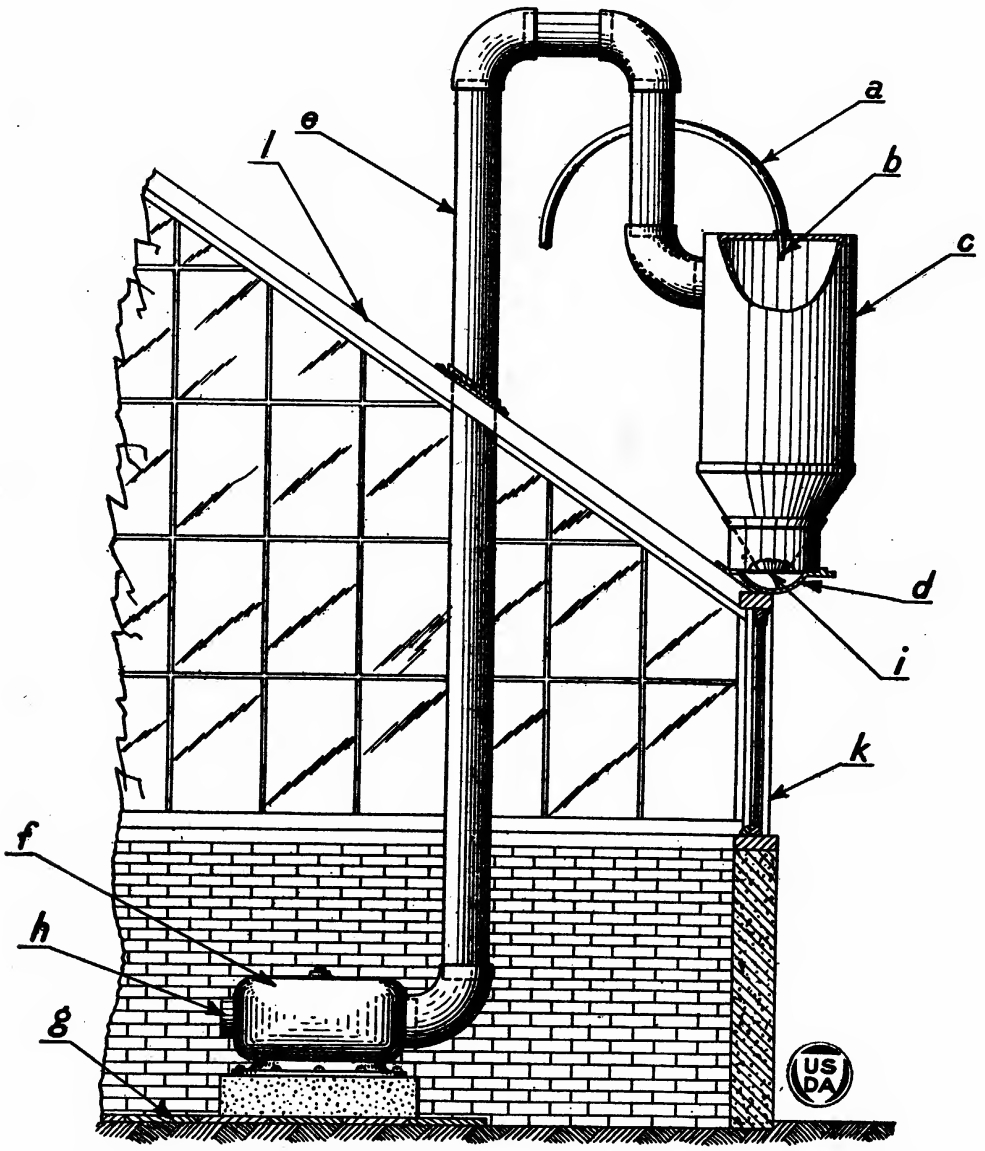

FIG. 1.—Diagram of air-washing apparatus for isolated room used for growing rust-infected seed: (*a*) Hose connection; (*b*) spray nozzle; (*c*) galvanized iron cylinder; (*d*) greenhouse gutter into which water from spray drained; (*e*) connection pipe from sprayer to blower; (*f*) electric blower; (*g*) floor of greenhouse; (*h*) mouth of blower where air entered the room; (*i*) air intake; (*k*) greenhouse wall; (*l*) greenhouse roof.

A number of writers have suggested the possibility of rust infection taking place from urediniospores on the surface of the seed or in the soil. To test this possibility, several flats were sown with wheat seed that had been covered with viable urediniospores of *P. glumarum tritici*. Chul wheat (C. I. No. 2406) was used for this experiment. About 300 plants were grown to maturity from seed thus treated. No rust infection appeared upon any of them at any time.

SUMMARY

(1) Observations show that stripe rust, caused by *Puccinia glumarum* (Schm.) Erikss. and Henn., may overwinter on the Pacific coast, both as mycelium and as urediniospores, on wheat and wild grasses. It was possible to collect viable urediniospores during every month from September, 1917, to July, 1918, at Corvallis, Oreg.

(2) Although the experiments which have been carried on are not absolutely conclusive, there is good evidence that the mycelium of stripe rust may overwinter at Moscow, Idaho, on *Hordeum jubatum* and *Bromus marginatus*.

(3) It has been found that the mycelium of stripe rust passes the dry summer months on the Pacific coast as dormant mycelium in the leaves of wild grasses. It is suggested that the climatic conditions which may prevail during the summer and fall of a given year determine whether or not an epidemic of stripe rust may develop upon winter wheat. The quantity of oversummering uredinia would be an important consideration in producing an epidemic on fall-sown wheat.

(4) Germination tests with urediniospores of stripe rust show that when the leaves of the infected host are kept in herbarium packets at ordinary room temperature the spores may remain viable at least 58 days. Urediniospores on leaves of wheat kept in open vials in a desiccator gave a slight percentage of germination at the end of 63 days. Urediniospores taken from wheat leaves, placed on glass slides and kept in a protected place in the laboratory, gave a trace of germination in 23 days.

(5) Urediniospores of *P. glumarum tritici* did not prove to be so resistant to desiccation as urediniospores of *P. graminis avenae*, *P. triticina*, or *P. holcini*.

(6) It has been shown that infection by urediniospores of stripe rust can not take place before the primary leaf of the wheat seedling has begun to expand.

(7) The period of incubation for the uredinial stage of stripe rust has been found to be 12 to 13 days under conditions favorable for infection. Low temperature and lack of sunlight may materially lengthen this period.

(8) The uredinia and telia of stripe rust occur commonly upon kernels of certain varieties of wheat. As high as 60 per cent of the kernels of certain varieties grown in a rust nursery were infected. Over 35 per cent of infected kernels have been found in wheat grown under ordinary field conditions.

(9) The germination of seed infected with stripe rust was only 50 per cent of that of the uninfected seed from the same seed lots.

(10) Forty rows, each 1 rod long, of rust-infected seed of several varieties of wheat were sown on October 10, 1917. No stripe rust developed on the plants from this seed before the next spring. Plants from clean seed sown at the same time became infected the next spring at about the same date as the plants from the infected seed.

(11) More than 4,700 plants were grown from rust-infected wheat seed in a specially constructed room in the pathological greenhouse at the Oregon Agricultural College, and no rust appeared upon any of them at any time.

(12) No infection appeared upon plants grown from wheat seed which had been covered with viable urediniospores of stripe rust before sowing.

(13) The results of the experiments here reported indicated that stripe rust can easily overwinter in the uredinial stage on the Pacific coast and

in the intermountain regions of the West. They further indicate that *P. glumarum tritici* is not transmitted from one wheat crop to the next by means of infected seed wheat.

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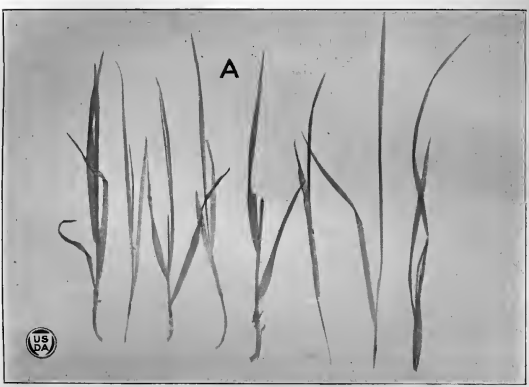
PLATE 1

Leaves of *Elymus glaucus* infected with *Puccinia glumarum*. These leaves were collected on September 20. Note telia on the stripes. A few uredinia are beginning to break through at x. This illustrates the method of oversummering on the host in the Pacific Coast region.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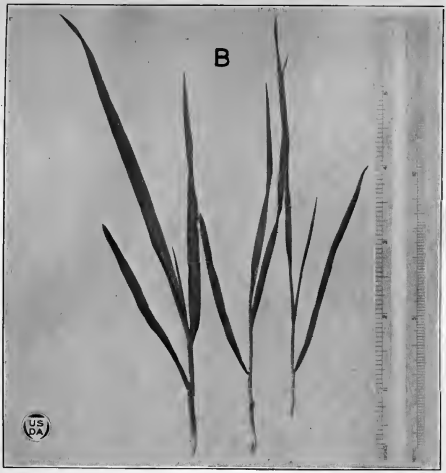

B

PLATE 2

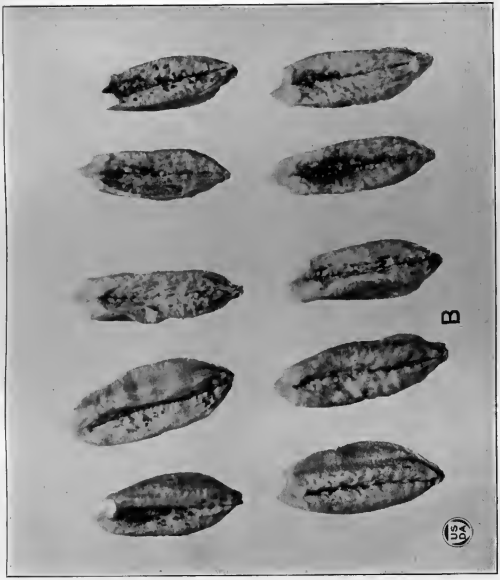
A.—Uredinia of stripe rust on seedlings of *Hordeum nodosum*. These plants became infected from older plants around which they were growing.

B.—Uredinia of stripe rust on seedlings of wheat which grew near older plants which were heavily infected. Seed from the same lot sown some distance from any infected plants produced seedlings free from the disease.

PLATE 3

A.—Two heads of Chul wheat grown in the greenhouse. The one on the right was inoculated with urediniospores of *P. glumarum tritici*. The one on the left was not inoculated. Infection just as severe has been noted to occur under natural conditions in the field.

B.—More or less shrunken kernels of Dale (Dale Gloria) club wheat showing both telia and uredinia of *P. glumarum tritici* (much enlarged).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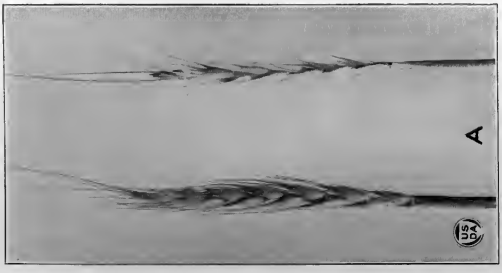
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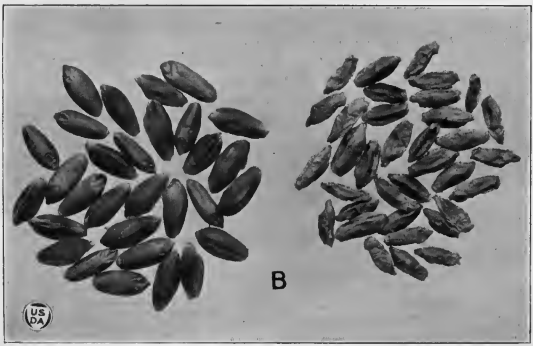
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PLATE 4

A.—Telia and uredinia of *P. glumarum tritici* on glumes of Chul wheat. This chaff was secured from straw after threshing. (Enlarged.)

B.—Two lots of Chul wheat. Those at the right are infected with stripe rust. Those at the left are healthy kernels from the same sample. (Enlarged.)

INFLUENCE OF SOME NITROGENOUS FERTILIZERS ON THE DEVELOPMENT OF CHLOROSIS IN RICE [1]

By L. G. WILLIS, *Chemist*, and J. O. CARRERO, *Assistant Chemist, Porto Rico Agricultural Experiment Station*

DEFICIENCY OF IRON AS A CAUSE OF CHLOROSIS

The chlorosis affecting certain species of green plants when they are grown on calcareous soils has been extensively studied by Gile (*6*)[2] and Gile and Carrero (*9*), who carried on the greater part of their experimental work with rice and with pineapples. They offered strong evidence to support their opinion that such chlorosis is caused primarily by the precipitation of iron in the soil by calcium carbonate and the consequent inability of the plant to obtain a sufficient supply of iron for the development of chlorophyll.

Mazé, Ruot, and Lemoigne (*16*) have recorded that in some of their cultures even the small quantity of 0.2 per cent of calcium carbonate was sufficient to render certain species of plants chlorotic by depriving them of enough iron for the formation of chlorophyll.

FACTORS GOVERNING THE AVAILABILITY OF IRON

REACTION OF THE CULTURE MEDIUM

Gile and Carrero (*8*) further showed that in some solution cultures of neutral or alkaline reaction rice became chlorotic, and that such condition was attributable to precipitation of iron as phosphate and hydroxid. In their more acid solution, which had a reaction equivalent to a P_H of 3.1, these investigators noted an apparently higher degree of availability of all forms of iron used. In all these solutions nitrogen was supplied in the form of nitrates.

Working more recently with wheat in sand culture with nitrogen furnished in the form of calcium nitrate, McCall and Haag (*18*) have shown that solutions having P_H values ranging from 4.02 to 7.00 produced chlorotic plants, the color of which was not restored by the addition of ferric nitrate to the culture. The addition of very small quantities of sulphuric acid, however, resulted in marked improvement in the color of the plants, which became normal green at harvest time. These investigators were of the opinion that the indirect evidence supported the assumption that chlorosis in their less acid cultures was due to lack of available iron or to faulty metabolism resulting from immobility of iron in the plant.

SOLUBLE PHOSPHATES

Factors in addition to the reaction of the culture medium are known to influence the development of chlorosis. Crone (*2*) advanced the hypothesis that soluble phosphates in solution cultures caused chlorosis of plants. Takeuchi (*23*), on the other hand, using wheat as a culture plant, showed that the chlorosis observed by Crone was probably due to the precipitation of iron as a phosphate in the culture medium. Later, Sidorine (*22*), working with corn, offered evidence to show that chlorosis,

[1] Accepted for publication July 11, 1922.
[2] Reference is made by number (italic) to "Literature cited," p. 639–640.

due to lack of assimilable iron for the plant, may be induced either by an excess alkalinity or by the presence of large quantities of soluble phosphates in the nutrient. It is not improbable, however, that chlorosis that is due to precipitation of iron as phosphate could be obviated by proper adjustment of the reaction of the medium, though the degree of acidity necessary to accomplish this would be greater than it would be were the iron precipitated as hyroxid.

EXCRETION OF SOLVENTS FROM PLANT ROOTS

The reaction of the mass of a medium is not, however, necessarily the critical condition governing the availability of iron. Mazé, Ruot, and Lemoigne (*16*) attributed the resistance to chlorosis of some of their culture plants that were grown in alkaline media to the ability of the plants to excrete organic acids from their roots. These acids formed complex molecular compounds of iron that were soluble in the presence of calcium carbonate, and thus made available sufficient iron for the needs of the plant.

The solvent action of root excretion on insoluble matter which was in contact with roots was recognized by Takeuchi (*23*), who recommended the agitation of the precipitate in solution cultures to prevent chlorosis resulting from lack of iron. Mazé (*15*), too, stated that a marked influence was exerted on the plants in his solution when the roots reached the bottoms of the flasks or when the solutions were agitated and the precipitate settled on the plant roots. These observations indicate, as one significant difference between the reactions of plants grown in solution cultures and of those grown in soils, that, in the first instance, contact between the insoluble nutrients is not maintained and the solvent action of the root excretions is dissipated, and that, in the second instance, the influence of such material, being localized, exerts its maximum effect.

The excretion from plant roots of organic acids other than carbonic acid has, however, never been satisfactorily demonstrated. The results obtained by Kossowitsch (*14*) show that the elimination of carbon dioxid is apparently a normal function of the roots of many, if not of all, species of plants. It does not, therefore, seem probable that the variation in the susceptibility to chlorosis of different species of plants can be due under normal conditions to the excretion of a specific solvent.

It may be that variations in the quantities of carbon dioxid which are evolved from the roots of plants of different species determine the susceptibility of each plant to chlorosis when the plant is grown in less acid media; and as the source of this compound must be the carbon that is assimilated through the agency of chlorophyll, the activity of which in turn is dependent upon an adequate supply of iron, it is possible that in chlorotic plants the intensity of chlorosis depends, to a large extent, upon the balance existing between the potency of the agencies depressing the solubility of iron in the soil and the solvent power of the carbon dioxid that is eliminated from the roots.

EFFECT OF UNASSIMILATED RESIDUES OF FERTILIZER SALTS

It was shown by Kossowitsch (*13*) and by Mazé (*15*) that there might be a modification of the reaction of a culture as a result of plant growth and that, when ammonium sulphate was used as the source of nitrogen, the utilization of the basic radicle resulted in the release of the acidic radicle which, rejected by the plant at its roots, tended to produce an

acid condition of the medium. Similarly, when nitrates were used, the reaction of the medium was changed but in the opposite direction.

The effect of these residues on the insoluble nutrients in the soil had been demonstrated by Shulov (*21*), who found that when sulphate of ammonia was used as a source of nitrogen the insoluble phosphates in the soil were rendered more available to plants than when nitrogen was supplied as sodium nitrate.

Mazé, Ruot, and Lemoigne (*17*), too, suggested that the chlorosis, which developed in corn with which they experimented, was caused by the precipitation of iron by calcium carbonate that was liberated in the culture solution when the plant assimilated the acidic radicle of a calcium salt.

More recently, Jones and Shive (*10*) have shown that, in solution cultures of wheat plants, the availability of ferric phosphate, as indicated by the susceptibility of the plants to chlorosis, was governed by the degree of acidity of the medium which increased or decreased as a result of plant growth according to whether ammonium sulphate or calcium nitrate was used as a source of nitrogen.

It is probably true that in a solution culture in which there is no close contact between the plant roots and the precipitated material the reaction of the entire medium must be sufficiently acid to render the iron soluble in order to prevent chlorosis, and that the hydrogen-ion concentration at the threshold of chlorosis is the critical reaction governing the availability of the form of iron in that particular culture. In the denser media, however, in which the roots are in contact with insoluble material and in which circulation of the nutrient solution is retarded and sedimentation of precipitated matter is prevented, there may be, as a consequence of plant growth and the rejection by the plant of nonassimilable compounds, a very significant modification of the composition and reaction of the nutrient, the extent and intensity of which it is not easy to determine. Under such conditions it is evident that only that part of the culture which is in contact with the plant roots is the true medium in which the plant grows, and that the reaction of the unassimilated residue of the nutrient salts may be of more significance than is the reaction of the mass of the medium.

EFFICIENCY OF NITRATES AND AMMONIUM SALTS AS NUTRIENTS FOR RICE

It has long been known that rice (*Oryza sativa*) differs from the majority of other economic plants in that it does not seem adapted to the utilization of nitrate nitrogen as a fertilizer when it is grown under swamp conditions. Ammonium salts, on the other hand, have generally been found to serve as a suitable source of nitrogen, but Daikuhara and Imaseki (*3*) have noted that the difference between the efficiencies of the two forms of nitrogen disappears when rice is grown on soil that is not submerged.

Many attempts have been made to explain this characteristic preference of the rice plant for ammonium nitrogen. Nagaoka (*19*) suggested that three factors were involved in interfering with the utilization of the nitrates: (1) Loss of nitrogen by denitrification; (2) the formation of poisonous nitrites in the process of denitrification; and (3) the absence in the plant of sufficient sugar to transform the nitric nitrogen absorbed into protein.

Daikuhara and Imaseki (*3*) showed that the last theory was untenable when they proved by analysis that the sugar contents of paddy rice and of upland rice were approximately equal. Their results, however, indi-

cated that there was a great tendency to loss of nitrate nitrogen from leaching and denitrification in the submerged soil. They also observed that plants which were treated with nitrate became pale yellow in the early period of growth but that they recovered later. This was attributed to the physiological effect of poisonous nitrites which were formed by the reducing action of certain bacteria.

Kelley (*11*), too, found that rice made a weak yellow growth in pots when it was supplied with nitrates as a source of nitrogen, but that the plants usually assumed a deep green color as they neared maturity and apparently made normal growth thereafter. In one series of sand cultures to which sodium nitrate was added, however, it was observed that although repeated plantings were made, the seedlings in every instance stood for some days, turned yellow, and died. These results were considered to indicate that nitrates were unable to nourish the young seedlings properly, but Kelley also attributed injury causing the rice plants to turn yellow to the action of nitrites in the culture. Perciabosco and Rosso (*20*), however, showed that nitrites in culture solution were absorbed by rice without evidence of injury.

More recent work by Trelease and Paulino (*25*) shows that ammonium salts are more efficient sources of nitrogen for rice than are the nitrates. The results of Kellner (*12*) and of Espino (*4*), however, indicate that, while young rice plants require ammonium nitrogen for normal development, the older plants utilize the nitrates as well as they do ammonium salts. Gile and Carrero (*8*), on the other hand, have reported results obtained by the use of culture solutions in which nitrogen was supplied in the form of sodium and potassium nitrates; and while they did not claim that theirs was an optimum solution for rice, they stated that when the solution was sufficiently acid to insure the availability of enough iron, the rice made normal growth and was equal in size to exceptionally large field-grown plants. These investigators also observed that the yield of plants might be diminished by a deficiency of iron which was not great enough to affect the color of the leaves.

EXPERIMENTAL DATA

On account of the effect which the unassimilated residues of the different forms of nitrogen have on the reaction of plant culture media and also because of the relation existing between the reaction of the medium and the appearance of chlorosis, it seemed advisable to reconsider the question of the comparative efficiency of nitrates and ammonium salts for rice. It was therefore decided to make a study especially to determine the relationship between the two classes of compounds and the chlorosis due to the unavailability of iron for the plant.

For the earlier investigations the soil chosen was an acid red clay which, in a preliminary test, had been found to be very deficient in nitrogen and phosphorus. Plants that were grown on this soil were apparently immune to chlorosis regardless of the quantity of lime that was added to alter the reaction. In the response of plants on this soil to different forms of nitrates and ammonium salts no differences attributable to the form of nitrogen used were noted. The results, however, were rendered of doubtful value on account of an abnormality which was apparently the straighthead disease of rice that is described by Tisdale and Jenkins (*24*).

Further tests were made in which there was used an alkaline sandy soil from a river bank having the following composition:

Analysis of moisture-free soil

Constituents.	Per cent.
Material insoluble in HCl (sp. gr. 1.115)	68.73
Ferric oxid (Fe_2O_3)	9.43
Aluminic oxid (Al_2O_3)	12.32
Manganese oxid (MnO)	.17
Lime (CaO)	2.02
Magnesia (MgO)	1.50
Potash (K_2O)	.20
Soda (Na_2O)	.38
Phosphorus pentoxid (P_2O_5)	.07
Sulphur trioxid (SO_3)	Trace.
Carbon dioxid (CO_2)	.37
Nitrogen (N)	.034
Loss on ignition	5.47
Total	100.29

Preparatory to being used, the soil was put through a process to crush the lumps, and it was then passed through a screen (4 meshes to the linear inch). After being thoroughly mixed, 45 pounds of the air-dried material were then weighed into each of the required number of glazed earthenware pots having a 5-gallon capacity. No provision was made for drainage.

All treatments were added in solutions with sufficient water to saturate the soil, and the pots were placed on cars permitting of the carriage to a screened inclosure for exposure during fair weather and to a glass house at night and during rains.

Seed of the Wataribune variety was used for experimental plantings, a strain of which had been carefully selected for several generations for this purpose. Sufficient seed was planted to furnish about three times as many plants as were required, and the excess seedlings were removed so that the most thrifty plants would be well distributed in the pot. Plantings were made immediately after the treatments were added, and the soils were kept wet to the point of saturation until the plants were about 2 inches high. The soils were then flooded and kept covered to a depth of 1 or 2 inches until the plants were cut.

In a comparison made of several compounds to determine the best source of phosphorus for the soil, orthophosphoric acid (H_3PO_4) was found to give satisfactory results. This form was, therefore, used in all the experimental work to obviate any interference by inert material which might mask the effect of other treatments.

EXPERIMENT I.—COMPARISON OF AMMONIUM SULPHATE AND SODIUM NITRATE TO DETERMINE THEIR INFLUENCE ON THE DEVELOPMENT OF CHLOROSIS IN RICE

Twenty-two pots of soil were given treatments of phosphoric acid and potassium sulphate to furnish each pot with 5 gm. of P_2O_5 and 2 gm. of K_2O. The nitrogen compounds were added in sufficient quantity to supply 1 and 2 gm. of nitrogen to the pots which received the lighter and the heavier treatments, respectively.

Of the pots which received each treatment, one-half were sprayed by means of an atomizer every two days with a 1 per cent solution of ferrous sulphate, preliminary tests having shown that ferrous sulphate was as efficient for spraying purposes as was ferric chlorid or ferric citrate. Five plants were selected from the seedlings to remain in each pot. Table I gives the plan of the experiment and the significant results obtained from it.

TABLE I.—*Chlorosis of rice on a calcareous soil as influenced by fertilization with sodium nitrate and ammonium sulphate*

SPRAYED WITH FERROUS SULPHATE

Pot No.	Nitrogen added as—		Analysis of solutions.							Weights of harvested plants.		Appearance of plants when cut.
			At planting.				At harvest.					
	$NaNO_3$	$(NH_4)_2SO_4$	Hydrogen-ion concentrations.	Parts per million of nitrogen present as—			Hydrogen-ion concentrations.	Parts per million of nitrogen present as—		Fresh.	Moisture-free.	
				NO_3	NO_2	NH_3		NO_3	NO_2	NH_3		
	Gm.	*Gm.*	P_H				P_H				*Gm.*	*Gm.*	
1	2		7.6	213	0.6	0.7	7.4	80	0.2	0.1	133	24.9	Yellow spotted with dark green.
2	2		7.8	288	.7	.7	8.1	80	.1	0	119	23.6	Do.
Average			7.7	251	.7	.7	7.8	80	.2	.1	126	24.3	
3		2	7.7	0	0	2.4	7.7	0	0	0	277	51.7	Dark green.
4		2	7.6	0	0	1.8	7.6	.6	0	.1	242	47.3	Do.
Average			7.7	0	0	2.1	7.7	.3	0	.1	260	49.5	
5	1		7.6	144	1.4	1.2	7.9	0	0	.1	110	23.8	Light green spotted with dark green.
6	1		7.6	163	1.4	.6	8.1	0	0	0	118	24.2	Do.
Average			7.6	154	1.4	.9	8.0	0	0	.1	114	24.0	
7		1	7.8	0	0	.3	8.1	0	.1	.1	(a)	(a)	
8		1	7.7	.2	0	1.5	7.9	0	.2	.1	171	34.6	Dark green.
Average			7.8	.1	0	.9	8.0	0	.2	.1	171	34.6	

NOT SPRAYED

9	2	.....	7.6	250	1.5	0	8.4	90	0	0	85	14.1	
10	2	.....	7.9	325	1.1	0	8.3	140	0	0	120	21.2	Yellow.
Average	.....	.....	7.8	288	1.3	0	8.4	115	0	0	103	17.7	
11	.....	2	7.8	0	0	.6	8.0	0	0	.1	320	59.5	
12	.....	2	7.9	.2	0	.4	8.0	0	0	0	247	47.7	Dark green.
Average	.....	.....	7.9	.1	0	.5	8.0	0	0	.1	284	53.6	
13	1	.....	7.7	150	1.3	0	7.9	0	0	0	128	25.3	
14	1	.....	7.8	125	1.5	0	7.9	0	0	.1	118	24.5	Light green.
Average	.....	.....	7.8	138	1.4	0	7.9	0	0	.1	123	24.9	
15	.....	1	7.9	0	0	1.2	7.9	0	0	.1	161	32.4	
16	.....	1	7.9	.1	.6	1.8	8.0	0	0	.1	145	30.6	Dark green.
Average	.....	.....	7.9	.1	.3	1.5	8.0	0	0	.1	153	31.5	
17	2	.....	7.9	225	1.2	.2	8.3	140	.3	1.0	(b)	(b)	
18	2	.....	7.8	250	1.2	0	8.3	240	.2	1.2	(b)	(b)	
Average	.....	.....	7.9	238	1.2	.1	8.3	190	.3	1.1	.....	.....	
19	.....	2	7.8	0	0	.5	8.3	.6	0	1.0	(b)	(b)	
20	.....	2	7.8	.2	.1	.6	8.4	1.2	0	1.6	(b)	(b)	
Average	.....	.....	7.8	.1	.1	.6	8.4	.9	0	1.3	.....	.....	

[a] Lost.
[b] Not planted.

The plants in this series of pots were cut while they were in the boot stage, it having been observed that those in soil cultures that had been rendered partly chlorotic by means of fertilizer treatment generally recovered their normal green color at about this period.

The results of this test show the influence on the development of chlorosis of the form of nitrogen used as a fertilizer and that the nitrate furnished was probably active in causing chlorosis, the effect being more marked on those pots receiving 2 gm. of nitrogen in that form than it was on those pots to which 1 gm. was applied. It would seem, therefore, that the calcareous soil was not the sole causative factor in similar cases of chlorosis, although this point has not been definitely established by the results obtained from the experiment.

That chlorosis was due to lack of sufficient iron for chlorophyll formation was evidenced by the development of a green color where the leaves were wet with the solution of ferrous sulphate. The nature of the leaf surface, however, was such that it was not possible to wet the entire area with the spray solution, and the plants therefore presented a mottled appearance. For this reason it is not certain whether the plants that were fertilized with sodium nitrate would have made a growth equal to that of the plants which received the ammonium sulphate had the spraying been more efficient as a remedy for chlorosis.

Observations made in this test do not show whether the plants which received sodium nitrate were chlorotic as a result of the form of nitrogen used or on account of the basic residue; whether the chlorosis, if caused by the basic residue, was due to the alkalinity of that residue or to the specific action of the sodium ion; nor whether there was a depression in plant growth additional to that caused by chlorosis.

A wide variation was observed in the size of the individual plants in the same pot and also in the tendency of plants of different sizes to recover from chlorosis. Apparently, absolute uniformity of cultural condition was not maintained in the experiment and the variations present governed the severity of the chlorosis.

Samples of the soil water for analysis were drawn from the mass of the soil by means of a pipette. The method of sampling was not ideal for the accurate determination of the composition of the nutrient solution, but it was chosen because it permitted the least disturbance of the plant roots. The determinations of hydrogen-ion concentration that were made by the colorimetric method of Clark and Lubs (*1*) are believed to be reasonably accurate, but the results showing the concentration of the different forms of nitrogen are only approximately correct.

The failure of the analysis to show the presence of appreciable quantities of ammonia is probably due to the absorption of that nutrient by the soil. Such removal of nitrogen from solution did not, it is thought, in any way interfere with the ability of the plant to absorb a sufficient amount for efficient utilization.

Evidence of the loss of some nitrogen through denitrification is shown by the decrease of nitrate nitrogen in the uncropped pots No. 33 and 35 during the period in which the plants were growing in the other pots. This loss, however, is thought to be of little significance in a comparison of the effects of the two forms of nitrogen.

The small quantity of nitrate nitrogen which accumulated in pots No. 34 and 36 as a result of the nitrification of ammonium sulphate may indicate that, as Fraps (*5*), Kelley (*11*), and Kellner (*12*), and others have shown, nitrification does not take place in submerged soils; or it

may indicate that denitrification processes were rapid enough to prevent the accumulation of considerable amounts of nitrate nitrogen. It is assumed, however, that in this experimental work the nitrification of ammonium sulphate was so greatly inhibited that the plants which were grown in pots treated with that compound derived the greater part of their nitrogen from the ammonium radicle. Nitrogen in the form of nitrite did not appear in significant quantities in any pot.

EXPERIMENT II.—A COMPARISON OF AMMONIUM SULPHATE AND AMMONIUM PHOSPHATE TO DETERMINE THEIR INFLUENCE ON THE DEVELOPMENT OF CHLOROSIS IN RICE

No direct method having been devised to eliminate the effect of the basic residues of the nitrate fertilizers in this soil, in order to test the effect of nitrate nitrogen on the development of chlorosis it was planned to determine whether or not resistance to chlorosis was characteristic of plants grown with ammonium compounds.

Thirty-four pots were prepared in the same manner as were those in experiment I. Potassium sulphate (K_2SO_4) was applied in sufficient amounts to furnish 2 gm. of potash (K_2O) to each pot, and phosphoric acid (H_3PO_4) and ammonium sulphate [$(NH_4)_2SO_4$] were used as sources of phosphorus and nitrogen in one-half of the number of pots, while monoammonium phosphate ($NH_4H_2PO_4$) was used to supply the same nutrient elements in the other half. Nitrogen in the amount of 1.25 gm. was added to each pot, and phosphorus was furnished in the amounts shown in Table II. The ammonium phosphate treatments were supplemented with ammonium sulphate to maintain the uniform quantity of nitrogen per pot. Plantings were made as in the former experiment, and five plants were selected from the seedlings to remain in each pot. The spraying was conducted as in experiment I. The results are given in Table II.

TABLE II.—*Chlorosis of rice on calcareous soils as influenced by fertilization with ammonium phosphate and ammonium sulphate*

Pot No.	Nitrogen added as—		Phosphoric acid added as—		Yield of dry matter.	Hydrogen-ion concentration of soil water at harvest.	Number of plants.	Appearance of plants.
	$NH_4H_2PO_4$.	$(NH_4)_2SO_4$.	$NH_4H_2PO_4$.	H_3PO_4.				
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Ph.*		
SPRAYED PLANTS CUT 42 DAYS AFTER PLANTING								
1	.....	1.25	.....	0.1	4.1	7.7	5	Green.
2	.....	1.25	.....	.5	6.0	7.7	5	Do.
3	.....	1.25	.....	1.0	9.6	7.7	5	Do.
4	.....	1.25	.....	2.5	9.7	7.7	5	Do.
5	0.02	1.23	0.1	.....	4.8	7.6	5	Yellow spotted with green.
6	.10	1.15	.5	.....	4.9	7.7	5	Do.
7	.20	1.05	1.0	.....	4.4	7.6	5	Do.
8	.50	.75	2.5	.....	2.4	7.6	5	Do.
UNSPRAYED PLANTS CUT 42 DAYS AFTER PLANTING								
9	.....	1.25	.....	0.1	2.9	7.5	5	Green.
10	.....	1.25	.....	.5	3.9	7.8	5	Do.
11	.....	1.25	.....	1.0	6.9	7.6	5	Do.
12	.....	1.25	.....	2.5	5.0	7.7	5	Do.
13	0.02	1.23	0.1	.....	1.3	7.7	5	Yellow.
14	.10	1.15	.5	.....	2.9	7.7	5	Do.
15	.20	1.05	1.0	.....	2.0	7.6	5	Do.
16	.50	.75	2.5	.....	.9	7.6	5	Do.
17	.....	1.25	.....	.....	4.0	7.7	5	Green.
SPRAYED PLANTS CUT 77 DAYS AFTER PLANTING								
18	.....	1.25	.....	0.1	60.1	7.2	5	Green.
19	.....	1.25	.....	.5	52.8	7.3	5	Do.
20	.....	1.25	.....	1.0	65.2	7.3	5	Do.
21	.....	1.25	.....	2.5	65.3	7.3	5	Do.
22	0.02	1.23	0.1	.....	51.0	7.3	5	Do.
23	.10	1.15	.5	.....	55.0	7.3	5	Striped.
24	.20	1.05	1.0	.....	60.6	7.3	5	Green.
25	.50	.75	2.5	.....	47.9	7.3	5	Do.
UNSPRAYED PLANTS CUT 77 DAYS AFTER PLANTING								
26	.....	1.25	.....	0.1	59.5	7.3	5	Green.
27	.....	1.25	.....	.5	46.0	7.4	5	Do.
28	.....	1.25	.....	1.0	53.5	7.4	5	Do.
29	.....	1.25	.....	2.5	57.5	7.5	5	Do.
30	0.02	1.23	0.1	.....	33.6	7.4	5	Yellow.
31	.10	1.15	.5	.....	27.9	7.3	5	Do.
32	.20	1.05	1.0	.....	9.5	7.7	1	Do.
33	.50	.75	2.5	.....	4.3	7.7	2	Do.
34	.....	1.25	.....	.....	54.6	7.5	5	Green.

In the original plan this test was to be conducted so that results could be obtained in duplicate, but 42 days after they were sown the plants under the influence of the different treatments showed such marked differences that it was deemed advisable to cut one-half the series in order that any tendency toward recovery might not deprive the writers of data showing the effect on plant growth of extensive chlorosis. At the time of the first cutting the plants in both pots in which the treatments had been duplicated were fairly uniform in size and appearance, so the probable error of the results obtained from the first cutting is small.

The weights of the plants that were cut when they were in bloom 72 days after planting are only approximately representative of the effect of the treatment because of the extreme variability in the size of the plants in many of the pots. In general, the variation was greatest in those pots showing the most severe cases of chlorosis, and it was apparently due to the unexplained difference in the tendency of individual plants toward recovery and normal growth. In pots No. 30 and 32 the chlorotic condition was so severe that some of the plants became diseased at the bud before they made any appreciable growth. The yields recorded for these pots represent only those plants which survived and made considerable growth after recovery.

As was true in experiment I, the effect of spraying with ferrous sulphate was only partly successful in overcoming chlorosis on account of the nature of the leaf surface. That either the iron applied was diffused throughout the leaf or that the effect of the partial development of green color was to aid the plant toward natural recovery was apparent soon after the first series of plants was cut when the spotted surface of the leaves of the remaining sprayed plants developed a striped appearance. By reference to Table II it may be seen that, regardless of the difference in fertilizer treatments, the plants that were sprayed were quite uniform in appearance, although the plants grown with ammonium phosphate were not equal in weight to those grown with ammonium sulphate.

The results obtained from experiment II show that the conditions causing the development of chlorosis were not necessarily induced by the presence of nitric nitrogen; nor were they traceable to the presence of an unassimilable basic radicle in the fertilizer salts used, in this case the indication being that chlorosis may have been caused by the precipitation of iron in the soil or in the plant by the phosphate ion liberated when the ammonium ion was assimilated at the more rapid rate.

The explanation is not definitely justified by the data at hand because it was not possible, owing to the small quantity of material available, to obtain analytical results showing the relative quantities of nitrogen and phosphorus assimilated. Moreover, it is not certain that in a calcareous soil ammonium phosphate would remain as such for any great length of time. The chlorosis which occurred in the presence of ammonium phosphate was much more severe than that which resulted from the use of sodium nitrate, but the plants grown with the former compound responded much better to spraying with ferrous sulphate and apparently tended toward a more complete recovery in the later stages of growth than did those grown with sodium nitrate.

EXPERIMENT III.—THE COMPARATIVE EFFICIENCY OF NITRATE AND AMMONIUM NITROGEN AS FERTILIZER FOR RICE WHEN THE INTERFERENCE OF CHLOROSIS IS DIMINISHED

In experiment I it was found (1) that the application of sodium nitrate to rice in a calcareous soil was attended by a chlorotic condition of the rice plant, and (2) that spraying with a solution of an iron salt was only partly effective in remedying the condition.

To determine, then, the comparative suitability of nitrate and ammonium nitrogen on this soil, an attempt was made to take advantage of certain individual rice plants which are less affected by the conditions causing chlorosis than are others, and also of a characteristic of the variety of rice used, the characteristic being that the rice tillers freely and produces culms and yields which are not closely dependent upon the maintenance of a definite number of plants of uniform size per unit area.

Eighteen pots were prepared and treatments were added in the same manner as they were in the previous tests, 2 gm. of nitrogen being furnished each pot in the forms of calcium nitrate, and ammonium sulphate. Calcium nitrate was chosen in preference to sodium nitrate to minimize, if possible, the intensity of the effect of the basic ion. Ammonium nitrate was used as a physiologically neutral form of nitrogen intermediate between the nitrates with basic residues and ammonium sulphate with acidic residues.

Potassium sulphate in sufficient quantities to furnish 2 gm. K_2O and phosphoric acid in quantities to supply 4 gm. P_2O_5 were added to each pot. Nitrogen treatments were replicated in six pots so that cuttings might be made at different stages of plant growth. It was planned to make the first cutting of plants representing each treatment at about the time that the plants which had received calcium nitrate were showing evidence of recovery from chlorosis, selecting for samples of the rice grown with calcium nitrate the plants in those pots in which chlorosis was most severe, so that those which were least affected and which might make the quickest recovery could be allowed to mature to afford a better comparison of the yields as governed by the efficiency of the different forms of nitrogen.

The second cutting was made when the heads were beginning to emerge from the boot, at which time all the plants in the test showed a remarkable uniformity in degree of maturity.

Seeding was made as in the other experiments. Ten seedlings were allowed to remain in each pot until the pots which had received calcium nitrate could be arranged in the order of their intensity of chlorosis. Two pots representing each treatment were then selected to be held for the first cutting, 10 plants being allowed to remain in each pot, and 4 seedlings were removed from each of the other six pots. Table III gives the results of the experiment.

TABLE III.—*Effect of nitrogenous fertilizers on the appearance and yield of rice plants at three stages of growth*

Pot No.	Form of nitrogen.	Age of plants when cut.	Original.				First stage.						Second stage.						Mature.					
			Analysis of soil water.				Analysis of soil water.				Yield of dry matter.	Appearance of plants.	Analysis of soil water.				Yield of dry matter.	Appearance of plants.	Analysis of soil water.				Yield of dry matter.		Appearance of plants.
			P_H.	P. p. m. nitrogen as—			P_H.	P. p. m. nitrogen as—					P_H.	P. p. m. nitrogen as—					P_H.	P. p. m. nitrogen as—			Straw.	Heads.	
				NO_3	NO_2	NH_3		NO_3	NO_2	NH_3				NO_3	NO_2	NH_3				NO_3	NO_2	NH_3			
		Days.									*Gm.*						*Gm.*						*Gm.*	*Gm.*	
1	$Ca(NO_3)_2$	40	7.4	240	0.1	0	7.9	32	0	0	7.1	Small; chlorotic.	7.9	3.5	0.1	0.7									
2	...do	40	7.4	216	.2	0	7.9	100	0.1	0	6.1	...do	7.9	2.5	.1	0									
3	...do	40	7.5	216	.1	0	8.0	40	.1	0	2.2	...do	7.8	0	0	.4									
Average			7.4	224	.1	0	7.9	57	.1	0	5.1		7.9	2.0	.1	.4									
4	$Ca(NO_3)_2$	93	7.4	264	.1	0	7.8	20	0	0		Chlorotic	7.8	.5	.2	0	39.2	Green; chlorotic.							
5	...do	93	7.4	240	.2	0.1	7.9	80	0	0		...do	8.1	0	.2	0	52.2								
Average			7.4	252	.2	.1	7.9	50	0	0		...do	8.0	.3	.2	0	45.7								
6	$Ca(NO_3)_2$	114	7.4	264	.1	0	7.9	26	0	0		Large; chlorotic.	7.5	0	0	.5		Normal	8.1	1.2			53	57	Normal.
7	...do	114	7.4	252	.1	0	7.9	34	0	0			7.6	0	0	.5		...do	7.8				58	59	Do.
8	...do	114	7.4	240	.1	0	7.7	30	0	0			7.8	.8	.2	.6		...do	8.0				36	54	Do.
Average			7.4	252	.1	0	7.8	30	0	0		...do	7.6	.3	.1	.5			8.0	.4			49	57	
9	NH_4NO_3	40	7.5	130	.2	0	8.1	0	0	0	14.3	Normal	7.6	0	.2	0									
10	...do	40	7.5	80	.1	.1	7.9	0	0	0	15.5	...do	7.9	2.5	.1	0									
Average			7.5	105	.2	.1	8.0	0	0	0	14.9		7.8	1.3	.2	0									
11	NH_4NO_3	93	7.5	120	.2	0	7.8	0	0	0		Normal	7.8	0	0	.3	62.2	Normal							
12	...do	93	7.5	130	.1	0	8.0	0	0	0		...do	8.2	0	0	.4	56.9	...do							
Average			7.5	125	.2	0	7.9	0	0	0			8.0	0	0	.4	59.6	...do							
13	NH_4NO_3	114	7.5	130	.1	0	7.8	0	0	0		Normal	7.7	0	.1	.4		Normal	8.1	.5			59	61	Normal.
14	...do	114	7.5	140	.1	.2	7.9	0	0	0		...do	7.8	0	0	.7		...do	7.8	.4			57	57	Do.
15	...do	114	7.5	120	.3	.1	7.9	0	0	0		...do	7.8	0	0	.7		...do	7.9	1.5			49	49	Do.
16	...do	114	7.5	130	.1	0	7.9	0	0	0		...do	7.8	0	0	.7		...do	8.0				38	38	Do.
Average			7.5	130	.2	.1	7.9	0	0	0			7.8	0	0	.6			8.0	.6			51	51	

TABLE III.—*Effect of nitrogenous fertilizers on the appearance and yield of rice plants at three stages of growth*—Continued

Pot No.	Form of nitrogen.	Age of plants when cut.	Original.				First stage.						Second stage.						Mature.							
			Analysis of soil water.				Analysis of soil water.				Yield of dry matter.	Appearance of plants.	Analysis of soil water.				Yield of dry matter.	Appearance of plants.	Analysis of soil water.				Yield of dry matter.		Appearance of plants.
			PH.	P. p. m. nitrogen as—			PH.	P. p. m. nitrogen as—					PH.	P. p. m. nitrogen as—					PH.	P. p. m. nitrogen as—			Straw.	Heads.	
				NO_3	NO_2	NH_3		NO_3	NO_2	NH_3				NO_3	NO_2	NH_3				NO_3	NO_2	NH_3			
		Days.								*Gm.*						*Gm.*							*Gm.*	*Gm.*	
17	$(NH_4)_2SO_4$.	40	7.5	0.4	0	0	8.0	0	0	0	23.0	Normal	8.1	0	0.1	0									
18	...do	40	7.5	1.4	.1	.1	8.2	0	0	0	23.2	...do	7.7	7.0	0	0									
Average			7.5	.9	.1	.1	8.1	0	0	0	23.1		7.9	3.5	.1	0									
19	$(NH_4)_2SO_4$.	93	7.4	0	0	0	8.1	0	0	0		Normal	7.9	0	0	0	67.5	Normal							
20	...do	93	7.4	0	0	0	7.9	0	0	0		...do	7.9	0	0	0	67.0	...do							
Average			7.4	0	0	0	8.0	0	0	0			7.9	0	0	0	67.3								
21	$(NH_4)_2SO_4$.	114	7.5	.4	0	0	8.0	0	0	0		Normal	7.9	0	0	.7		Normal	7.8	1.3			49	50	Normal.
22	...do	114	7.5	1.6	.1	0	7.9	0	0	0		...do	7.9	0	0	.7		...do	7.7	1.0			57	59	Do.
23	...do	114	7.5	0	.1	.2	7.9	0	0	0		...do	7.5	0	0	.5		...do	8.0	.8			55	56	Do.
24	...do	114	7.5	0	0	0	7.9	0	0	0		...do	7.5	0	0	.8		...do	8.0				61	55	Do.
Average			7.5	.5	.1	.1	7.9	0	0	0			7.7	0		.7			7.9	.8			56	55	

In this test the rice that was fertilized with calcium nitrate was more or less chlorotic in all pots until after the first cutting was made. The plants that were furnished with ammonium nitrate were chlorotic for only the first few days of their growth. Plants that were fertilized with ammonium sulphate were not chlorotic at any stage of development.

There was a wide variation in the degree of chlorosis of the different plants which had been made chlorotic as a result of fertilizer treatment. It was therefore found advisable in the first cutting to include with the two pots treated with calcium nitrate a third pot in which the chlorosis was so severe that it was doubtful whether the plants would survive for a later cutting. At the time of the second cutting some of the plants in pots No. 4 and 5, which had been given calcium nitrate, had become green, and the others were only partly chlorotic. The plants from each of these pots were therefore divided according to their color into two samples to be weighed and analyzed.

As was found in experiment I, ammonia nitrogen was apparently almost completely absorbed by the soil very soon after the addition of the ammonium sulphate, and nitrites were not found in significant quantities at any time. This may have been due to the nonproduction of the latter form of nitrogen, to its rapid assimilation by the plants, or to conversion to other forms of such amounts as were produced.

The concentration of nitrates in the soil water had decreased considerably at the time of the first cutting, and when the second samples were taken the waters were nearly free of nitrates—a phenomenon which was coincident with the recovery of a majority of the plants from chlorosis and which may have been indirectly a contributory factor influencing recovery.

In regard to the average yield of mature heads per pot, the pots which were treated with calcium nitrate were practically equal to those which received ammonium sulphate, while the yield from the pots to which ammonium nitrate had been added was somewhat lower than that made by the others. The wide differences in yields from individual pots that were treated with ammonium nitrate may indicate, however, that the yield was influenced by some detrimental factors which did not cause other visible symptoms of injury; and it is possible that the relative inefficiency of the form of nitrogen used was not the cause of the low average yield.

The basis of selection of the pots in which the soil had been treated with calcium nitrate and from which the plants were to be harvested at maturity is open to criticism. It is thought, however, that the three pots in which the plants made rapid and complete recovery from chlorosis are comparable with the same number of pots of any other treatment, and that they are, for purposes of determining the influence of the fertilizer on the yield, more truly representative of the treatment than would have been an average which included some chlorotic plants. With this point in view it is evident that, under the conditions of this experiment, the nitrates, as represented by calcium nitrate, served for the nutrition of the rice plant as suitably as did ammonium sulphate when the factors causing chlorosis were inoperative.

ANALYTICAL DATA

As to the cause of chlorosis in the series of pots which received calcium nitrate, some interesting analytical data showing some of the differences between plants of the first and second cutting in experiment III and between green and chlorotic plants of the same cutting are given in Table IV.

TABLE IV.—*Composition of rice plants as influenced by nitrogenous fertilizers*

40 DAYS AFTER PLANTING

Pot No.	Dry matter per pot.	Increment dry matter per pot.	Nitrogen.	Nitrogen in increment.	Iron.	Iron in increment.	Ash.	Ash in increment.	Silica.	Silica in increment.	Silica-free ash.	Silica-free ash in increment.	Iron in ash.	Iron in increment of ash.	Iron in silica-free ash.	Iron in increment of silica-free ash.
	Gm.	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	7.1	.....	3.54	.....	0.0223	.....	19.05	.....	11.78	.....	7.27	.....	0.1171	.....	0.3067	.....
2	6.1	.....	3.59	.....	.0175	.....	21.10	.....	13.36	.....	7.74	.....	.0829	.....	.2262	.....
3	2.2	.....	.....	.....	.0333	.....	26.72	.....	19.84	.....	6.88	.....	.1246	.....	.4840	.....
Average	5.1	.....	3.56	.....	.0220	.....	20.96	.....	13.55	.....	7.40	.....	.1049	.....	.2973	.....
9	14.3	.....	3.33	.....	.0294	.....	17.48	.....	9.85	.....	7.63	.....	.1680	.....	.3853	.....
10	15.5	.....	3.38	.....	.0342	.....	17.45	.....	9.77	.....	7.68	.....	.1960	.....	.4453	.....
Average	14.9	.....	3.36	.....	.0319	.....	17.46	.....	9.81	.....	7.66	.....	.1827	.....	.4162	.....
17	23.0	.....	2.90	.....	.0331	.....	17.13	.....	8.61	.....	8.52	.....	.1932	.....	.3885	.....
18	23.2	.....	3.09	.....	.0345	.....	17.05	.....	9.00	.....	8.05	.....	.2023	.....	.4286	.....
Average	23.1	.....	3.00	.....	.0338	.....	17.09	.....	8.81	.....	8.28	.....	.1978	.....	.4082	.....

93 DAYS AFTER PLANTING

4	[a] 28.4	.....	0.81	.....	0.0190	.....	14.43	.....	8.74	.....	5.69	.....	0.1317	.....	0.3339	.....
4	[b] 10.8	.....	.81	.....	.0184	.....	18.68	.....	13.10	.....	5.58	.....	.0987	.....	.3297	.....
5	[a] 48.8	.....	.86	.....	.0161	.....	13.00	.....	7.67	.....	5.33	.....	.1238	.....	.3021	.....
5	[b] 3.4	.....	.95	.....	.0208	.....	22.57	.....	15.63	.....	6.94	.....	.0922	.....	.2997	.....
Average	45.7	40.6	.84	0.36	.0174	0.0169	14.47	13.66	8.94	8.36	5.53	5.30	.1202	0.1237	.3146	0.3190
11	62.2	.....	.79	.....	.0128	.....	14.93	.....	10.26	.....	4.67	.....	.0857	.....	.2941	.....
12	48.6	.....	.93	.....	.0110	.....	15.14	.....	10.63	.....	4.51	.....	.0726	.....	.2439	.....
Average	55.4	40.6	.85	[c]	.0120	.0047	15.02	14.09	10.42	10.62	4.60	3.47	.0799	.0334	.2609	.1356
19	67.5	.....	.90	.....	.0138	.....	15.02	.....	10.17	.....	4.85	.....	.0919	.....	.2846	.....
20	67.7	.....	.84	.....	.0127	.....	14.71	.....	10.03	.....	4.68	.....	.0863	.....	.2714	.....
Average	67.6	44.5	.87	[c]	.0132	.0025	14.86	13.70	10.10	10.77	4.76	2.93	.0888	.0184	.2773	.0854

[a] Green plants. [b] Chlorotic plants. [c] Loss.

The samples analyzed represent the entire plant above ground. The dry matter produced under the influence of each treatment was found to vary considerably, especially in those pots which received calcium nitrate, and the results of the analyses are therefore weighted in proportion to the amount of dry matter produced in the respective pot for the calculation of the average.

ANALYTICAL METHODS

The analytical methods used were essentially those prescribed by the Association of Official Agricultural Chemists.[3]

Nitrogen was determined by the Gunning method, sodium sulphate being used in place of potassium sulphate. In order to determine the ash and its constituents, the material was charred over a low flame, the sample was weighed, leached with dilute hydrochloric acid, and then filtered and washed on a tarred Gooch crucible having a pad of ash-free filter paper. The insoluble residue consisting of silica and carbon was dried and weighed and the carbon was burned off. The ash percentages were calculated from the weight of the original incinerated material by subtracting therefrom the loss of weight due to the ignition of the carbon.

Carbon dioxid in the ash was not determined on account of the small quantity of material available. The quantity of silica reported is the sum of the weights of insoluble silica and of that in the hydrochloric acid extract which was obtained by evaporation and dehydration. Iron was determined by permanganate titration after it was reduced with zinc and by the Reinhardt method. The results obtained by each method were in agreement.

DISCUSSION OF RESULTS OF ANALYSIS

Table IV discloses some results which may have a positive significance in showing the influences causing the chlorosis in experiment III. Table III shows that the nitrates had practically disappeared from the soil water at the time of the second cutting, and Table IV shows that a marked decrease in the percentage of nitrogen in the plant occurred during the period between the first and second cutting, those plants which had received nitrogen as calcium nitrate having gained 0.36 per cent of nitrogen in the increment of dry matter, while the plants in the other two series showed a loss of total nitrogen.

If, therefore, the production of basic residues by the assimilation of the nitric fertilizer salts produced in the soil a condition that prevented the absorption of iron, or in the plant a condition which prevented the utilization of iron for the development of chlorophyll, the influence must have been removed before the plants in experiment III were ready for the second cutting, and at a time that was approximately coincident with the recovery of the plant from chlorosis.

A comparison of the average results of the iron determinations indicates that, in those plants which received calcium nitrate, the first cutting of the plants had less iron in the dry matter, in the ash, and in the silica-free ash than was found in the same components of the green plants grown with the other treatments. In the second cutting the chlorotic plants had the higher content of iron in the dry matter; and while the

[3] WILEY, H. W., ed. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. As compiled by the committee on revision of methods. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.), 272 p., 13 fig., 1908. Reprinted 1912.

percentage of iron in the ash was higher in the green plants, this was largely due to a high proportion of silica in the ash of the chlorotic plants. The percentage of iron in the silica-free ash was about the same in the green and in the chlorotic plants.

Such observations would indicate that the chlorosis in these pots was not due to a deficiency of iron, but Gile and Ageton (7) explained a similar result in their work as possibly being due to the fact that the tissues of chlorotic plants represent an earlier period of growth than do the tissues of green plants of the same age and that they therefore require a greater quantity of iron to prevent chlorosis. It would not be impossible, however, for iron in a chlorotic plant, though present in sufficient quantity, to be rendered inactive for the development of chlorophyll by abnormal conditions in the plant.

It is shown that the green plants had an ash content which was less than that of the chlorotic plants, but the high ash content of the latter may have been due to the depression of carbon assimilation and to a consequent small quantity of carbonaceous matter in the plant. The high silica content of the chlorotic plants can best be explained in the same way, and variations in the amount of this constituent in the plant ash are apparently of no importance.

In regard to the silica-free ash, the plants of all treatments had a general uniformity of compositions at the same stage of growth but gave some evidence that the use of calcium nitrate as a fertilizer depressed the absorption of this constituent up to the time of the second cutting. It is hardly possible, however, that the silica-free ash content of the plant can be considered as indicative of the way in which nitrate fertilizers cause the development of chlorosis, because the analysis of plants in the second cutting from the nitrate pots showed that there was no consistent difference between the chlorotic and the green plants in this regard.

As was shown of the nitrogen content, the rate of absorption of the ash constituents, with the exception of silica, tended toward diminution as the plant passed through the stage in which it began to recover from the condition causing chlorosis. It does not appear, however, that the analytical results presented offer an evidence that variation in the ash content of the plant at different periods of growth has any bearing on chlorosis.

SUMMARY

The development of chlorosis of rice on calcareous soils may be governed by the nature of the nutrients supplied, those compounds represented by sodium nitrate, calcium nitrate, and ammonium phosphate, which, in themselves or by virtue of an unassimilable ion, are the cause of the precipitation of iron, being associated with chlorosis, while the plants which have been supplied with ammonium sulphate in which the unassimilable radicle may serve as a solvent for iron may be thrifty and of normal color.

It has not been shown by the experimental methods used whether the basic material in the soil or the basic residues of the nitrates were primarily the cause of the conditions determining the appearance of chlorosis, nor whether the acidic residue of ammonium sulphate served to prevent chlorosis in the calcareous soil. Inasmuch, however, as it was shown in the first experiment that the degree of chlorosis attending the use of sodium nitrate as a nutrient was in proportion to the quantity

of that salt furnished, it seems possible that the basic residues of the nitrates were primarily the cause of the chlorosis and that the alkalinity of the soil was a secondary factor.

The reaction of the soil in which the plant roots are in contact with the insoluble supply of iron is shown to be of less significance in determining the availability of iron for the plant than are the effects of the products rejected by the plant roots.

Chlorosis of rice grown on the soil used was especially severe in young plants, and plants which were not too seriously affected showed a tendency to recover as they approached maturity. This phenomenon of recovery was coincident with a decrease in the rate of absorption of nitrogenous compounds, a fact which substantiates the evidence that the nitrate fertilizers were causative of the chlorotic condition, although it is possible that an increase in the elimination of carbon dioxid from the roots had some influence in aiding the plant to recover its normal color by making more iron available in the soil.

The observation that nitrates are less suitable than ammonium salts for the fertilization of young rice plants may be based on the influence on the plants at the period of greatest absorption of nitrogen of the unassimilated residues of the fertilizer used rather than on the inferiority of nitric nitrogen as a nutrient. It seems probable also that the injury which has heretofore been ascribed to the toxic effect of the nitrites, derived from nitrates by reduction, was but a manifestation of chlorosis which was caused by the action of the basic residues of the nitrate salts used as nutrients.

The nitrogen of calcium nitrate may be suitable equally with that of ammonium sulphate in the physiological processes of the rice plant when it is used under cultural conditions in which the reaction of the unassimilated residue of the nitrate does not interfere with the absorption and utilization of iron.

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SOME GRAMINICOLOUS SPECIES OF HELMINTHOSPORIUM: I [1]

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INTRODUCTION

Although the genus Helminthosporium includes a large number of forms thriving saprophytically on the bark, leaves, and stems of both woody and herbaceous plants, it has become familiar to plant pathologists and perhaps, in a large measure, to students of fungi generally, through a relatively moderate number of parasitic species. Undoubtedly the most widely known of these parasitic forms are those affecting graminaceous hosts, as considerable losses to important cereal crops, including especially barley, corn, rice, oats, wheat, and sorghum, in various parts of the world, have continued for several decades to encourage a desire for knowledge leading to some sort of effective control. Besides these economically important forms, many parasitic species of Helminthosporium have been recorded as thriving on various members of the grass family, but have remained more or less obscure because either the hosts affected were of little economic value, or, being important, the parasitism occasioned little or no observable damage.

However, as is not uncommon in the case of large genera, publication of an increasing number of descriptions of presumably new species, thriving on related or even identical hosts, has injected a large degree of uncertainty into the specific taxonomy. In many instances, writers have failed to compare their organisms with congeneric forms, or have used for such comparison herbarium material which had already undergone the degenerative changes in structure incident to the death of the spores. It was in an effort to define the more distinctive differences between the forms parasitic on barley and oats, and those found on a few of the more common uncultivated or wild grasses that the present study was undertaken. This paper, which it is hoped may be followed by others dealing with the very considerable variety of species of Helminthosporium growing on grasses in the United States, is offered as a comparative mycological account of some of the more readily available species. No attempt is made here to deal with the intimate pathological aspects, as these have been for some years the subject of intensive study by other workers, both in this country and abroad.

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[2] In the work reported in the present paper, the writer received help from a number of investigators not mentioned in the text. Acknowledgments are due especially to Mrs. Agnes Chase and Dr. A. S. Hitchcock for the identification of various grasses; to Dr. Theodor Holm for help in the preparation of the manuscript; to Dr. C. S. Gager for permission to use the facilities of the Brooklyn Botanic Garden, where the studies were in large part carried out; and to Dr. A. G. Johnson of the Office of Cereal Investigations for helpful suggestions and criticisms.

APPEARANCE OF AFFECTED PLANTS

Although the symptoms produced by a species of Helminthosporium on some particular host are generally quite well defined, the different members of the genus considered collectively bring about a considerable variety of changes. These may be briefly considered under a number of categories.

SPOTBLOTCH, FOOTROT, EYESPOT

Perhaps the most easily recognized type of lesion is represented by the discoloration resulting from the attack, for example, of *Helminthosporium sativum* P. K. & B. on barley (Hordeum spp.), wheat (Triticum spp.), and quack grass (*Agropyron repens* [L.] Beauv.). Here each foliar infection produces a fairly well-defined, more or less longitudinal spot ranging in color from light brown to nearly black. An entirely similar type of leaf injury is characteristic of the diseases of Kentucky bluegrass (*Poa pratensis* L.) and of barnyard grass (*Echinochloa crus-galli* [L.] Beauv.), attributable to two congeneric parasites that are newly described in this paper as *H. vagans* and *H. monoceras*, respectively. When the leaf sheaths also are affected, the discoloration often becomes increasingly diffuse downward, so that the base of the stem may be quite uniformly discolored—a condition that in the case of wheat, where it is usually complicated with more or less injury to the roots, has become widely known as footrot. Similar dark foliar spots characterize the incipient attack of *H. leersii* Atk. on white grass (*Leersia virginica* Willd.), of *H. giganteum* H. & W. on goose grass (*Eleusine indica* [L.] Gaertn.), and Bermuda grass (*Cynodon dactylon* L.), and of *H. oryzae* B. de H. on rice (*Oryza sativa* L.). Later, however, the central areas lose their dark color, the older and larger spots being finally represented by a dark brown ring surrounding a central straw-colored area. The term "eyespot," which has been applied (*73*)[3] to a disease of sugar cane (*Saccharum officinarum* L.) caused by *H. sacchari* Butl., is perhaps most accurately descriptive of the latter type of foliar lesions.

NETBLOTCH

A second category of discoloration very characteristic, though less common, is caused by *Helminthosporium teres* Sacc. on barley, as well as by a fungus on *Festuca elatior* L. described in this paper as *H. dictyoides*. The affected leaves, while still green and living, show abundant brownish discoloration in irregular pattern, within which may be recognized a network of darker longitudinal and transverse linear streaks. With the withering of the leaf these reticulate markings become less pronounced, and are finally more or less completely obliterated.

STRIPE

Helminthosporium gramineum Rabh. causes an unusual type of injury to barley, the leaves previous to heading time becoming variegated with yellow bands extending frequently the whole length of the leaf. On the premature death of the plants the foliar organs split along these longitudinal markings, giving them a ragged or shredded appearance. This type of injury has been shown to be contingent on continued development of the parasite in the growing tissues in a manner not unlike the development of certain smut fungi.

[3] Reference is made by number (italic) to "Literature cited" pp. 731–739.

WHITE BLAST

Helminthosporium turcicum Pass. produces on maize (*Zea mays* L.) a type of injury that is probably more common than might be supposed, as it is likely to escape detection. The green color of the affected tissue disappears completely, leaving a chlorotic area that increases in size until it may be several inches long and perhaps one inch in width. Owing to the large size of the corn leaf blade, the desiccated areas, which may be surrounded, moreover, by a narrow, brownish, marginal zone, contrast sharply with the surrounding green tissue and are quite readily recognizable as due to the agency of a parasite. However, with the larger number of graminaceous hosts, having much smaller leaves, proportionately large segments of the blade are involved at once; withering usually starts at the tip and proceeds downward, thus simulating the appearance of withering due to drought. It may be mentioned that the parasitic nature of species of Helminthosporium not associated with dark discoloration or conspicuous pathological changes in the mechanical properties of the plant tissues involved manifestly can not be ascertained definitely by observation alone. *Helminthosporium dematioideum* Bub. and Wrob. on the leaves of sweet vernal grass (*Anthoxanthum odoratum* L.) may be cited as an example of a considerable number of fungi, the relation of which to their host or substratum is certainly not as obvious as might be desired.

OTHER TYPES OF INJURY

Differing quite markedly from all of these forms of injury is that caused by a species of Helminthosporium attacking young plants of a species of Paspalum, provisionally identified as *Paspalum boscianum* Fluegge, which will be further discussed as *H. micropus*. The first evidence of infection appears as a water-soaked area, the tissue of which has lost all its rigidity. The condition suggests an injury such as might have been brought about by the application of a few drops of boiling water. The affected area frequently dries and shrivels, often leaving the surrounding tissue quite normal; or if the affected spot is large enough to interrupt the vascular communications the more distal portion of the leaf blade may gradually wilt without any further advance of the fungus.

In the attack of *Helminthosporium ravenelii* Curtis on *Sporobolus indicus* (L.) R. Br. it is quite impossible to notice either wilting or discoloration. The fructifications of the fungus grow directly out of the inflorescence in a dense, dark, brownish green, velvety layer, the latter being so conspicuous and abundant as to have suggested the term "smut grass" as common name for the host. The infection undoubtedly is altogether local, for even when the larger portion of the panicle has been overgrown with the fungus, the exposed parts present an appearance not greatly different from that of an entirely healthy inflorescence. The fungus discussed in this paper as *H. oryzae*, when developing on the inflorescence of rice, shows an approach to *H. revenelii* in the crowded habit of its sporophores.

TAXONOMIC CHARACTERISTICS

The definition of the genus Helminthosporium Link (*85*) as recognized in the large works of Saccardo (*128, v. 4, p. 402*) and Lindau (*83*) has been very generally adopted by mycologists. The genera Brachysporium

Sacc. (*128, v. 4, p. 403*) and Napicladium Thüm. (*150*) are distinguished from Helminthosporium, the former in possessing relatively short spores, the latter in having spores tapering toward the apex. Naturally, these distinctions are based on no fundamental differences, and may readily be supposed to have been advanced primarily to serve in dividing in some way a large group of organisms into a number of smaller groups. As it is not always possible to determine definitely whether the spores of a certain species are in the main to be regarded as longish, or short, or tapering, the advantage accruing from erection of these genera on artificial distinction has been at least partly counteracted by the confusion occasioned by different writers assigning the same form to different genera. Indeed, certain of the forms included in the present paper have been assigned by some writers to one or the other of the genera allied to Helminthosporium, and a few other forms might perhaps be thus assigned with equal cogency.

The imperfect fructifications characteristic of the genus Helminthosporium consist of sporophores emerging from the substratum singly or in clusters, or more rarely arising as a dense, velvety layer. After attaining a certain length and becoming usually one-to-several-septate, depending somewhat on the species, a spore is developed at the tip. After the latter has reached a certain degree of maturity the sporophore continues to grow near the point of attachment of the first, pushing aside the first spore, and producing another spore a little farther on. A number of spores are thus produced, which, with the exception of the last one, usually fall off or are blown off in nature, their places of attachment being marked by a series of geniculations bearing a dark scar at the apices. In most foliicolous species, the sporophores as collected in the field show branching in only relatively infrequent instances, although a few forms exhibit a more readily demonstrable tendency toward ramification. In *H. ravenelii* branching of the sporophore is, however, very abundant and accounts in a measure for the dense, velvety texture of the crust. Length, width, color, frequence of septation, and general habit of the sporophore, while of considerable value in the diagnosis of some species and consequently always to be given consideration in specific descriptions, in general leave much to be desired in distinctiveness.

The mode of emergence from the epidermis of the host is usually not especially characteristic. In the case of those grasses having small leaves provided with a relatively delicate epidermis emergence usually takes place in an altogether miscellaneous manner, either from the stomata or between epidermal cells. In the case of those grasses of which the epidermis is mechanically somewhat stronger, as in barley or barnyard grass, the sporophores, especially in the beginning before the leaf tissue becomes distorted by shriveling, show a tendency to emerge from the stomata, although emergence between epidermal cells is never uncommon. The only instance observed by the writer where the sporophores appear to be entirely confined to the stomata is that of *Helminthosporium turcicum* on corn leaves, the localization here being readily attributable to the character of the epidermis, which in this host is of unusual mechanical strength, the stomata on the other hand being large and uniformly distributed. Sporophores may appear singly, or in clusters ranging in numbers from 2 to 5, 6, or even 7. Small clusters usually are proliferated nearly simultaneously. However, where the number runs up to half a dozen, some of the sporophores are usually younger,

having been developed after the first two or three had perhaps commenced to proliferate spores.

By far the most distinctive characteristics for taxonomic purposes are to be found in the spore. Within the genus these structures show an unusual range of difference with regard to size. Those of *Helminthosporium giganteum*, for example, which are among the largest reproductive bodies produced by any group of fungi, exceed those of the smaller species like *H. dematioideum* about twice in width and ten times in length, although the latter can not be regarded as especially small. The length of the spore, particularly in the case of those species in which this body is nearly colorless and straight cylindrical like *H. tritici-repentis* Died. *H. bromi* Died., *H. teres*, and *H. giganteum*, is subject to great variation, the length of the smaller ones often scarcely exceeding their width. For this reason the magnitudes approaching the maximum are to be regarded as the more characteristic of the species. The width of the spores of any particular species on the other hand is much more apt to be nearly uniform, regardless of considerable differences in length.

With respect to coloration, the spores represented in the genus exhibit all gradations between the subhyaline condition present, for example, in *Helminthosporium giganteum* and *H. tritici-repentis*, to the dark olivaceous hue characteristic of *H. sativum* and a considerable number of closely related congeneric forms. Most of the species are intermediate between these extremes—yellowish, yellowish brown, and brown. A few species have spores with subhyaline end cells, all the other cells being uniformly dark; or the middle cells may be dark, with the color becoming perceptibly fainter toward the ends.

The spores of most forms of Helminthosporium are characterized by some peculiarity in shape by which they can be often recognized with a high degree of certainty. The general contour may be straight cylindrical as in *H. avenae* Eidam, curved ellipsoidal as in *H. sativum*, or tapering toward the tip as in *H. dictyoides*. The basal end is usually most characteristic. In *H. teres*, the proximal cell is hemispherical; in *H. bromi*, hemi-ellipsoidal; in *H. tritici-repentis*, elongate conical with rounded apex, thus crudely suggesting in outline the top or bottom aspect of a snake's head. In *H. monoceras*, the basal half tapers gradually toward the basal end, which is abruptly rounded off; in *H. turcicum* the tapering is more abrupt and continues to the insertion of the hilum. The same is true in *H. micropus* except that here the extreme basal end is drawn out somewhat, the contour being slightly concave near the hilum. In this connection, it may be stated that the hilum—that is, the more or less calloused region at the base of the spore, marking the place of attachment to the sporophore—may be represented by a conspicuously protruding modification as in the two species last mentioned; or, as is much more frequently true, it is visible as a dark spot situated altogether within the contour of the spore wall. In a few species the hilum is not at all conspicuous, as in *H. giganteum*, where it appears as a small wedge-shaped basal prolongation, with a delicate single-contoured confining membrane.

As a good deal of the taxonomic literature based on dead herbarium material gives a wrong impression of the structure of the spore, particularly with reference to its walls and septa, a few remarks may be justified here. The developing spore remains nonseptate during the earlier stages of growth, usually until its definitive size has been largely

attained. Cross walls then appear, the first formed segments being further reduced in size by later successive divisions. Although at the time a segment has just undergone division, the appearance under the microscope is that of a thin line extending across the spore, the protoplasts thus delimited soon round up along the zones where cross walls are in contact with the external wall. The latter in the hyaline-spored forms seems to undergo no readily perceptible increase in thickness; in the dark-spored forms the subsequent apparent increase in thickness is quite considerable. In the process of maturation the individual segments appear to deposit a membrane of their own, the matured spore thus consisting of the original outer spore wall, inclosing a row of more or less independent segments, like peas in a pod. In some species, as for example, *Helminthosporium leersii*, where the outer wall is relatively delicate, these segments may be removed readily from their enveloping membrane by tapping or gently pressing on the cover glass. The process often results in no injury at all, each segment being capable of germinating independently and promptly.

The mode of germination characteristic of a species usually bears a more or less apparent relation, both to the shape of the spore and the structure of its wall. In most of the forms with cylindrical hyaline spores, where the peripheral wall is everywhere uniformly thin, germ tubes are produced more or less indiscriminately from all segments, basal, apical, and intermediate. In the species with tapering spores, as *H. dictyoides*, for example, one germ tube usually arises from the distal cell, and one or two from the larger basal cell, the intermediate segments rarely participating directly in the process. The germ tubes are not polar in position, but arise from undifferentiated regions usually laterally or obliquely at some distance from the hilum or the extreme apex. The fully matured spores of *H. sativum* and closely related species have two perceptibly thin places in the peripheral wall, these being located at the tip and at the base of the spore, in the latter case occupying a narrow zone adjacent to and surrounding the hilum. These areas may be clearly seen, for example, in *H. monoceras*. Here normal germination takes place by the production of one germ tube from the thin-walled region at the tip, or from that near the hilum, or more often from both; never from the intermediate segments, unless, as has been mentioned, the latter have been partly exposed or completely liberated from the enveloping wall by manipulation or mechanical injury. In some other forms like those later discussed under the names *H. halodes*, *H. rostratum*, and *H. oryzae*, germination of entirely mature spores takes place as in *H. sativum* and *H. monoceras*, but not infrequently newly proliferated, subhyaline spores, the walls of which have not become thickened, can be seen to produce, in addition to the two polar germ tubes, one or more tubes from the middle cells as well.

A modification of the septa, visible in the spores of some species, and undoubtedly present in others, may be not without significance in germination. If the cross walls of large, subhyaline-spored forms, like *Helminthosporium teres* or *H. giganteum*, are closely examined a small circular spot having perhaps one-fifth or one-fourth the diameter of the spore may usually be distinguished. It is difficult to determine whether this represents an open communication between the segments or merely two opposed pits in the adjacent segment walls. The appearance of these walls in plasmolyzed dead spores would suggest that the latter alternative is the more probable one, a suggestion that is supported by the fact that

each segment suffers death independently of the others. There can be little doubt, however, that this modification provides means of communication between adjacent living segments. Such a communication would manifestly be of importance especially in those species in which germ tubes are produced only from the two end cells. There are, it may be mentioned, peculiarities in the germination of some species that can not be definitely ascribed to the structure of the septa or of the peripheral wall, or indeed to any demonstrable structural feature; as, for example, the production of three germ tubes from the basal cell of *H. dematioideum*, or the proliferation of clusters of tubes from the end cells or middle cells in *H. giganteum*.

It is interesting to note that if leaves bearing actively sporulating fructifications of forms possessing thin-walled, hyaline spores, like *Helminthosporium teres* or *H. bromi*, are examined under the microscope by reflected light, it will be seen that the mature spores lying about in some abundance are badly distorted and their walls utterly collapsed. When these spores are mounted in water, most of them instantly become turgid, and at a suitable temperature begin to germinate within 30 minutes, showing that the collapsed condition is by no means an indication of death. Dead spores or dead segments of partly living spores, also present in a preparation, recover their former size and shape very largely, although not wholly, but are readily recognized by their abnormally swollen membranes and coarsely granular contents made familiar by the drawings and descriptions of many authors, who unfortunately regarded them as normal. Although some authors report germination of spores exhibiting such swollen walls presumably in all segments, either as a result of age or the application of reagents, the writer has never observed an instance of viability in such material.

Germination is usually accompanied by conspicuous protoplasmic changes beginning in the segments concerned, and finally involving all the segments of one spore. The contents lose their uniform hyaline structure, becoming minutely granular or uniformly vacuolate, or more usually both granular and vacuolate. The germ tubes and mycelium in general vary somewhat with the different species. All the species seem to thrive on the culture media commonly employed in laboratories, which fact, together with the large size of the spores, makes the members of the genus among the fungi most easily isolated and cultivated. Not all of the species, however, can be made to sporulate in pure culture, those possessing hyaline spores—*Helminthosporium bromi*, *H. tritici-repentis*, etc.—being especially refractory in this respect. The majority of forms with dark spores, on the other hand, sporulate quite readily and even abundantly, although the spores thus produced may depart somewhat in shape and size from those produced under ordinary field conditions. This is particularly true of *H. sativum*, where the spores, instead of being long and slender-allantoid, become shorter, thicker, and nearly straight. When media containing a large amount of nutrient is employed, an abundant development of mycelium usually takes place and sporophores are produced in great numbers. Growth, however, soon comes to a standstill, leaving the sporophores short, and bearing only a few spores, the latter frequently being abnormally short. In such cases more satisfactory results can be obtained by the use of media containing little nutrient, as corn-meal-decoction agar or tap-water agar. If such cultures are protected from evaporation, the relatively small number of sporophores will continue to grow for several months, producing scores of

fairly characteristic spores in a dense racemose cluster. Sporophores thus produced may show a tendency to branch, not usually observable in material collected in the field.

A process quite analogous to germination may be observed in the proliferation from the spores of *Helminthosporium gramineum*, of short sporophores in the regions where ordinarily germ tubes appear. These bear a variable number of secondary spores that may usually be distinguished from the primary spores by their smaller dimensions. When this takes place while the latter are still attached to the sporophore, the whole apparatus may bear a partial resemblance to an Alternaria fructification. A somewhat similar condition appears to obtain quite normally also in *H. catenarium*, the fungus parasitic on wood reedgrass (*Cinna arundinacea* L.), where, however, the secondary spores more often are borne directly at the tip of the primary one, which thus comes to bear two hila. When grown in culture, this species develops ramifying fructifications in which a series of spores alternating with short sporophoric hyphae bear short sporophoric processes from the end cells. In fructifications of this type, the distinction between sporophore and spore is at least partly obliterated, the two types of structures merging into one another. This miscellaneous type of growth may be observed when *H. teres*, *H. cyclops*, and even *H. sativum* are grown on artificial media. This growth is apparently encouraged by moist conditions, although, as has been indicated, it appears to occur in nature in the case of *H. catenarium* and *H. gramineum*.

It may not be out of place to mention a peculiarity in the growth of the mycelium of most species of Helminthosporium which, although perhaps not confined to this genus, may not be without significance. If, for example, a number of spores of *H. bromi* are mounted in water and two come to lie in contact with each other, it will be seen that usually one or more pairs of germ tubes are proliferated from approximately opposite positions and immediately anastomose, thus uniting the two spores by several short hyphal connections. (Pl. 8, Dc.) This tendency toward anastomosis is expressed even more strongly in the mycelium that develops somewhat below the surface of agar cultures, and may be studied conveniently by cultivating the fungus on poured plates, the anastomosis occurring abundantly near the lower surface of the agar. Some of the cells involved in these hyphal fusions swell into subglobose bodies and proliferate short irregular processes of inflated segments, the whole resulting in small, dark brown, knotty masses of mycelium. Some of these continue to increase in size, developing into the subspherical sclerotia, readily visible to the naked eye, the profuse occurrence of which in culture is a distinguishing mark of this species. Although the writer has not succeeded in cultivating these sclerotia further, there can be little doubt that they represent immature perithecia, as they are entirely similar to the immature perithecia found on leaves of the natural host in fall. The inference is further strengthened by the fact that other species of Helminthosporium, *H. teres* and *H. triciti-repentis*, of which ascigerous conditions are known, show these structures in abundance. The writer is inclined to believe that in whatever species such sclerotia or abundant anastomoses (resulting in the production of complexes of lobulate segments) are found to occur, perithecia may be sought with considerable prospect of success.

Ascigerous forms have been reported for a relatively small number of species of Helminthosporium parasitic on grasses. These are all readily referable to the genus Pyrenophora Rab. or to Pleospora Fries, depending on the presence or absence of bristles on the perithecium—a basis for generic distinction, which, while regarded by Winter (*159, p. 493*) with considerable justification as inadequate, has been recognized as valid by Saccardo (*128, v. 2, p. 238*) and Lindau (*82, p. 429*). *Helminthosporium bromi, H. tricici-repentis*, and *H. teres* have associated with them in this country ascigerous forms belonging to the genus Pyrenophora that occur in great abundance on dead host material in spring, *Pyrenophora teres* (Died). (=*Pleospora teres* Died.) being found on the spike and culm of barley straw, *Pyrenophora tritici-repentis* (Died.) (=*Pleospora tritici-repentis* Died.) on the culm, and in slight measure on the decaying leaves, of quack grass, while *Pyrenophora bromi* (Died.) (=*Pleospora bromi* Died.) is very abundant on the leaves of awnless brome grass, *Bromus inermis* L. In the latter two species the perithecia usually reach perfect development, asci and ascospores reaching maturity early in May near Madison, Wis. Material collected at that period exhibited abundantly the preliminary swelling and circumscissile rupture of the ascus preceding the simultaneous expulsion of the spores from near the base of the dehiscing structure—a mode of discharge observed by Porter (*110*) in species of Pleospora, and in the species under consideration by Diedicke (*28*) as well as by Atanasoff (*2*).

Pyrenophora teres, on the other hand, probably does not form altogether normal ascospores, if the observations made in the spring of 1919 and 1920 may be taken as typical. The ascus may remain undeveloped, showing no trace of young ascopores; or ascospores may be delimited, but become arrested in their development before or after septation has occurred; or certain segments of the spore may develop normally, the others eventually collapsing, giving rise to the unsymmetrical spores shown in Plate 3, D. As Diedicke (*29*) and Noack (*95*) have shown, the developing perithecia or sclerotia begin to proliferate condiophores with the advent of suitable conditions; and a perithecium involved in the production of conidiophores is not likely to show any further development of its ascospores. The production of conidia usually is large in the case of the sclerotial form on barley; moderate in the case of *Pyrenophora tritici-repentis;* and small in the case of *Pyrenophora bromi.* The latter species is probably the only one in which the production of ascospores plays an essential part in the resumption of growth in spring; for, as a rule, in the forms on quack grass and barley, the conidia produced on the sclerotium or immature perithecium would appear to play a relatively larger part in effecting early dissemination on the young host plants. In the case of the forms with dark, thick-walled spores, as for example, *H. sativum* and *H. vagans*, with which neither sclerotia nor perithecia are known to be associated, the conidia are found to germinate readily in spring, since exposure in winter does not bring about any very decided diminution in viability. Conidia of *H. sativum, H. oryzae*, and *H. ravenelii* will germenate well one year after their formation, whereas those of *H. bromi, H. tritici-repentis*, and *H. teres* are mostly dead within one or two months. The absence of an ascigerous stage from the life history of the former species, and its presence in the latter, are probably not without significance in relation to the longevity of the conidia.

HELMINTHOSPORIUM GRAMINEUM RAB.

Brachysporium gracile (Wallr.) Sacc. var. *gramineum* (Rab.) Sacc. 1886, *in* Sylloge fungorum, v. 4, p. 430.
Napicladium hordei Rostrup 1893, *in* Sygdomme hos landbrugsplanter foraarsagede af Snyltesvampe, p. 130–132.
Helminthosporium gramineum (Rab.) Erikss. 1885, *in* Fungi par. scand. exs., no. 187.
Heterosporium gramineum of Oudemans, not Rabenhorst.

The binomial *Helminthosporium gramineum* was applied by Rabenhorst[4] to a fungus occurring on leaves of barley (*Hordeum vulgare* L.) collected at Poppelsdorf, Germany, June, 1856, and distributed as No. 332 of the Herbarium Mycologicum. The pieces of leaves and stem that constitute the specimen deposited in the Herbarium of the Office of Pathological Collections are too small to show any possible characteristic pathological effect; and the fructifications of the fungus are in a condition that would appear to make its identification with any particular one of the three congeneric species now known to occur on barley a matter of great uncertainty. Rabenhorst regarded the fungus as related to *Helminthosporium gracile* Wallr. but differing from the latter in that its spores were solitary, elongated-cylindrical, and 3 to 6 septate. Apparently, in accordance with this view, Saccardo (*128, v. 4, p. 430*) at first reduced the form to a variety of Wallroth's species, which, moreover, he transferred to the genus Brachysporium. Later, however, he listed it as an independent species (*128, v. 10, p. 615*).

In 1886, Eriksson[5] distributed as *Helminthosporium gramineum* (Rab.) Erikss. specimens of diseased barley collected near Stockholm, Sweden, during the preceding season. The label includes a short revision of the specific diagnosis:

> Hyphi conidiophori solitarii vel 2–4 aggregati, subflavi 1–5 septati, denique saepe angulato anfracti. Conidia subflava, recta, elongato cylindracea, 1–5 septata, 50–100 μ longa, 14–20 μ lata.

Although this characterization applies better to the parasite causing the stripe disease than to that responsible for net-blotch, Eriksson apparently did not distinguish between the two. At any rate, the specimen in the herbarium of the Office of Pathological Collections shows lesions of both diseases; and in an account (*37*) of the "leaf spot disease" (*blad fläckensjukdom*) he reported most of the plants to have been affected more or less, while a relatively small proportion (1 to 5 per cent) were affected so badly that no heads were developed. It may readily be supposed that the less severely diseased plants were mainly affected with net-blotch instead of with stripe, a supposition supported by the description of the foliar lesions as elongated dark brown spots with light margins.

Von Post (*111*), working independently of Eriksson, in 1886 published an account of the "brown stripe disease" (*brunrandsjukdom*) of barley, that had been very destructive at Ultuna, Sweden. The longitudinal yellowish streaks, characteristic of all the leaves of attacked individual plants, suggestive of the variegation of ribbon grass, and later changing to brown or yellowish brown; the dying of the plants before the development of spikes, the systemic distribution of the fungus (indicative of seed transmissal and seedling infection) provide conclusive evidence that this investigator was dealing exclusively with the stripe disease.

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[4] RABENHORST, G. L. KLOTZSCHII HERBARIUM VIVUM MYCOLOGICUM SISTENS FUNGORUM PER TOTAM GERMANIAM CRESCENTIUM COLLECTIONEM PERFECTAM . . . Ed. II, Century III, no. 332. 1856.
[5] ERIKSSON, J. FUNGI PARASITICI SCANDINAVICI EXSICCATI NO. 187. *Helminthosporium gramineum* (Rabh.) Erikss. Stockholm, Sweden. July, 1885.

The conidial form that von Post observed developing on the brown areas of affected leaves he identified with *Helminthosporium gramineum* Rab. as defined and distributed by Eriksson.

Rostrup (*123*), in 1888, described a disease characterized by symptoms and development similar to those described by von Post, which had been doing considerable damage in certain sections of Denmark, and which he attributed to a new species of Napicladium, *N. hordei.*

In 1892, Pammel (*102*) reported a destructive disease of barley from Iowa, which obviously was identical with that described by von Post, being evidenced long before heading time by the presence on all the leaves of affected individual stools, of pale yellow streaks extending from base to tip, premature death, and subsequent tearing of the foliage into shreds. Pammel identified his fungus with the one distributed by Eriksson as *Helminthosporium gramineum* Rab.

Publications by Ritzema Bos (*120*) and Frank (*43*) are evidence that the same type of malady appeared and caused losses during the next few years in Holland and in Germany. However, Ritzema Bos' figures of the foliar lesions as well as of the 8-septate spore, and the description of the symptoms in Frank's earlier account (*42*) leave a suspicion that, like Eriksson, these authors were dealing not with stripe alone, but also with net blotch, and failed to distinguish between the two diseases and causal organisms.

Rostrup (*125*) appears to have been the first investigator to recognize that barley was affected by two different diseases caused by two distinct related fungi. However, as he associated Rabenhorst's binomial with the cause of the less destructive "leaf spot disease" (*bladpletsyge*) instead of with the "stripe disease" (*stribesyge*), and attributed the latter to a different although related genus of fungi, the prevailing taxonomic confusion was not immediately settled. Indeed, it was not until the appearance of Ravn's detailed papers (*115*, *116*) that the ambiguity, which Eriksson's publications had originally introduced, was disposed of effectively. Ravn assigned the parasite causing stripe to *Helminthosporium gramineum* Rab., reduced *Napicladium hordei* Rostrup to a synonym, and distinguished the fungus very clearly, with regard to morphology and pathogenicity, from the congeneric forms causing net blotch of barley and leaf spot of oats.

While Ravn's papers (*115*, *116*) thus left no further occasion for confusing *Helminthosporium gramineum* with *H. teres*, it did nothing to distinguish it from *H. sativum*, a species later described from the United States, but the occurrence of which as a third congeneric form parasitic on barley has not been recognized in Europe. As will be pointed out in another connection, the European literature is not devoid of ambiguous accounts, of which Massee's treatment (*90*) of barley leaf blight may be taken as an example, indicating that *H. sativum* is certainly not altogether absent, but usually is mistakenly recognized as *H. gramineum.* And a similar condition obtains in the writings of investigators in America and other countries.

Yet after allowances for erroneous diagnoses are made, stripe remains one of the most important and widespread diseases of barley. In Europe it has been reported not only from Sweden (*111*), Denmark (*115*), Germany (*42*), and Holland (*120*), but also from England by Prain and Percival (*112*) and by Biffen (*13*), from Ireland by Johnson (*72*), and from Russia by Jachewski (*66*). According to the records of the Plant Disease Survey, it is found in most of the States of the Union, apparently wherever

barley is grown to any extent, the damage varying from a relatively small amount to approximately one-fifth of the crop. From Canada the disease has been reported by Güssow (*48*) and by Fraser (*45*). Hauman-Merck (*52*) noted the prevalence of *Helminthosporium gramineum* on barley in Argentina to an extent unknown in Central Europe. Yoshino (*161*) and Ideta (*65*) have recorded the disease in Japan, where Hori (63), in 1918, observed an unusually severe outbreak. Chou (*20*) notes *Pleospora graminea* Died. on barley among the pathogenic fungi prevalent in the locality of Nanking in China. In India, the stripe disease has been studied by Butler (*19*), who states that the damage caused in that country is less than that recorded in European literature.

The symptoms of the stripe disease were described correctly by von Post (*111*), Pammel (*102*), and Rostrup (*124*), and in an especially detailed manner by Ravn (*115*). The first evidence of infection may often be recognized in the unfolded leaves of young seedlings by the presence of small pale spots, although generally at this stage the manifestations of abnormality are not pronounced. Later, usually about 6 weeks after sowing, after a number of leaves have become fully developed, these organs begin to show longitudinal, etiolated, yellowish stripes, often extending from the base of the blade to the tip. A single broad blade may show from 5 to 7 of these stripes although a smaller number is much more common. Not infrequently the stripes are interrupted and are thus replaced by numbers of elongated yellowish streaks. In any case, the similarity to ribbon grass then constituting one of the most striking characteristics of the disease, is soon modified by the appearance of dark brown discoloration in longitudinal elongated streaks. These brown discolored streaks are most apt to occur especially at the margins of the yellowish stripes, the tissues of which have now become dry and brittle, but often extend also as brown lines beyond the base of the blade, into the upper portions of the sheath. Often the brown streaks are separated from the remaining healthy parts by a yellowish zone within which the chlorophyll has largely disappeared. At this intermediate stage, especially when the brown streaks are short and relatively wide, the disease is apt to be mistaken for netblotch or spotblotch. Later the likelihood of a wrong identification is again diminished, for finally the remaining green tissues are also involved, the yellow and brown discolorations gradually fade into a uniform dark gray or brownish gray, the dead, rather brittle tissues split freely, and a dark efflorescence consisting of the abundant fructification of the parasite makes its appearance on the longitudinal foliar strips. (Pl. 1, A.) As the mechanical rigidity of the diseased leaf tissue becomes greatly lessened during the later stages, the leaf blades or their shredded remains (Pl. 1, Ab-e) usually droop or hang down in a characteristic way.

The effect of the parasite on the stem is equally pronounced. The elongation of this organ incident to heading, especially of the uppermost internodes, is considerably reduced or sometimes almost entirely suppressed. As a result the height of the plants is correspondingly reduced, diseased specimens generally not attaining much more than half the height of healthy individuals. Most frequently the spike never becomes visible, but remains entirely enclosed in the basal portion of the upper leaf sheath. In other instances, the inflorescence is thrust into the upper part of the upper leaf sheath, and, failing to emerge normally, protrudes partly from the opening on the side of the latter. (Pl. 1, A.) This abnormal mode of emergence generally involves some distortion of the awns, as

these tend to remain wedged in the sheath, from which, indeed, they may fail to become completely liberated. The condition thus brought about is in a measure characteristic of the disease, although Hegyi (*57*) reports a similar type of malformation due to the attack of aphids and to a period of cold weather at a critical time just before heading. In a relatively small proportion of plants, the inflorescence emerges from the sheaths, but for a smaller distance than in normal plants. However, even then the ovaries rarely develop anything beyond abortive seeds, the enveloping lemmas and paleas showing a decided brownish tinge. Viable seed certainly is not generally produced, the few instances recorded by Ravn being apparently more or less exceptional.

In southern Wisconsin, the life of diseased plants usually comes to an end between June 20 and June 25, depending to a large extent on the earliness or lateness of the season. Although at first readily distinguishable in the field, the dead plants are soon hidden as a result of the continued growth of the healthy individuals. By harvesting time, ordinarily soon after the middle of July, they have usually collapsed to such an extent that their remains are not found without special search. Owing to the production of immense numbers of fructifications during the several intervening weeks, particularly on the dead foliar parts, the spores of the fungus may be distributed to the floral parts of healthy plants. The experimental work of Ravn showed that the reappearance of the disease in successive seasons is due to the resultant contamination or infection of the seed with spores of the fungus. When seed thus naturally inoculated germinates, the seedling tissues are immediately infected. As the growth of the fungus keeps pace with that of the host, the parasite maintains itself in the growing point and, indeed, in all parts of the plant, even when at the time no outward symptoms are visible. The development of the disease, including its eventual manifestation toward flowering time, thus presents a striking analogy to that of the systemic smut diseases, and is brought about by a similar mode of parasitism of the fungus concerned. To this manner of development also must be attributed the fact observed by von Post (*111*), Rostrup (*124*), Pammel (*102*), Ravn (*115*), and others that the affected single individual plants or affected stools are quite uniformly diseased in all their parts, and are, with possible rare exceptions, totally destroyed. The use of terms like "moderate," "slight," "more or less," while not inappropriate in describing the extent to which a barley crop may be affected by stripe, is altogether inapplicable in describing the severity of attack of individual plants, and can in such connection be interpreted only as evidence indicating an erroneous diagnosis.

While in morphology *Helminthosporium gramineum* exhibits no striking characteristics, the writer is of the impression that its similarity to other congeneric species, particularly to those occurring on the same host, as well as on other cereals, seems to have been somewhat exaggerated. It is certainly conspicuously different from *H. sativum*, and is usually not so difficult to distinguish by microscopical examination of its fructifications from *H. teres* and *H. avenae* as Ravn's statements might lead one to believe. The sporophores occur usually in clusters of 2 to 6, fascicles of 3 to 5 being common (Pl. 1, Ea–Ee), whereas the corresponding clusters of *H. teres* (Pl. 2, Ea–Ec) and *H. avenae* (Pl. 4, De–Dg) rarely include a larger number than 3. Although, just as in the latter two species, the basal segment is considerably distended, the width of the sporophores of *H. gramineum* is perceptibly smaller in the

distal portion, measuring usually approximately 6 μ as compared with 7 to 9 μ for those of *H. teres* and *H. avenae*.

The spores vary from subhyaline when newly proliferated to yellowish brown when fully mature, never, however, becoming dark olivaceous, like those of *H. sativum*. They are (Pl. 1, Ba–h) typically straight or very slightly curved; subcylindrical, but quite frequently widest in the basal portion and tapering more or less toward the apex. Both apical and basal ends are rounded off, abruptly presenting a hemispherical contour. The peripheral wall is relatively thin as in other species having subhyaline or light-colored spores. The septa vary from 1 to 7 in all the material the writer has had occasion to examine, and are only infrequently associated with perceptible constrictions (Pl. 1, Bf, h), while in the spores of *H. teres* (Pl. 2, Ca) constrictions are more common and often moderately pronounced. Germination takes place promptly when the spores are mounted in water, usually within 30 minutes. Germ tubes are proliferated usually from both end segments and from several intermediate segments, the numbers produced from the basal segment varying from 1 to 3, while the other segments rarely give rise to more than one (Pl. 1, Ca–d).

A phenomenon undoubtedly related to germination is the production in nature of secondary spores or short secondary fructifications from primary spores, that in this species occurs to a greater extent than in the other congeneric forms parasitic on cereals, and which suggests comparison, for example, to *H. catenarium*. The secondary spores are not generally produced directly on the apex, but on a sporophoric process arising from the apex (Pl. 1, Dc, e) or obliquely from the apical cell (Pl. 1, Da, d) or less frequently laterally from the basal segment (Pl. 1, De). Not infrequently the process remains short and gives rise to only a single spore (Pl. 1, De), while again it grows out into a sporophore bearing half a dozen spores (Pl. 1, Dc). Evidently such secondary development may take place after the primary spore has become detached from the sporophore on which it was borne, as it usually is associated with a collapse of some or all segments of the primary spore involved (Pl. 1, Da). The secondary spores (Pl. 1, Dca–cc, Dda) may be nonseptate or show 1 to 3 cross walls; they are, in general, decidedly smaller than the primary ones, the minimum dimensions approximating 11 by 20 μ (Pl. 1, Dca–cc, Dda). As the smaller individuals of the one order can not be readily distinguished from the larger individual of the other, all gradations may be found between these magnitudes and the maximum dimensions of the primary spores, 20 μ for width, 105 μ for length. The maximum measurement for spore length of *H. gramineum* is thus barely two-thirds as large as that of *H. teres*—a fact of great importance in identifying the species by microscopical examination.

Diedicke (*29*), in 1903, reported from Germany the discovery of an ascigerous stage of *Helminthosporium gramineum* in a species of Pleospora, which he found to correspond fairly well to *P. trichostoma* (Fr.) Winter. However, as the latter appeared to include a number of forms parasitic on different hosts but not interchangeable in their host relations, he regarded it as a collective species, and separated it first into a number of biologic races (*28*) which later he recognized as individual species (*29*). The perithecial form collected on old barley straw in a field that had been affected severely with the stripe disease was accordingly referred to a newly derived species, *P. graminea*. On inoculating barley leaves with sclerotia of the fungus, typical symptoms of stripe

are reported to have appeared in a number of cases, thus presumably establishing its identity. Diedicke's findings were confirmed by Noack (*95*) who, working independently, studied apparently the same ascigerous fungus collected on barley stubble in Hesse, Germany. Using conidia, ascospores, and sporulating material of host leaves, Noack secured infection by inoculating the first leaf of barley seedlings when it had attained a height of only several centimeters; and also on plants about 4 weeks older. The symptoms induced in the young seedlings were systemic, whereas on the older plants local infections manifested by the appearance of brownish spots were obtained.

The work of both Diedicke and Noack is open to the criticism that it was not done in connection with parallel infection experiments carried out with *Helminthosporium teres*, and that consequently these authors were, perhaps, not so capable of distinguishing between the pathological effects of the two related fungi as might have been desired. It may be mentioned that their fungus was obviously very similar to the form discussed in this paper as *Pyrenophora teres* (Died.) collected by the writer near Madison, Wis., where it occurred in abundance in spring on barley stubble apparently regardless as to whether or not the crop of the preceding year had been seriously affected with stripe. Recently, Paxton (*106*) has reported the occurrence of the mature perithecial stage of *H. gramineum* on two-year-old barley straw in California. Transfers of ascospores to corn-meal agar resulted in the production of conidia, which when used to inoculate barley gave rise to typical stripe lesions. According to Van Poeteren (*109*), perithecia of the parasite have been found on the glumes of germinated grains of barley, the seedlings of which exhibited the symptoms of stripe. This author suggests incubating seed in a moist warm atmosphere for three days in order to determine in advance from the appearance or nonappearance of perithecia whether it will give rise to diseased or healthy plants.

Of all the diseases due to species of Helminthosporium, the stripe disease undoubtedly has been made the subject of the largest amount of investigation aiming at effective methods of control. As the effective inoculum is presumably very largely, if not entirely, borne on the seed, the disinfection of the latter by the use of various fungicides and by the application of heat has not been without a considerable measure of success. Copper sulphate was found effective by Riehm (*119*) when applied in 1 or 0.5 per cent solution for 30 minutes; by Lind and Ravn (*81*) when applied in 0.5 per cent solution for 4 hours; and by Müller and Molz (*93*) when applied in the same solution for 16 hours. Lind and Ravn (*81*) obtained satisfactory results by the application of formaldehyde in 0.2 per cent solution for 6 hours; Schander (*129*) in 0.2 per cent solution for 30 minutes; and Johnson (*70*) in 0.16 per cent solution for 3 hours at 20° C., in 0.25 per cent solution for 2 hours at 10°, and 0.25 per cent solution for 1 hour at 25°. According to Lind and Ravn (*81*) treatment with mercuric bichloride in 0.1 per cent solution for 1 to 2 hours gives good results. Riehm (*119*) found 0.2 per cent mercury chlorphenol applied for 15 minutes effective. The hot-water treatment of seed for the control of stripe was found by Lind and Ravn (*81*) less effective than chemicals and of value only for lightly infected seed; Johnson (*68*), however, found a modified treatment with hot water 5 hours cold, and 15 minutes at 52° C., quite valuable. A variety of intermittent hot-water treatments have been devised, consisting in dipping the seed in hot water for relatively brief periods alternating with

longer periods of exposure to cooler water. Müller and Molz (*93*), as well as Lind and Ravn (*81*), found hot-air treatment worse than useless for stripe, as it tended to increase the proportion of diseased plants; Atanasoff and Johnson (*3*), on the other hand, found that such treatment markedly reduced the disease without materially injuring the seed. As the disinfection of barley seed usually involves a number of diseases, the choice of any particular treatment often is contingent on its effectiveness in controlling smut, netblotch, spotblotch, and the bacterial blight as well as stripe.

Owing to the successful control of stripe by seed treatment, not much attention has been devoted to other methods of combating the disease. The various reports already mentioned, of the occurrence of an ascigerous stage, and the statement by Paxton that 16-year old herbarium specimens of cultivated barley affected with *Helminthosporium gramineum*, when placed in a moist chamber, produced conidiophores and viable conidia from the dormant mycelium, suggest the possible value of measures involving sanitation. Indeed, Frank (*43*), Weiss (*157*), and later Jaczevski (*66*) recommended rotation of crops, though perhaps more on general principles than because of possible experimental evidence or knowledge of the presence in fields of an overwintering stage playing an important part in establishing the parasite on successive crops. Ferraris (*40*) recommended burning the stubble in addition to seed disinfection. The removal and destruction of diseased plants found effective in Scotland (*134*) would obviously constitute a method of eradication too laborious to be seriously considered in the United States. The observations of various investigators, notably of Hori (*63*) and of Lind and Ravn (*81*), show that the proportion of diseased plants is increased when the seed germinates in soil at a low temperature. Müller and Molz (*93*), however, did not find late sowing in a warmer seedbed advisable; for, although less disease developed, the delay brought about a decrease in the yield.

Von Post (*111*) observed considerable differences in susceptibility to stripe between varieties of barley. Ravn (*115*) found the six-rowed varieties as well as the *erectum* types more heavily attacked, in general, by *Helminthosporium gramineum* than the *nutans* types, a condition exactly the opposite of that he found to obtain with reference to attack by *H. teres*. Kiessling (*76*), while unable to confirm Ravn's findings regarding the comparative susceptibility of the nodding and the erect types, noted pronounced differences in the proportions of diseased plants present in the stands of different varieties. It is worthy of note that some of Kiessling's strains, representing genetically pure lines, manifested with fair consistency moderate susceptibility, while other pure lines exhibited a high degree of resistance. This author urged, very justifiably, that the plant breeder ascertain the measure of resistance to stripe inherent in any varieties of barley with which he may be dealing and reject those lines showing marked susceptibility.

HELMINTHOSPORIUM TERES SACC.—PYRENOPHORA TERES (DIEDICKE)

Helminthosporium hordei Eidam 1891, in Der Landwirt, Bd. 27, p. 509.
Pleospora teres Died. 1903, in Centralbl. f. Bakt. Abt. 2, Bd. 2, no. 2, p. 52–59.

The binomial *Helminthosporium teres* was applied by Saccardo (*126, pl. 833*) to a fungus collected on leaves of barley near Padua, March, 1881, and figured in the "Fungi Italici" as having 3-septate conidio-

phores arising in a group of five from a green substratum and bearing at the tip a single conidium. The latter were represented as dark green structures, thick walled, 4 to 5 septate, ellipsoidal or subcylindrical, tapering perceptibly toward the rounded ends. A part of a leaf, showing a green region interspersed with uniformal brown elliptical areas marked with short, black, longitudinal lines, was doubtless intended to represent the pathological habit of the fungus. In 1882 (*127*), a brief diagnosis of the species was published:

Maculis oblongis amphigenis, olivascentibus; hyphis fasciculatis, 100–130=12, cylindraceis basi subincrassatis, fuligineis; conidiis acrogenis cylindraceis, rectis utrinque rotundatis, 110–115=18, 4–5 septatis, non constrictis, obscure olivaceis.

Neither the figures nor the text make it possible to identify definitely Saccardo's fungus with any one of the three species of Helminthosporium parasitic on barley. The number of sporophores in a fascicle, and the number of septa in the spore, suggest the form causing stripe; the thick wall, tapering ends, color, and absence of constrictions in the contour of the spore, suggest that causing spot-blotch; while the excessive width of the sporophore and the relative straightness of the spores suggest that responsible for net blotch. Although size of spores and appearance of lesions clearly point away from the stripe fungus, the former is nearly equally applicable to the other two species, while the latter is scarcely characteristic of any.

Nearly a decade later Briosi and Cavara[5], in an account of the fungus causing leaf spot of oats, recognized it as a form of Saccardo's species. Oudemans (*100*), after examining Rabenhorst's specimens of *Helminthosporium gramineum*, concluded that the latter fungus was identical both with the one redescribed by Eriksson (*37*) under the same name and with *H. teres* Sacc. Ravn (*115*), however, did not accept Oudemans' views in their entirety, but in distinguishing two diseases of barley applied Rabenhorst's binomial to the fungus causing stripe, and Saccardo's to the causal organism of barley "Helminthosporiosis" or net blotch. In making these dispositions he took into consideration the destructive character of the parasite described by Rabenhorst in contrast to the local foliar lesions which, while figured and described very poorly by Saccardo, he was nevertheless able to identify with those characteristics of "Helminthosporiosis" by an examination of Saccardo's original specimens. The Italian mycologist, moreover, confirmed Ravn's opinion that his *H. teres* was the species responsible for "Helminthosporiosis."

The disease itself had not escaped earlier observation by other students. As was pointed out in another connection, Eriksson's specimens of *Helminthosporium gramineum* Rab. showed typical lesions of net blotch as well as of stripe; and his note on the "bladfläckensjukdom" indicates that the former was moderately abundant in the field. Kirchner (77), in 1891, had published an account of the "brown-spottedness" (Braunfleckigkeit) of barley leaves, observed in southern Germany during the preceding two seasons. The spots were described as blackish brown in color, narrow, often over 1 cm. in length, and surrounded by a narrow yellow zone while the leaf is still green. With the multiplication of the spots the leaves were observed to wither and give rise to the fructifications of the fungus. Material was submitted to Eriksson, who pronounced the fungus identical with that distributed by him, although

[5] BRIOSI, G. et J. CAVARA. I FUNGI PARASSITI DELLE PIANTE COLTIVATE OD UTILI. No. 80, Pavia, Italy. 1889.

Kirchner called attention to the fact that up to eight septa were present in the spores. This fact, together with the relatively mild nature of the disease, indicates that he was most probably dealing with net blotch. In 1898 Rostrup (*125*) had discussed a disease of barley which he designated as a "leaf-spot disease" (*Bladpletsyge*), characterized by the presence, largely on the lower, less vigorous leaves, of elongated dark brown spots, surrounded by a narrow yellow margin. This malady, which was not observed to be very destructive, Rostrup attributed to *H. gramineum* Rab. Apparently the same trouble had been investigated later also in Silesia by Eidam (*35*), who attributed it to a new species of Helminthosporium, *H. hordei*, which he found was not transmissible to oats and consequently different from the congeneric parasite causing leaf spot of the latter host.

The disease is evidently widely distributed in the United States, the records of the Plant Disease Survey containing reports of its occurrence in 21 States, including all the barley-growing districts of any importance. In this connection it may be mentioned, however, that although often responsible for appreciable loss, net blotch is of relatively minor economic importance compared to stripe or spot blotch. It is true that in Bakke's paper (*6*), which contains the first published record of the disease in this country, *Helminthosporium teres* is represented as probably the most serious parasite of barley. As Bakke failed to distinguish between the disease under consideration and the much more serious trouble attributable to *H. sativum*, the exaggerated account of the destructiveness, as well as the somewhat inaccurate morphological treatment of *H. teres*, both of which unfortunately appear to have been incorporated in Butler's handbook (*19*), are readily explained.

The course of development of netblotch has been described in detail by Ravn (*115*). In the vicinity of Madison, Wis., where in 1919 the writer had occasion to observe the progress of the disease, the regular crops exhibited the characteristic symptoms in small measure during the earlier part of the season. Later the lesions sparingly present on scattered leaves were completely obliterated by the blotches due to *Helminthosporium sativum*, that began to appear in great profusion at the heading stage about June 20. A much more favorable opportunity to study the disease presented itself with its development on volunteer plants from the latter part of August until the beginning of November, when netblotch was present in considerable abundance, to the practical exclusion of both stripe and spotblotch. The writer is informed that a somewhat suppressed manifestation of the trouble on the regular crop, and its extensive distribution on the volunteer crop, is, in general, not altogether uncharacteristic of its seasonal occurrence in our northern latitudes.

According to experiments reported by Ravn (*115*), the disease is propagated by seed infected or contaminated by the fungus. When such seed germinates at a relatively low temperature, as, for example, 10° to 15° C. or less, there results a local infection of the first seedling leaf which he designated as primary Helminthosporiosis. This is in contradistinction to secondary Helminthosporiosis resulting from infection by conidia developed on the dead tissues involved in the primary or in subsequently developed secondary lesions. On the other hand, when germination took place at a temperature of 20° C. or above, primary Helminthosporiosis failed to develop even though the seed was infected or contaminated. That such thermal relations are effective

in the development of primary lesions in the field was shown by the virtual absence of such lesions from the first leaf of plants resulting from sowings made in July and the first half of August, in contrast to a high percentage of infection secured from sowings made in March and April. The prevalence of netblotch on volunteer barley in the northern latitudes of our Middle Western States, where the temperature in midsummer ordinarily is at least as high as in Denmark, is accordingly on *a priori* grounds not to be attributed to seed infection. And, indeed, the abundance of the lesions on the first few leaves of a large proportion ofvolunteer seedlings in the fields kept under observation by the writer in 1919 indicated secondary infection due to spores from stubble and other remains of the regular crop.

Barley leaves affected with netblotch usually are not difficult to distinguish from leaves attacked by stripe or spotblotch. The presence of the parasite in the living foliar tissues is manifested by the appearance of dark brown spots or streaks which at first may be barely perceptible, measuring perhaps not above 1 mm. in length, but later increasing considerably in size, although not frequently measuring more than 20 to 25 mm. in a longitudinal direction. The increase in width usually is relatively small. A variegated appearance simulating that of the foliage of ribbon grass, due to alternately placed green and yellow stripes, is never produced, although the brown streaks characteristic of the later intermediate stages of stripe may sometimes be approximated in leaves affected by *Helminthosporium teres*. The most distinctive feature of the discolored areas involved in netblotch lesions is to be found, however, in the irregular distribution of the brown pigment, the latter being accentuated in very narrow lines, some oriented longitudinally, others transversely or obliquely to the exis of the leaf. As a result, a more or less irregular dark brown reticulate pattern may be distinguished within the areas of diffused brown. (Pl. 2, A, B.) Indeed, the pigmentation of the interstices within the reticulate pattern may become so reduced that the discoloration is present almost exclusively as a sharply defined network of brown lines. (Pl. 2, B.) The resulting macroscopic appearance, utterly different from that characteristic of stripe or spotblotch, and duplicated (as far as the writer is aware) only in leaves of meadow fescue affected by *H. dictyoides*, led Johnson (3) to apply to the disease the descriptive name "netblotch," which has since been generally adopted in the United States. The parts of the leaf immediately adjacent to the discolored tissue usually shows more or less etiolation that becomes manifest in the appearance of a narrow yellowish zone surrounding the spots. (Pl. 2, B.) Sometimes, especially in incipient lesions in which the reticulate character is pronounced, the measure of etiolation is apparently small, and the dark brown netlike lines are found in leaf tissue apparently very little changed. Later, however, all the lesions show yellow margins, which eventually become extended until the whole leaf blade is involved and withers from the tip to the base. (Pl. 2, Ad, Ae.) At this stage the spots, which may number several score on a single leaf and often become more or less confluent, begin to fade from a dark brown to a more diffused dull brownish gray. Not much later the fructifications appear as a light efflorescence extending usually from the brownish spots over the surrounding yellowish gray portions of the dead foliar organ.

The injury due to *Helminthosporium teres*, like that occasioned by most of its foliicolous congeners is thus the result of the destruction of

leaf tissue. As long as weather conditions are suitable, each successive leaf to be unfolded sooner or later becomes infected and in time may be killed. Plate 2, A, showing a volunteer seedling with five leaves, the first (Pl. 2, Ae) entirely withered, the next three (Pl. 2, Aa–b) exhibiting increasingly earlier stages, and the fifth (Pl. 2, Aa) apparently healthy, drawn from material collected September 2, 1920, represents a rather severely affected specimen. It may be mentioned that after September 15 the production of new lesions was much slower, with the result that, on November 3, the upper leaves of the plants, then about 24 inches tall, were entirely free from the disease, although fresh lesions could be found on the leaves lower down, while the lowermost withered foliage was covered with an abundance of profusely sporulating conidial fructifications.

The brown or olivaceous conidiophores (Pl. 2, Ea–d), emerging from the stomata or between epidermal cells, singly or in groups of 2 or 3, vary usually from 120 to 200 μ in length, and above the swollen basal cell, from 7 to 9 μ in diameter. They are thus somewhat stouter than those of *Helminthosporium gramineum* and *H. sativum*, besides being less closely septate, the septa occurring at intervals varying usually from 15 to 60 μ, and averaging approximately 35 μ. The writer has not observed them in fascicles of 5 and 6, indicating that their occurrence in groups of such number is at least less common than in the case of the stripe fungus. On the other hand, the conidiophores of *H. teres* appear to be quite indistinguishable from those of *H. avenae* in all respects.

Although *Helminthosporium teres* has been confused with both *H. gramineum* and *H. sativum*, the spores of the netblotch fungus are certainly not readily mistaken for those of the latter species. The writer's material, collected near Madison, Wis., October 28, 1919 (after a protracted period of damp, cloudy weather under conditions apparently nearly optimum for sporulation) showed these structures (Pl. 2, Ca–f) to vary from 30 to 175 μ in length and from 15 to 22 μ in width. In respect to spore length, therefore, the species is altogether superior to the two congeneric forms occurring on barley and inferior to *H. bromi* in approximately the same degree as the latter is inferior to *H. giganteum*. From 1 to 10 septa were found present, the septa, after the delimited segments have partly rounded up, being associated usually with perceptible constrictions in the contour of the spore. As the constriction at the proximal septum is especially pronounced and constant, and the basal, as well as the apical end, is rounded off in a hemispherical form, the basal segment is given a subglobose shape as characteristic for the species as are the basal segments of *H. tritici-repentis* and *H. bromi*.

The spores are subhyaline in color, like those of the latter two species, when newly proliferated, but become greenish fuliginous or yellowish when older, usually, however, not assuming a tinge quite as dark as the brownish yellow of fully matured spores of the stripe fungus, and consequently never approximating the dark olivaceous color of those of *H. sativum*. Associated with this light color is a thin peripheral wall, the drawings of Saccardo (*126*) and some other authors showing the outer wall as a thick structure being apparently based on dead material. While the spores of *H. gramineum* usually are entirely straight, those of the netblotch fungus not infrequently exhibit slight irregular crooks (Pl. 2, Cf), in which respect as well as in the general subcylindrical shape and location of the hilum within the contour of the basal end, a similarity to *H. bromi* and *H. tritici-repentis* again is evident.

When the spores are mounted in water, they germinate promptly, germ tubes being produced laterally or obliquely from intermediate as well as end segments, the protoplasm changing from a uniform to a regularly vacuolate structure. (Pl. 2, Da–d.) Like those of related species they are relatively short lived, a considerable proportion of the segments dying during the first 10 to 15 days of exposure, and probably few surviving after two months. It appears very probable that Bakke's account (7) of the longevity of *Helminthosporium teres* was based on material not of this species, but of *H. sativum.*

On media containing little organic food material, like tap-water agar (tap water 1,000 cc. agar-agar 20 gm.), or Beyerinck's agar (distilled water 1,000 cc., ammonium nitrate 0.5 gm., dipotassium phosphate 0.2 gm., magnesium sulphate 0.2 gm., calcium chloride 0.1 gm., ferrous sulphate trace, agar-agar 15 gm.), aerial growth, although sparse, often consists almost entirely of conidial fructifications bearing spores similar in structure and color to those developed in nature, but usually much shorter and containing only 2 to 3 septa. A strong tendency toward production of secondary spores is apparent also in artificial cultures, giving rise to conditions like those illustrated by Ravn (*115*).

On media containing a large amount of organic food material, a profuse white aerial growth results, consisting partly of fluffy mycelium, and partly of more or less compact erect columnar masses. The submerged mycelium shows abundant anastomosis with the formation of numerous complexes composed of dark brown inflated or lobulate segments. (Pl. 9, Fa–b.) The latter apparently represent incipient stages of sclerotia which on sterilized barley straw may become readliy visible to the naked eye, often exceeding 0.5 mm. in diameter. Ravn, although failing to observe any indication of ascus formation in these cultivated bodies, nevertheless interpreted them as immature perithecia of a Pyrenomycete, probably related to *Pleospora polytricha* (Wall.). This interpretation was justified by Johnson's (*69*) discovery in Wisconsin of the ascigerous stage of the netblotch parasite in a fungus he referred to the genus Pleospora, the specific identity of the stages in the pleomorphic forms being supported by apparently conclusive inoculation and cultural studies. The same ascigerous form was collected by the writer on barley straw and stubble from fields near Madison, Wis., late in March, 1919, as well as in March and April of the following year. As Diedicke (*29*) and Noack (*95*) recognized a similar ascigerous form as the perfect stage of *H. gramineum* in Germany, it may not be superfluous to mention that the Pyrenophora fructifications found on barley stubble in Wisconsin do not seem to be confined to weak culms such as might conceivably represent the remains of plants affected with stripe, but are found very abundantly on culms which, because of their manifest normal size and attachment to a perfectly developed head, can not easily be supposed to be derived from "striped" plants. (Pl. 3, A.) Previous to the publication of Noack's paper, Diedicke (*29*) had suggested the binomial *Pleospora teres* for the ascigerous stage of *H. teres*, at that time unknown, and consequently somewhat hypothetical.

The perithecia on barley straw (Pl. 3, B) are of the same type as those of *Helminthosporium bromi* and *H. tritici-repentis.* In general, however, they are perceptibly larger, measuring usually about 0.5 mm. in diameter. Although the lateral wall may sometimes taper toward the top, a distinct ostiolar beak usually is not present, and in most instances the ostiole is represented by a mere opening in the apical portion of the fruiting body. Setae may be altogether absent, or present in moderate number not

usually near the ostiole but on the lower portion of the wall. They (Pl. 3, Ca) differ from the sporophores (Pl. 3, Cb, Cc) usually produced in large numbers on the upper surface of the fruiting body in spring, in being dark olivaceous rather than of a brown color, in being more closely septate, and in tapering toward the tip to about one-half their basal diameter. Although material of *Pyrenophora tritici-repentis* and *P. bromi* collected near Madison, Wis., in the spring of 1919 and 1920, showed asci and ascospores in excellent condition, the development of corresponding structures in *P. teres* did not go beyond a more or less abortive stage. In most instances the asci remained small; if ascospores were delimited, they usually failed to grow to normal dimensions, and frequently showed no cross walls. Plate 3, D represents approximately the least abnormal conditions found in the season of 1920, showing each of the asci with several obviously normal spores, the remainder being either more or less misshapen, or having one or several or all of the segments collapsed. The space in the interior of the imperfectly developed fruiting body not occupied by asci, is filled with colorless vertically oriented, more or less filamentous pseudoparenchyma. As has been indicated in another connection, the failure of the asci to develop normally may be attributed to the advent of weather conditions in the spring encouraging the production of the numerous conidiophores (Pl. 3, B), the initiation of which appears to involve a cessation in the development of the internal parts of the perithecium. As abundant moisture and a relatively high temperature appear to favor the conidial stage, it is not illogical to expect that a long protracted period of cold, dry weather in spring might result in the production of more nearly normal asci.

Judging from the more satisfactory material examined, the asci of *Pyrenophora teres* are subcylindrical throughout most of their length, the proximal portion tapering toward the short stipitate base, and the wall of the apical end modified by a ring-like thickening. They measure 30 to 36 by 220 to 250 μ, and contain 8 spores in distichous arrangement. Normal specimens of the latter are light brown in color; measure approximately 18 to 22 by 52 to 60 μ; and show three transverse septa associated with perceptible and often pronounced constrictions. One or both middle segments may, in addition, be divided by a longitudinal wall. The protoplasmic contents appear more or less granular and vacuolate. Germination takes place promptly when the spores are mounted in water, in a manner entirely similar to that of the two related ascigerous forms discussed in this paper.

It is difficult to estimate the measure of importance to be attributed to the ascospores and conidia developed by the perithecia in reestablishing the fungus at the beginning of successive seasons. The abundance of these fructifications, however, indicates that they constitute a source of inoculum that ought not bo be overlooked, and certainly provides an additional reason for crop rotation, or such sanitary measures as turning under or otherwise disposing of the stubble of the preceding season. Most of the investigations relating to the control of netblotch have been carried out as subsidiary to control studies of the more destructive diseases affecting barley, particularly stripe, loose and covered smuts (*Ustilago nuda* and *U. hordei*), and spotblotch. In general, the seed treatments effective against stripe and spotblotch have also been found to reduce materially the number of primary infections of netblotch. Nevertheless the literature does not seem to indicate any treatment for the disease quite equalling in efficacy, for example, the various formalde-

hyde treatments that have been devised against stripe, or the hot-air treatment described by Atanasoff and Johnson (*3*) against spotblotch. Moreover, as the plant is subject to attack at any stage of growth, and the fungus can spread from a small number of diseased seedlings to other plants, reduction of primary lesions by methods aiming at the disinfection of the seed is of relatively less value than in the case of diseases, the occurrence of which is contingent on seed contamination and infection during the germination period.

HELMINTHOSPORIUM AVENAE EIDAM

Helminthosporium teres Sacc. forma *avenae-sativae* Briosi & Cavara 1889, *in* I fungi par. delle piante colt. od utili, no. 80.
Helminthosporium avenae-sativae (Br. & Cav.) Lindau *in* Rabenh. Krypt. Fl. V. Deutschland. Ed. 2, Bd. 1, Abt. 9, p. 34.
Helminthosporium avenae (Br. & Cav.) Ravn, 1900, *in* Bot. Tidsskr. v. 23, p. 212–213.
Helminthosporium gramineum of Ritzema Bos, Frank, Fraser not Rabenhorst.

In 1889 Briosi and Cavara[6] distributed specimens of a fungus collected near Pavia, Italy, where it was found parasitic on the leaves of oats, *Avena sativa* L., producing narrow, oblong, longitudinally elongated, olivaceous foliar spots, with dark margins. The infection was described as starting ordinarily at the tip of the leaf, where the first spots appear, and from whence the mycelium gradually invades the leaf parenchyma, until the entire blade withers and dies. The injury to the foliage thus occasioned was reported to interfere with the full development of the seeds. Briosi and Cavara designated the fungus itself as *Helminthosporium teres* Sacc. forma *avenae-sativae*, differing from the typical species by the greater length of the conidiophores, the occurrence of the latter singly instead of in fascicles, and the somewhat smaller dimensions of its spores. In the brief diagnosis of the form, accompanied by figures, the conidiophores are described as scattered, stout, cylindrical, many-septate, fuliginous, measuring 150 to 200 by 9 to 12 μ; the spores as acrogenous, olivaceous, cylindrical, or slightly swollen in the middle, rounded at the ends, 4 to 6 septate, and measuring 80 to 110 by 15 to 16 μ. Their representation of the septa and spore wall as thick structures indicates that Briosi and Cavara used dead material for their studies, a circumstance to which may be attributed, perhaps, the inexact description of the color of the spore, and their failure to mention the more distinctive features of the fungus.

Eidam (*35*), in 1891, published an account of a leafspot of oats occurring in Silesia and affecting usually the first leaf, but sometimes also the second and third leaves of the host. He recognized the causal parasite as a new species, *Helminthosporium avenae*, distinct from his *H. hordei* (= *H. teres* Sacc.) because of the negative results obtained in his attempts to infect barley with the former, and oats with the latter. Ritzema Bos (*121*) later described an attack upon oats by *H. gramineum* Rab., the resulting foliar spots differing from those occurring on barley in being short, somewhat round, and associated with a reddish color of the diseased leaves. Ravn (*115*), as the result of comparative cultural and biometrical studies of the parasites causing stripe, netblotch of barley, and the "Helminthosporiosis" of oats, concluded that the latter represents an independent species.

It is of some slight nomenclatorial interest to note that Ravn, apparently in the belief that Briosi and Cavara's priority in recognizing the

[6] Op. cit., p. 657

parasite as a separate taxonomic entity necessitated the perpetuation of their form name as the specific name, adopted the combination *H. avenae* (Br. & Cav.). The last portion of the form name—*sativae*—he omitted purposely on the ground that it was unnecessary. Lindau (*83, p. 34–35*), presumably because of the patent irregularity in altering a name in such manner, recognized Briosi and Cavara's form name without alterations as the proper specific name, and consequently listed the fungus as *H. avenae-sativae* (Br. & Cav.). However, as the use of an earlier varietal name to replace a specific name (when the variety is raised to specific rank) is not sanctioned by present usage, it is obvious that both Ravn's and Lindau's combination are equally unauthorized, and that the proper combination is evidently the one established by Eidam.

In this connection, it may be mentioned that Cooke (*23*), in 1889, described as *Helminthosporium avenaceum* Curtis Herb., a fungus occurring on straw in the United States (oat straw according to Saccardo) the conidia of which were characterized as cylindrical to subfusoid, pale honey-colored, and measuring 75 to 85 by 15 μ. Harkness (*50*) mentioned *H. avenaceum* Curt. as having been found on Avena, at San Francisco, in April. Atkinson (*4*) records having collected a fungus to which he applies the same name, in Mississippi, on June 26, 1891. It is not impossible that these authors were dealing with the fungus originally described from Italy, inasmuch as the brief diagnosis published by Cooke is not greatly at variance with that of Briosi and Cavara, and in purely morphological details nearly as satisfactory for the parasite causing leaf spot of oats as the latter. Since in none of the American writings was the fungus associated with any lesions in the living plant, such possible identity can not readily be established. The question is further complicated by the occurrence of forms of Helminthosporium, associated with sclerotia appearing saprophytically in considerable quantity on oat straw in spring, and evidently representing immature perithecia of Pleospora or Pyrenophora. The writer has investigated two such forms collected near Madison, Wis., in 1919 and 1920, one of which seems to be similar to or identical with *H. avenae*, while the other is entirely different, its small, dark olivaceous, strongly curved, 5-septate spores germinating by the production of 2 polar germ tubes. Further details may be published in a later paper.

In 1895, Harvey (*51*) published a brief account of a disease of oats found in Maine during the preceding two seasons, manifested apparently by premature yellowing of the foliage, and the subsequent production of dark brown spore masses that appeared as small dark dots or lines upon the affected leaves. The fungus in question was a species of Helminthosporium, which Ellis, to whom material was sent, identified as *Helminthosporium inconspicuum* C. & E. var. *brittanicum* Grove. Unfortunately, however, as the only statement regarding the host relationship of Grove's variety in the diagnosis given by Saccardo (*128, v. 4, p. 411–412*) refers to fading grass leaves without any mention of species or genus, it is quite impossible, with the paucity of morphological detail, to identify it with any one of a considerable number of fungi.

Ellis's identification might be supposed to indicate that the Maine fungus corresponded in some measure with the diagnosis of the Warwickshire form:

Effusum bruneolum, hyphis subflexuosis, vix nodulosis, 4–5 septatis, pallide brunneis, 160–180=10 μ; conidiis elongis, diaphanis, endochromate brunneolo diviso, dein 3–5 septatis, 60–100=18–22 μ.

That the correspondence is not especially close is indicated by Harvey's statement that the spores of his fungus were somewhat shorter, measuring 40 to 80 by 15 μ, and sometimes less frequently septate, namely, 1 to 5 times. And his figure of the spore, showing this body as an ellipsoidal, comparatively closely septate structure does not suggest any close resemblance either to *H. inconspicuum* C. & E. or to *H. teres*, between which Grove's variety was reported as occupying a median position. Nor is it any more suggestive of *H. avenae*. On the assumption that only one parasitic species of Helminthosporium occurs on cultivated oats, Harvey's fungus might perhaps nevertheless be supposed to be identical with the one distributed by Briosi and Cavara. An examination of specimens of diseased oats collected near Bloomington, Ill., in June, 1920, bearing conidiophores and conidia of a type somewhat different from that generally found characteristic of the oat leaf-spot fungus, has, however, made the writer inclined to believe that such an assumption might probably prove to be incorrect.

The disease caused by *Helminthosporium avenae* is widely distributed. It was reported early from Germany (*35*), Austria (*56*), Denmark (*115*), Belgium (*88*), Holland (*121*), and Italy. Fraser (*45*), in 1913, found the "stripe disease of barley" severely affecting oats in Quebec. Anderson (*1, p. 105*) reported *H. avenae* from Alaska. According to records of the Plant Disease Survey the Helminthosporium leaf spot of oats appears to have been observed in New York, Pennsylvania, Indiana, Wisconsin, Louisiana, Iowa, Minnesota, Nebraska, Montana, Idaho, and Washington. Undoubtedly, it occurs at least in all the northern States to a greater or less extent; the writer, for example, has found it quite abundant in Connecticut and Maine during the season of 1921, apparently wherever the host was cultivated. Yoshino (*161*) reported *H. avenae* as occurring in Japan, and Butler (*19*) records leaf spot as being very common in India, especially on young plants.

Ravn's investigations have shown that, in general development, the disease follows the same course as netblotch of barley. The infection of the seedling during the germination period results in the production of primary lesions on the first leaf; from the primary lesions the fungus spreads to the foliage, is disseminated later by successive generations of spores, and finally the maturing fruit is infected or contaminated to propagate the trouble the next season. The local symptoms of infection, on the other hand, however, are quite different. Instead of numerous spots exhibiting an irregular reticulate pattern of short accentuated lines or streaks, the affected oat leaves rarely show more than 3 or 4 brown spots. (Pl. 4, A.) It is true that the colored figure in Butler's manual shows a large number of lesions on a single leaf. Such a heavily spotted condition certainly has never been observed by the writer, and, perhaps, may be associated with a more severe manifestation of the disease in India.

The spots may be broad and irregular, or long and narrow; in any case, the margins are frequently poorly defined, merging gradually into yellow, reddish, or orange shades which eventually spread over a large portion of the leaf blade. The gradual extension of diffused yellow and reddish discoloration appears to be coincident with the progress of the fungus in the affected tissue. In the absence, usually, of extensive brown conspicuously abnormal spots, the morbid decline of the leaf, due to the development of the parasite, simulates withering occasioned by weather conditions or maturation much more closely than in any of the three Helminthospo-

rium diseases of barley. As a result, the damage to the oat crop due to leafspot, although undoubtedly not of major importance, is much more likely to be considerably underestimated than that caused by the majority of related diseases of other cereals. Thus, during the latter part of June, 1920, the oats observed by the writer growing in isolated patches in the eastern half of Long Island bore only meager evidence of being affected by leafspot. Nevertheless, four weeks later, microscopical examination revealed the fructifications and spores of *H. avenae* on more than half of the mature plants, sometimes in considerable quantity.

After the death of the affected leaf, the red and orange pigmentation very largely disappears, being replaced by a pale-yellow or gray color, and even the brown discoloration usually loses some of its intensity. At this point the conidiophores of the fungus make their appearance. As Ravn has pointed out, these structures (Pl. 4, Da-g) are very similar to those of *Helminthosporium teres* in all respects—color, dimensions, septation, and mode of emerging from the epidermis—although exhibiting sometimes a slightly greater tendency toward occasional branching (Pl. 4, Dc, De). The spores also resemble those of *H. teres* very closely, having approximately the same range in size and number of septa; and showing the same irregularly cylindrical shape, hemispherical ends, subhyaline to light fuliginous coloration, and mode of germination by the production from intermediate as well as end segments, of laterally or obliquely oriented germ tubes.

The writer was unable to confirm Ravn's finding that the conidia of *H. avenae* slightly exceed in length those of *H. teres*. In general, the dimensions of the two species appeared quite equal, and whatever slight inequality in length and width was observable was rather in favor of *H. teres*. It must be mentioned, however, that the fresh material used by the writer in the study of the two forms was not developed under comparable conditions, that of *H. teres* having been collected late in October during a damp, cool period seemingly especially favorable for sporulation; while the material of *H. avenae*, collected late in July, had manifestly developed at midsummer temperature. Perhaps the two fungi might better be regarded as biological forms of the same species, in the same sense in which such forms are recognized in *Puccinia graminis* and in the mildews. Whatever slight morphological differences, demonstrable in the conidial stage by biometrical methods, may exist between the two fungi, they could scarcely be of a larger magnitude proportionally than the differences between various biological forms of, for example, the stem rust fungus. A study of the ascigerous form of *H. avenae*, which, as has been suggested, the writer believes he has collected, although in very poor condition, ought to cast some light on its taxonomic relationship. For the present, it is advisable to follow Eidam and Ravn in regarding the parasite on oats as distinct from *H. teres*. Certainly the idea, proposed by Briosi and Cavara, of placing it as a morphological "forma" of *H. teres* is not tenable.

It may not be amiss to call attention to an error apparently caused by a partial misinterpretation of Ravn's paper, and more particularly of his widely copied but, perhaps, insufficiently representative figures of the conidia and conidiophores of *Helminthosporium gramineum*, *H. teres*, and *H. avenae*. In some general treatises, the impression is conveyed that the possible morphological difference between *H. avenae* and *H. teres* is approximately of the same order as the difference between *H.*

teres and *H. gramineum*. The differences between the latter two species are, in reality, altogether much larger. For example, while the largest spores of the fungi causing netblotch and leafspot of oats, seen by the writer, have measured between 170 and 175 μ, the longest spores of the stripe fungus have not been found to exceed 105 μ in length. The number of septa in the spore of the stripe fungus very rarely exceed 7, while in the other two species 8 and 9 cross walls may be found quite readily and even 10 or 11 occur in a small proportion of instances. Production of secondary spores, common in *H. gramineum*, is rare in *H. avenae* or *H. teres*. In short, *H. gramineum* is not closer to either *H. avenae* or *H. teres* than a moderate number of other congeneric species.

As leafspot usually is not a serious disease, and in the United States not very conspicuous in its manifestations, little attention has been paid to its control. Atanasoff and Johnson (*3*) have found the hot-air seed treatment effective in reducing the disease. The value of methods of control based on seed disinfection would appear to be contingent, at least to some extent, on the absence of a possible sclerotial or ascigerous stage, or the relative ineffectiveness of such a stage as a factor in the propagation of the fungus.

HELMINTHOSPORIUM TRITICI-REPENTIS DIEDICKE—PYRENOPHORA TRITICI-REPENTIS (DIED.)

Helminthosporium gramineum Rab. f. sp. *tritici-repentis* Diedicke *olim* 1902, *in* Centbl. Bakt.[etc.] Abt. 2, Bd. 9, p. 317–329.
Pleospora tritici-repentis Died. 1903, *in* Centbl. Bakt.[etc.] Abt. 2, Bd. 11, p. 52–59.
Pleospora trichostoma (Fr.) Wint. f. sp. *tritici-repentis* Noack 1905, *in* Ztschrif Pflanzenkr., v. 15, p. 193–205.

Helminthosporium tritici-repentis as recognized by Diedicke (*28*), first, as a biological or form species of *H. gramineum* Rab. and later (*29*), as an independent species, distinct from *H teres*, *H. bromi*, *H. gramineum*, and *H. avenae*. It appears to be widely distributed, its perithecial form occurring very abundantly in our northern latitudes on the dead culms of quack grass, *Agropyron repens*. The visible effects resulting from the attack of the fungus on growing plants of quack grass are usually not at all conspicuous, any dark discoloration like that induced by the parasitism of *H. sativum* on the same host, being absent. The affected leaf gradually loses its green color and withers from the tip downward, changing at the same time first to a pale yellowish, and later to a gray color. As the foliage of the host developed after the earlier stages of growth is relatively rigid, the mechanical distortion associated with the death of any except the lower and more delicate leaves (Pl. 5, A) usually is not very noticeable.

According to Diedicke, the disease resembles barley stripe in affecting the whole plant, usually suppressing the development of the inflorescence, or preventing its emergence from the enveloping sheath. Although this opinion is not without some plausibility, anatomical evidence regarding the distribution of the fungus in the tissues of the plant would appear to be necessary before the disease can be regarded as systemic in the same sense as stripe. Some differences in the manifestations of the two diseases certainly are apparent. The symptoms do not become evident simultaneously in all the leaves of individual quack-grass plants, but are manifested first in the basal leaves, and later may appear in successively higher foliar organs. Nor do diseased leaves of *Agropyron repens* exhibit anything similar to the longitudinal variegation char-

acteristic of the foliage of barley infected with *H. gramineum*. The conidial fructifications of *H. tritici-repentis*, moreover, make their appearance scattered sparsely here and there over the surface of the dead leaves, not crowded on the affected parts like those of the stripe fungus. It is evident, therefore, that at least in some respects, the development of *H. tritici-repentis* on the host presents closer analogies to types like *H. dematioideum* and *H. siccans* than to *H. gramineum*.

The dark olivaceous usually 3 to 6 septate sporophores of *Helminthosporium tritici-repentis* (Pl. 5, Da-f) emerge from the stomata, or, more frequently, from between epidermal cells. Above the somewhat swollen basal segment, they vary in width from 7 to 9 μ, being thus perceptibly inferior in this dimension to the homologous structures of *H. bromi*. In length they vary from 80 to 220 μ. The conidia (Pl. 5, Ba-m) are typically subhyaline, straight-cylindrical, from 1 to 9 times septate, from 12 to 21 μ in diameter, and 45 to 175 μ in length. As in *H. teres*, the septa usually are associated with slight but perceptible constrictions in the contour of the thin peripheral spore wall.

The most distinctive peculiarity in the spores of this species is found in the shape of the basal segment, the proximal portion of which usually tapers abruptly in the manner of a cone to be rounded off or flattened near the hilum. In profile the basal segment thus is remotely suggestive of the horizontal aspects of the head of a snake, while the distal end usually is rounded off in a hemispherical form (Pl. 5, Ba, e, j, k). It may not be superfluous to add that departures from this type are not infrequent. The 2 or 3 terminal segments may taper appreciably toward the distal end (Pl. 5, Bb, c, d, m) or the width of the different segments may vary considerably (Pl. 5, Bg, m) or the axis of the spore may be somewhat curved (Pl. 5, Bc). Germination begins within an hour after the spores are mounted in water. Each segment is capable of producing a germ tube, the larger spores (Pl. 5, C) thus producing from 6 to 8 or more germ tubes, although usually one or several cells may remain seemingly inert.

The ascigerous stage of this species of Helminthosporium, as mentioned before, occurs in great abundance on the dead culms of *Agropyron repens* and to a small extent also on the leaves, particularly on the sheaths. (Pl. 6, Ba-b.) Although the black perithecia are readily discernible in autumn, their subsequent maturation appears to be rather slow. In the vicinity of Madison, Wis., mature perithecia have been collected from early in April until well toward the end of May. When developing in the looser tissue of the leaf, especially after some decay has taken place, the imbedded portion usually is distinctly subspherical, from 0.2 to 0.35 mm. in diameter, and tapers into a short, well-defined beak as shown in Plate 6, A. However, when developing in the harder tissue of the culm, the perithecium usually is much more irregular in shape and the beak less readily recognizable as a special modification. In any case, the beak usually bears near the ostiole a number of dark-brown sterile setae which may be straight or flexuous, continuous or several times septate, occasionally branched, and varying in number from several to over a score. As Diedicke pointed out, under certain conditions a large number of conidiophores and conidia may be produced from the upper side of the perithecium, the further development of the ascospores, if not complete, then often being checked altogether. This tendency toward conidial production usually is well expressed in the case of those perithecia found on the upright culms; on the other hand, when the host material is loosely

covered with leaves or other rubbish, it often is entirely suppressed. There can be little doubt that the environmental factors of temperature and moisture are of primary importance in this connection.

Diedicke recognized this perithecial form as a new species, *Pleospora tritici-repentis*, which he distinguished from *Pleospora bromi* largely because of the smaller dimensions of its perithecia, asci, and spores, and because of the failure of reciprocal inoculations to produce infection. Noack (*95*) did not accept the taxomonic dispositions made by Diedicke but regarded the perithecia on *Bromus asper* and *B. inermis* as not different morphologically from those on *Agropyron repens*, or from the perithecia on barley presumably associated with the stripe fungi. He consequently reduced the parasites on quack grass and on the two species of Bromus to biological forms of *Pleospora trichostoma* (Fr.) Wint., which, like Diedicke earlier, he identified as the ascigerous stage of *Helminthosporium gramineum*. The fungi under consideration were accorded a dubious status in the general works published subsequently, like those of Lindau (*83*) and of Stevens (*139*).

A comparison of material collected by the writer near Madison, Wis., in the spring of 1920, showed the general correctness of Diedicke's statements regarding the relative sizes of the perithecia and asci of *Pyrenophora tritici-repentis* and *Pyrenophora bromi*. As the diameter of the perithecia of the parasite on quack grass appears to vary from 0.20 to 0.35 mm. the inequality in size between the fruiting bodies of the two forms certainly is not pronounced. In *Pyrenophora tritici-repentis*, especially when developing on decaying leaves, the ostiolar beak usually is narrower, less massive, and consequently a somewhat more distinctive structure than in *Pyrenophora bromi*. The asci (Pl. 6, C) of *Pyrenophora tritici-repentis*, measuring usually from 170 to 215 by 43 to 50 μ, are similarly somewhat smaller than those of *Pyrenophora bromi*.

It may be mentioned that measurements of asci in fully matured living material are dependent to a considerable extent on the amount of moisture present, as these structures under dry conditions usually are found contracted tightly over the spores, while under moist conditions they swell until the space inside of the fruiting bodies is completely occupied. In any case, on being crushed out of the perithecia, they undergo very considerable swelling preliminary to the rupture of the ascus wall and the discharge of the eight ascospores. The latter, of a brownish color and measuring usually 18 to 28 by 45 to 70 μ, are uniformly three times transversely septate, often with one or both of the middle segments further divided by a longitudinal wall. The septa are associated with constrictions in the peripheral wall of the spore, which frequently is found enveloped in a gelatinous covering. (Pl. 6, Da, Db, Eb.)

Germination takes place promptly by the production of a germ tube from several or all segments. (Pl. 6, Da–b.) On media ordinarily employed in laboratories, like potato agar, a fairly compact mass of white aerial mycelium is produced, corresponding in all respects to the growth obtained by the use of conidia. Anastomosis with the resultant production of groups of inflated lobulate segments is abundant in the submerged mycelium, but large sclerotia or imperfect perithecia of a size readily visible to the naked eye, like those produced by *Helminthosporium bromi*, have never been observed in pure cultures of *H. tritici-repentis*.

The parasite on quack grass consequently is to be regarded not as a biological race of *Helminthosporium gramineum* but as an independent morphological species. While the ascigerous stage bears a strong resemblance to *Pyrenophora bromi*, as well as to a number of other congeneric forms found on graminaceous hosts, it is readily distinguishable at least from *Pyrenophora teres*. On the other hand the conidia which, like those of *H. teres* and *H. avenae*, show a range in length intermediate between the range of this dimension in *H. gramineum* and *H. bromi*, can not possibly be mistaken for the conidia of any of these species because of the characteristic contour of the basal segment.

HELMINTHOSPORIUM CATENARIUM, N. SP.

During the latter part of September, 1920, the writer kept under observation a stand of wood reed grass (*Cinna arundinacea* L.) near Brooklyn, N. Y., on the northern coast of Long Island. Although the season had not been a dry one, the grass, nevertheless, showed symptoms suggesting drought injury. The distal portions of most of the leaves had withered completely (Pl. 7, A) and, in some instances, the injury involved more than half of the blade. Less frequently, the foliar organs exhibited longitudinally elongated dry areas or spots within healthy green parts. No indication of any discoloration, either at the margins of the lesions or in the dried portions, was ever observed. As the disease presented many of the characteristics of white blast, quite common on sweet corn in the trucking district in the vicinity of New York City, some of the affected leaves were collected and examined in the laboratory. The microscope revealed an abundance of Helminthosporium fructifications on the affected leaves, particularly on those parts that had apparently been dead for some time.

As in other species of Helminthosporium occurring on graminaceous hosts possessing foliage with a relatively firm epidermis, the sporophores of the fungus on *Cinna arundinacea* are found to emerge very largely from the stomata. (Pl. 7, Ea-c.) Beyond being conspicuously thin walled, and rather unusually strongly geniculate at the points of attachment of the conidia, they exhibit no especially distinctive features.

The spores, which are colorless to light yellowish, however, show such a large measure of variability in shape and apparent development, that the fungus is easily recognized as one of the more aberrant and peculiar members of the genus. A considerable proportion of the spores are of moderate length, straight and tapering (Pl. 7, Ba, d, j), and perhaps could not be readily distinguished from those of *Helminthosporium dictyoides*, although the latter eventually become more deeply tinged with yellow. Usually only the shorter spores appear both straight and uniformly tapering. Generally, those in excess of 80 μ are very perceptibly curved or bent in an irregular manner, and, in addition, the diameter of the different segments varies to a very considerable extent. The longer spores often show a decided median constriction. (Pl. 7, Bb, e.)

Frequently, a secondary spore (Pl. 7, Bi) is found attached at the tip of a primary one (Pl. 7, Bh), being characterized by smaller dimensions and, if immature, by the absence of septa. That the formation of secondary spores is not an unusual occurrence is indicated by the presence of a dark conspicuous scar at the tip of many spores, quite similar to the basal hilum and often associated with a peculiar prolongation of the distal

portion of the terminal segment, giving the spore a general contour crudely suggesting that of a rifle cartridge with contracted tip. (Pl. 7, Bc, f.) It thus will be seen that while the smaller spores resemble those of *H. dictyoides*, and in a measure those of *H. gramineum*, the larger ones are of a length not attained by the spores of any of these species, approximating most closely that of the spores of *H. tritici-repentis*. (Pl. 7, Bb.) The spores of the parasites on *C. arundinacea* and on *Agropyron repens*, moreover, show additional similarity in respect to coloration and general shape, both being subhyaline, as well as more or less irregularly curved and of variable width. The spores of *H. tritici-repentis*, however, have not been observed to show an apical hilum or apical attenuated prolongation; nor has the fungus on wood reed grass exhibited the peculiar modification of the basal segment characteristic of *H. tritici-repentis*.

In pure culture, on potato agar or corn-meal agar, the parasite on *Cinna arundinacea* grows readily, producing a white mycelium, both in the form of erect compact tufts 2 to 5 mm. in height and usually developed at the point of inoculation, or of minute superficial flecks scattered sparsely over the surface. In any case, the hyphae usually remain sterile for a number of days before the spores begin to develop terminally. After this stage is reached the sporophore, instead of developing by alternately elongating and proliferating spores, as is usual in the genus, usually develops in the manner somewhat similar to that characteristic of members of the genus Alternaria. The tip of the primary spore (Pl. 7, Dba) may bud to produce a sessile secondary spore (Pl. 7, Dbc) and this may produce a tertiary spore in the same way. Not infrequently, however, the tip of the spore (Pl. 7, Dbc) grows out into attenuated segments (Pl. 7, Dae) having approximately the diameter of the primary sporophore and obviously of a similar nature. These segments usually never attain any great length before proliferating a terminal spore (Pl. 7, Dbf); and as growth continues, fructifications result, consisting of superimposed spores and sporophoric segments, that may exceed 0.5 mm. in height. As the basal and distal segments of the spores not infrequently produce short sporophoric branches directed at oblique angles to the main axis (Pl. 7, Dab, ac, ad, af) and bearing one or more spores (Pl. 7, Dbd, be, bg), the fructifications usually are further complicated by the presence of a number of lateral sporiferous processes. It may be mentioned in this connection that the distinction between spore segment and sporophoric segment is not always well defined, but often may be partly obliterated, the fructification then being represented by a process of segments varying from 6 to 18 μ in thickness, and disarticulating at certain constricted septa marked by the presence of hila.

As far as the writer is aware, the parasite on *Cinna arundinacea* has not been described in literature. Atkinson (*4*), it is true, reported *Helminthosporium turcicum* as occurring on the leaves of this host in Alabama. While it is not impossible that Atkinson may have been dealing with the same fungus that causes injury to maize, there would seem to be much more probability in the assumption that this writer was confronted with the fungus under consideration, although with the exception of the pathological effects occasioned by them, the similarity between the two parasites can hardly be said to be a close one. Because of its tendency toward the formation of chains of spores in nature as well as in pure culture, the fungus on wood reed grass is named *H. catenarium*.

DIAGNOSIS

Helminthosporium catenarium, n. sp.

Attacking the foliage of *Cinna arundinacea*, L., where it causes the premature death of large areas of tissue, the tip and margin being usually most commonly affected.

Sporophores brown or olivaceous; emerging usually from the stomata, singly or in groups of 2; measuring 5 to 8 by 60 to 200 μ; producing the first spore at a distance of 25 to 60 μ from base, and successive spores at intervals of 15 to 30 μ, the point of attachment marked by scars at the apices of pronounced geniculations.

Spores 1 to 10-septate, the septa sometimes associated with slight constrictions or irregularities in the contour of the thin peripheral wall; subhyaline to light yellowish, 14 to 18 by 30 to 200 μ, measured at their maximum diameter; the shorter ones usually straight, widest at the basal or second segment, tapering uniformly to approximately half the maximum diameter at tip; the longer ones often perceptibly crooked, irregular in diameter, frequently showing both a basal hilum and an apical scar, the apical scar marking the attachment of a secondary spore, and usually associated with a peculiar modification of the distal portion of the terminal segment, consisting in the prolongation of the latter at a uniform diameter representing the minimum width of the spore. Secondary spores or spores of a higher order of the same diameter, but usually considerably shorter, less frequently septate, or continuous. Both types germinating normally by production of 1 or 2 lateral germ tubes from basal segment, and a single lateral or oblique tube from terminal segment. Contour of basal end hemi-ellipsoidal, of distal end hemispherical; hilum and apical scar not protruding.

In pure culture on potato glucose agar, aerial mycelium white or dirty yellowish, present as dense erect tufts 2 to 5 mm. high at point of inoculation and as small flecks scattered sparsely over the surface; in either case consisting of sterile hyphae and an increasing number of conidial fructifications; the latter arising on the expanded terminations of hyphae not otherwise much modified, and consisting of a series of successively proliferated spores that may be either sessile or separated by intercalary, narrower sporophoric segments. The fructification frequently branching, as a result of the proliferation of lateral or oblique sporophoric processes from the basal or terminal segment of individual spores; and, less typically, sometimes consisting of miscellaneous processes of segments varying from 6 to 18 μ in thickness, and disarticulating at constricted septa marked by the presence of scars or hyla.

HABITAT.—Parasitic on *Cinna arundinacea* L. collected at Douglaston, N. Y., September, 1920.[7]

HELMINTHOSPORIUM BROMI DIEDICKE—PYRENOPHORA BROMI (DIED.)

Helminthosporium gramineum Rab. f. sp. *bromi* Diedicke *olim* 1902, *in* Centbl. Bakt. [etc.] Abt. 2, Bd. 9, p. 317–329.

Pleospora bromi Died 1903, *in* Centbl. Bakt. [etc.] Abt. 2, Bd. 11, p. 52–59.

Pleospora trichostoma (Fr.) Wint. f. sp. *bromi* Noack 1905, *in* Ztschr. Pflanzenkr. v. 15, p. 193–205.

The occurrence in Germany of a species of Helminthosporium parasitic on *Bromus asper* Murr. was recorded by Diedicke (*28*) in 1902. Krieger [8] later collected and distributed the same fungus on the leaves of *Bromus inermis* Leyss. It undoubtedly is on the latter host that the parasite is found most commonly in the United States, observations made by the writer in the vicinity of Madison, Wis., during the spring of 1920, pointing toward its general prevalence on this widely distributed host. Diedicke described the disease symptoms occasioned by the fungus so adequately that little can be added except in the way of corroboration.

Helminthosporium bromi is probably one of the earliest of all parasites affecting the grasses of our northern latitudes, to appear in spring, as

[7] Type specimens of all species described in this paper as new have been deposited in the following herbaria: Office of Pathological Collections, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.; Cryptogamic Herbarium, Harvard University, Cambridge, Mass.; herbarium, New York Botanical Garden, New York City; herbarium, Department of Botany, University of Wisconsin, Madison, Wis.

[8] KRIEGER, W. FUNGI SAXONICI. No. 1941 *Helminthosporium bromi* Died. [exsiccati]. 1903, 1905.

the first leaves that arise from the overwintering rootstock of *B. inermis* have barely completed their development before thay begin to show the scattered spotting characteristic of its attack. (Pl. 8, Ab–c.) Each spot originates as a minute dark brown or black speck about which the chlorophyll gradually appears to break down, producing a yellowish or nearly colorless halo. Both the central spot and the surrounding zone usually increase considerably in extent, especially in a longitudinal direction, until the former may occupy an area 2 mm. in width and 6 mm. in length. On badly infected leaves the yellowed zones frequently run together as shown in Plate 8, B. Even more moderate infection, however, leads to a premature withering of the leaf (Pl. 8, Ad), beginning at the tip and proceeding downward, until the whole structure may be involved; for although the sheath is much less frequently spotted, it is not entirely immune, especially while the plant is still very young. After the leaf has become withered the sporophores make their appearance, first on or near the darkened areas, but later quite generally over the entire leaf. Emerging singly or in clusters of two (Pl. 8, Ea–c), most frequently between epidermal cells without much reference to the stomata, they measure 7 to 10 μ in diameter and usually 100 to 150 μ in length, although sometimes attaining a maximum length of 250 μ. From 2 to 6 septa usually are present. The geniculations associated with the production of successive spores are not generally very pronounced.

In the account given by Diedicke (*28*) the spores are described as 4 to 6 septate, 108 to 150 μ long, 13 to 20 μ in diameter, and exactly similar to those described and figured by Ravn (*115*), the supposed similarity presumably applying to *Helminthosporium teres*. Such a statement of the morphological features of the spores of the two species, it is quite impossible to substantiate by a comparison of American material, which reveals instead very decisive differences. The contrast in length between the two species is especially conspicuous, the spores of *H. bromi* measuring from 45 to 265 μ in length, examples exceeding 200 μ in length being of not uncommon occurrence. (Pl. 8 Ca-f.) Some difference in diameter also is perceptible, although this is much less pronounced, the spores of *H. bromi* varying commonly from 14 to 26 μ. As the number of septa in the spores of both species varies from 1 to 10, it is apparent that the individual segments in the spores of *H. bromi* exceed in length those of *H. teres* in approximately the same measure as do the spores. In *H. teres*, moreover, the spore often is conspicuously constricted at the septa, a modification absent or less strongly pronounced in those of *H. bromi* (Pl. 8, De). Related to these features are the specific peculiarities in the contour of the basal cell that have been mentioned in another connection, this contour being approximately hemi-ellipsoidal in *H. bromi* and hemispherical in *H. teres*.

In the conidia of *Helminthosporium bromi*, the hilum is represented by a scar, not especially conspicuous, and included entirely within the contour of the peripheral wall. As the peripheral wall consists of a relatively thin membrane, it is not surprising that the conidia show little resistance to unfavorable conditions. Even in actively sporulating material, microscopic examination reveals one of several dead segments in most of the spores, and when material is kept in the laboratory the proportion of dead segments or dead spores is very greatly augmented in the course of a week. When mounted in water, viable spores germinate

very promptly, producing lateral or oblique germ tubes indiscriminately from middle and end cells, the number of tubes originating from any particular segment not usually exceeding two. Anastomoses of newly proliferated germ tubes are not infrequent, the germination of spores lying in juxtaposition, thus often yielding scalariform figures united by several hyphal connections. (Pl. 8, Db–c.) As has been pointed out previously, when the fungus is cultivated on potato agar media, anastomosis of hyphae is associated often with the production, below the surface of the substratum, of numerous groups of inflated segments. Of the latter, a small proportion develop into subspherical sclerotia, usually from 0.2 to 0.5 mm. in diameter, the presence of which in agar cultures (Pl. 9, C) is characteristic of the fungus, and which, judging from their size and structure, doubtless represent immature perithecia.

In Germany, Diedicke (*28*) noted the appearance on *Bromus asper* of the sclerotia or young perithecia as early as July 26. This date corresponds quite closely with their appearance in southern Wisconsin. When fully matured, they consist of a subglobose portion imbedded within the somewhat distended leaf tissue, usually from 0.3 to 0.4 mm. in diameter and tapering into an irregular ostiolar beak protruding approximately 0.1 mm. above the broken epidermis. A variable number of septate, tapering, sterile bristles usually are to be found near the tip of the ostiolar modification; and although it is not improbable that in some seasons conidiophores may be produced abundantly from the perithecium as in *Pyrenophora tritici-repentis* and *P. teres*, only a sparse production was observed during the season of 1920.

The development of the asci appears to be delayed until spring, beginning evidently with the warmer weather usually experienced in our northern latitudes late in February or early in March, and proceeds slowly, until toward the middle of April they readily discharge their spores when mounted in water. In all morphological details the asci closely resemble those of *Pyrenophora tritici-repentis*, although they usually seem to be somewhat larger. The difference, which is not pronounced, becomes, perhaps, most evident during the process incident to to spore discharge, as the asci of the parasite on brome grass appear to undergo perceptibly greater distention before the outer membrane is burst than the asci of the congeneric parasite on quack grass. Plate 9, D, representing an ascus partly distended, measuring 300 μ in length and 65 μ in diameter, shows the 8 spores in distichous arrangement immersed in granular epiplasm, the thickened ringlike modification at the apex, and the short well-defined stipe common to both species.

The ascospores, light brown in color and measuring 20 to 30 μ by 45 to 72 μ, are uniformly divided by 3 transverse septa. Longitudinal septa may be absent, or one or both of the middle segments may be further divided by a longitudinal wall. Germination takes place by the production of a germ tube from several or all of the segments. Cultures of the fungus derived from ascospores differ in no particular from those derived from conidia.

Because of the distinctive and extraordinarily large conidia characteristic of the parasite and its production of sclerotia on various kinds of agar media, the writer agrees with Diedicke in regarding the fungus as an independent species. In this connection it may be mentioned that Diedicke (*28*) first reported it as a biological species of the stripe fungus, a disposition which Noack (*95*), who recognized *Pleospora trichostoma* as the ascigerous stage of *Helminthosporium gramineum*, later sought to

maintain. The ascigerous stage, it must be admitted, is morphologically not readily distinguished from related congeneric forms. The fungus, in any case, would seem to be referable to Pyrenophora rather than to Pleospora, if, indeed, the former is to be maintained as a separate genus.

HELMINTHOSPORIUM GIGANTEUM H. & W.

Heald and Wolf, in 1911, described (*54*) and later also (*55*) figured a species of Helminthosporium on Bermuda grass (*Cynodon dactylon* L. = *Capriola dactylon* [L.] Kuntze) collected at Falfurrias, Tex., where it was found associated with a disease—

characterized by the presence of numerous yellowish or pale straw-colored spots, 0.5 to 1 mm. wide, by 1 to 4 mm. long, longitudinally elongated, and with a narrow brown border. The spots are generally absent from the leaf sheath, and when numerous they may become confluent on the lamina and thus cause somewhat extended dead areas.

The fungus, which was named quite appropriately *Helminthosporium giganteum*, was further characterized as follows:

The conidiophores are dark brown, many-septate, 9 to 12 by 200 to 400 μ, with a slightly bulbous base; the spores are elongated, cylindrical with slightly tapering ends, 5-septate, pale brown, densely granular contents, 15 to 21 by 300 to 315 μ.

The writer collected the same parasite on Bermuda grass at various times during the months of February, March, and April, 1921, near Fort Myers and Wauchula, Fla. During February and March, especially in the vicinity of Fort Myers, the fungus occurred in considerable abundance, old spots bearing sporophores being found scattered generally over the foliage of the host. Fresh lesions providing material suitable for study were almost entirely absent at that time, a circumstance attributable apparently to the condition of the host; for the latter, although green, was not actively growing and the foliage, moreover, was everywhere severely affected by *Puccinia cynodontis* Desm. and *Helminthosporium cynodonti* Marignoni. About the middle of April, several weeks after the resumption of active vegetative growth, the more recently unfolded leaves of *Cynodon dactylon* began to show newly developed lesions associated with conidiophores and conidia in a living state. At nearly the same time altogether similar foliar spots made their appearance on goose grass (*Eleusine indica* [L.] Gaertn.). Microscopical examination revealed no morphological difference in the fructifications present on the two graminaceous species.

On July 13, 1922, a stand of Bermuda grass near Bladensburg, Md., was observed to be attacked by the fungus with unusual severity. Many of the leaves had been killed altogether, and of those that remained functional nearly all bore scores of discrete or confluent spots, or extensive whitened areas which often involved altogether from one-fourth to one-third of the tissues of the individual foliar organs. Plants of *Agropyron repens* distributed in the Bermuda grass likewise bore a sprinkling of the foliar spots characteristic of the fungus, although in smaller quantity. On microscopical examination the similarity of the abundant fructifications on both Bermuda and quack grass collected in Maryland to those of the Florida parasite was readily apparent. *Agropyron repens* and *Eleusine indica*, therefore, represent additional hosts of the interesting fungus described by Heald and Wolf (*55*).

The foliar lesions bear a good deal of resemblance to those produced, for example, by *Helminthosporium leersii*. They first become visible as minute brown spots in green and otherwise healthy tissue. (Pl. 10, A.)

Soon these spots increase in length and width, the center in the meantime fading to almost white or light straw color. In this way the appearance described by Heald and Wolf (*54*) is brought about. (Pl. 10, A, B.) At this stage the lesions are of the simple "eye-spot" type. Under certain conditions, apparently when the parasite is thriving most luxuriantly, the diseased areas become greatly enlarged, sometimes occupying the entire width of the leaf blade of Bermuda grass for a distance of one centimeter or more. When such development occurs the dead areas usually show a number of roughly concentric brown markings, evidently corresponding to successive positions of the margin, and giving the areas a distinctively zonate aspect. With the multiplication of the smaller lesions and the extension of the larger ones, a large proportion of the leaves are killed outright long before the end of the season. Previous to this the sporophores of the fungus make their appearance singly or in pairs over the larger dead regions always at some distance from the margin of the surrounding green tissue. The conidiophores are distributed rather sparsely in comparison with the crowded spacing of the homologous structures of many congeneric forms, although not actually few in number.

The sporophores of *Helminthosporium giganteum* (Pl. 10, Ea, b) are the largest of those of any species studied by the writer, the measurements of the Florida material agreeing well with those given in the original specific description. The septa are mostly spaced with considerable regularity, at intervals varying commonly from 25 to 40 μ. Usually the scar marking the point of attachment of the first spore is found 140 to 250 μ from the base, and successive scars associated with geniculations, usually not pronounced, occur at intervals of approximately 40 μ.

The conidia (Pl. 10 Ca–d), which are produced in relatively small numbers, are easily the most massive of those of any species of Helminthosporium hitherto described, and are probably among the very largest produced by any group of fungi. Individual spores were found exceeding considerably in length even the generous measurements given by Heald and Wolf, the one figured in Plate 10, Cc, for example, measuring 385 μ in length and 21μ in diameter. The volume of a spore of such dimensions is several hundred times greater than the volume of spores of molds that are not by any means regarded as minute fungi, while on comparison with some of the smallest types, like species of Actinomyces, ratios approximately 1 to 300,000 may be obtained. The writer has found it possible, after staining with eosin, to make out with the naked eye, spores approaching the dimensions given.

Besides being the largest, the conidia of *Helminthosporium giganteum* also, perhaps, are the shortest-lived conidia of any species of Helminthosporium discussed in this paper. If the spores from a group of fructifications which are still actively proliferating new conidia are mounted in water, a large proportion will be found to contain one or more dead segments. After spore production in a group of fructifications ceases all of the conidia will contain dead segments, and in a relatively brief period, probably not exceeding two weeks, only a small number will still show living segments. To this lack of longevity, together with the production of spores in small numbers, the comparatively restricted occurrence of the parasite probably may be attributed.

The spores of *Helminthosporium giganteum*, while alive, are altogether hyaline, colorless, and filled with homogeneous protoplasm. The characterization of these structures by Heald and Wolf in the words, "pale

brown, densely granular contents," would seem to indicate that these authors very probably drew their description from dead material. The peripheral wall is thin as in other species with subhyaline spores, and sometimes shows a perceptible constriction at the septa, the latter varying in number usually from 2 to 6 and delimiting segments ranging up to 80 μ in length, and from 16 to 25 μ in diameter. The ends of the spores are rounded off abruptly, showing usually a hemispherical or hemiellipsoidal contour. The basal end is modified by the presence of a minute dark apicular projection at the apex of a small, faintly delimited, obtusely conical part, which, while the spore is still attached, fits into a depression in the center of the scar on the sporophore.

When the spores are placed in water they germinate very promptly, the germ tubes being proliferated sometimes singly and indiscriminately from end or middle segments (Pl. 10, Da, c); or more typically in groups of 3 or 4, usually from both basal and apical ends (Pl. 10, Db), or less commonly from one or more intermediate segments (Pl. 10, Dc). In any case the germ tubes are of unusual width, measuring 6 to 10 μ in diameter, and grow with remarkable rapidity. Germination is associated with a change in the protoplasm from an apparently homogeneous to an abundantly vacuolated structure. It may not be superfluous to add that the spores filled with "densely granular contents" never germinate, and segments exhibiting such structure, always together with a swollen peripheral wall, similarly remain inert.[9]

HELMINTHOSPORIUM DICTYOIDES, N. SP.

During the latter part of June, 1920, the writer observed a disease on meadow fescue (*Festuca elatior*, L.) which seemed to be widely prevalent in the region about Washington, D. C., scarcely any stand of this grass being entirely free from it. As meadow fescue, although not one of our most highly prized forage plants, is nevertheless of not inconsiderable economic importance, and is besides very generally distributed, an effort was made to determine whether the disease occurs also in other sections. In the vicinity of New York City, from early in July to the end of August, it was found to be so common on *F. elatior* that the very characteristic and conspicuous lesions could, in the absence of an inflorescence, be quite safely used to distinguish this species of grass from grasses having somewhat similar foliage as, for example, *Bromus inermis* or *B. secalinus* L. Especially severely affected material was found near Port Washington on Long Island, where the dampness of the atmosphere incident to proximity to the sea, may have favored the progress of the malady. Other collections of diseased material were made at Stamford, Conn., August 2, 1920; Norwood, Mass., November 7, 1920; Lisbon Falls, Me., July 24, 1921; Annapolis, Md., October 15, 1921; and at numerous stations in Maryland, Virginia, and the District of Columbia near Washington, D. C., from early in August until late in October, 1921. Indeed, the writer has never failed to find the disease present to a greater or less extent on meadow fescue in all the localities in the New England and Middle Atlantic States that he has had occasion to visit.

The symptoms of the trouble, a brief account of which was published in 1922 (*32*), are very similar to those induced by *Helminthosporium*

[9] Since this account was written the writer has had opportunity to study the mode of development of the fungus in greater detail and on a considerable number of additional hosts. The results have been partly indicated in a brief note (*33*), and will be published in more complete form in a later paper.

teres on barley. The newly affected green tissues show abundant brownish discoloration in irregular patterns, within which may be recognized a network of darker longitundinal and transverse linear streaks. (Pl. 11, A, C.) The minute reticulate design formed by the latter is, in well developed cases, more extensive and pronounced than in any specimens of barley affected with netblotch which the writer has ever seen. After a considerable portion of the leaf blade has been involved, it gradually withers and dies, the withering beginning at the tip and proceeding toward the base. (Pl. 11, A.) In 1920, in the neighborhood of New York City, such destruction of foliage continued throughout nearly the entire season, and caused an amount of damage that appeared to be far from trivial. Indeed, the writer is inclined to believe that the malady, which may conveniently be designated like that caused by *H. teres*, as netblotch, is the most serious parasitic trouble affecting meadow fescue in our northern latitudes.

On examining the withered portions of affected leaf blades, the cause of the disease is readily recognized as a species of Helminthosporium. To *H. teres*, however, the fungus shows no close similarity, the spores of the barley parasite having dimensions so much greater as to preclude any possibility of confusing the two forms. It shows a much greater degree of similarity to *H. gramineum*. The sporophores, as in the stripe fungus, are found in groups larger than in most congeneric species, the number in a group varying usually from 2 to 6 (Pl. 11, Ea-i). On the other hand, the basal enlargement characteristic of the sporophores of *H. gramineum* appears to be less pronounced in the homologous structures of the form on meadow fescue. The spores of the two fungi possess some characteristics in common, but exhibit quite distinctive specific differences as well. Thus they agree in color, in both species varying from subhyaline and colorless when newly proliferated, to distinctly yellowish when fully mature; and on measuring show an approximately equal range in length. However, the spores of *H. gramineum* are appreciably greater in diameter, and, while manifesting a tendency to taper toward the apex, do not depart very greatly from a straight-cylindrical type, whereas those of the parasite on *Festuca elatior* (Pl. 11, Ba-q) more frequently show a very pronounced diminution in diameter from the base to the tip, and in only relatively few instances approach an approximately cylindrical shape. In germinating the conidia of the fungus on meadow fescue do not generally produce germ tubes indiscriminately from both the end and the middle segments, but typically give rise to a lateral or oblique germ tube from one or both end segments (Pl. 11, Dc-k), although, less frequently, one or more germ tubes may be produced from a middle segment (Pl. 11, Da-b). It also may be mentioned that the spontaneous development from primary spores of short sporophores bearing secondary spores, characteristic of the barley stripe fungus, has not been observed in any material of the parasite on meadow fescue.

Another congeneric form with which the species of Helminthosporium on *Festuca elatior* might possibly be confused is the one described in this paper as *H. siccans* that occurs on *Lolium multiflorum* Lam. and *L. perenne* L. The latter on comparative examination, however, is readily distinguished by the appreciably larger dimensions, conspicuously darker coloration, and less tapering form characteristic of its conidia. These conidia when altogether mature possess, moreover, a considerably thicker peripheral wall, and, in germinating, typically produce two germ tubes from each of the end segments instead of one.

The literature contains, as far as the writer is aware, only two references to the occurrence of a species of Helminthosporium on *Festuca elatior*. The earliest is that of Diedicke (*29*), who recorded his discovery of a parasitic form that produced a local infection similar to the type of infection brought about by *H. bromi*, *H. teres*, and *H. avenae*. As this writer gave no further details concerning either the fungus or the disease, it is not possible to determine whether or not he was dealing with the parasite found widely distributed in the Middle Atlantic States. In a later reference by Pammel, King, and Bakke (*104*), is reported from Iowa the occurrence on *F. pratensis* (=*F. elatior* L.) of a species of Helminthosporium having spores similar to those of *H. sativum* and producing on its host a leaf spot closely resembling late blight of barley, although less severe. Material deposited by these authors in the Office of Pathological Collections, Bureau of Plant Industry, was examined by the writer. This examination revealed no reason why the fungus should not be referred to *H. sativum*. At any rate the Iowa fungus represents an entirely different organism from the one under consideration, which would appear to merit recognition as a new species. On account of the reticulate pattern characteristic of the foliar lesions the specific name *dictyoides* is suggested.

DIAGNOSIS

Helminthosporium dictyoides, n. sp.

Occurring on *Festuca elatior* L., on which it causes a moderately destructive disease of the foliage, with symptoms very similar to those of barley attacked by *Helminthosporium teres*; newly infected leaves showing irregular brownish areas marked with dark longitudinal and transverse streaks forming a delicate reticulate pattern. Affected leaves later withering, the withering beginning at the tip and progressing to the base of the blade.

Sporophores dark brown or olivaceous; emerging singly or in groups of 2 to 6 from stomata or between epidermal cells; measuring usually 6 to 8 μ in diameter and 70 to 150 μ in length; 3 to 6-septate, the septa generally occurring at intervals of 10 to 30 μ; producing first spore usually at a distance of 50 to 100 μ from base; points of attachment of successive spores marked by moderately or strongly pronounced geniculations.

Conidia subhyaline and nearly colorless when newly proliferated, to yellow when fully matured; typically straight; maximum diameter usually at basal segment, 14 to 17 μ; tapering uniformly and very perceptibly to apical segment, the latter in long spores frequently not exceeding 8 to 9 μ in diameter, in short ones usually of greater diameter; more rarely approximately cylindrical, or short ellipsoidal. Length 23 to 115 μ, usually 50 to 70 μ, 1 to 7 septate, typically 3 to 5 septate, the septa not associated with perceptible constrictions except occasionally, and then constrictions most frequently present only at the basal septum; length of segments 7 to 24 μ, typically 12 to 15 μ. Contour of basal end hemispherical, of apical end hemispherical or hemiellipsoidal; peripheral wall or exospore uniformly thin, and entirely including the hilum within its contour. Germinating typically by two germ tubes, one from each end segment and produced usually at right or oblique angle to axis of spore; rarely by one or more germ tubes from intermediate segments.

HABITAT.—Collected at Brooklyn and Port Washington, N. Y.; Stamford, Conn.; Norwood, Mass.; Lisbon Falls, Me.; Washington, D. C.; Kensington and Annapolis, Md.; and Falls Church, Va. Apparently found wherever the host occurs in the Middle Atlantic and New England States.

HELMINTHOSPORIUM SICCANS, N. SP.

In the latter part of June, 1922, the writer observed that the Italian rye grass, *Lolium multiflorum* Lam, in the experimental farm at Arlington, Va., was very generally affected by a leaf spot disease. The trouble was found to occur on the foliage of both young and more nearly mature plants, being manifested by the appearance of minute, longitudinally

elongated, dark brown spots, measuring usually 0.1 to 0.3 mm. in width and 0.2 to 1.0 mm. in length, although sometimes apparently as a result of coalescence, attaining dimensions several times larger. Many of the more severely affected leaf blades bore more than a hundred of these localized discolorations and frequently more than a dozen could be distinguished on the sheath, mostly near its juncture with the blade. (Pl. 12, Ab.) Owing to the large number of spots often present on a single foliar organ, an appearance somewhat suggestive of netblotch is brought about, but as distinct transverse markings have not been observed, the reticulate pattern characteristic of leaves of barley and meadow fescue attacked by *Helminthosporuim teres* and *H. dictyoides*, respectively, is not evident. It may be mentioned that the most distinctive sharply defined discolorations have usually been found on leaves of younger plants, while on the leaves of plants attacked after the heading stage the discoloration frequently appeared to be somewhat suppressed. In any case, however, the organs attacked soon turned yellow at the tip and withered, the withering eventually involving the sheaths as well as the blades.

Microscopical examination of leaves that had succumbed to the malady revealed an abundance of fructifications typical of the genus Helminthosporium emerging from the dead tissues. Although the symptoms of the disease caused by it are quite different from those of the stripe disease of barley, the fungus shows a strong resemblance to *H. gramineum*. The spores of the two species are nearly similar in shape, being usually straight and cylindrical or tapering toward the tip. The tendency toward tapering, to be sure, is more pronounced in the conidia of the parasite on Italian rye grass, which, moreover, when fully mature, are of a brown or brownish olivaceous color, appreciably darker than the yellow fuliginous hue characteristic of the spores of the stripe fungus. With this deeper coloration is associated a peripheral wall, decidedly thicker than the spore wall of subhyaline-spored types but inferior to that of species possessing olivaceous spores of the type of *H. sativum*. The conidia of the form on *Lolium multiflorum* are perceptibly longer than those of *H. gramineum*, and a proportional inequality obtains in regard to the spore segments of the two species.

Germination in water normally takes place in a manner very similar to the germination in *H. gramineum;* that is, by the production typically of one or two germ tubes from both apical and basal segments and a single tube from several or all intermediate segments, although occasionally two tubes may be proliferated from an intermediate segment. The production of sporophoric processes by conidia under natural conditions, while not uncommon, is not as frequent in the fungus on *L. multiflorum* as in the stripe fungus, and apparently usually comes to an end with the production of a single secondary conidium.

The form on Italian rye grass shows considerable resemblance to *Helminthosporium dictyoides* on meadow fescue, not only in general pathological habit but in morphological details as well. The conidia are largely of the same straight cylindrical or tapering form. They are noticeably larger, however, more frequently septate, and when fully mature darker and provided with a thicker peripheral wall. The mode of germination also shows some difference, the parasite on *Lolium multiflorum* being usually more profuse in the production of germ tubes. With the form described in this paper as *H. stenacrum* occurring on *Agrostis stolonifera* the fungus is not readily confused, being distinguished

by its smaller dimensions, thicker peripheral wall, and absence of attenuated apical prolongation.

Although not much importance can be attributed to the length of the conidiophores as a diagnostic characteristic, it may not be superfluous to mention that in the species under consideration these structures show a rather usually wide range in this dimension. This variability apparently is less due to differences in length of intervals between the points of insertion of successive spores than to pronounced differences in length of the sterile portion below the insertion of the first conidium. It is not difficult to suppose that changes in environmental conditions are responsible for either greater or lesser development of the sporophore preliminary to the proliferation of the first conidium, resulting in conditions like those illustrated in Plate 12, Fe-j, on the one hand, and like those figured in Plate 12, Fa-d, on the other.

A fungus quite indistinguishable from the form on Italian rye grass was found to occur abundantly in Virginia and Maryland on the closely related host, *Lolium perenne*. An examination of numerous specimens of diseased perennial rye grass collected near Annapolis in May, 1921, and in the vicinity of Washington, D. C., during the months of May and June, 1922, revealed no constant or significant morphological characteristics by which the form parasitic on this forage crop could be distinguished from the parasite on Italian rye grass. In the absence of any cross-inoculation work the writer is inclined to regard the fructifications on the two hosts as belonging to the same species of Helminthosporium. It must be noted, however, that the attack of the fungus on *L. perenne* is usually not associated with the conspicuous spotting of the leaves readily observed on affected foliage of *L. multiflorum*, the discoloration being generally less evident and in many instances scarcely demonstrable. In the latter event, the withering of the foliage due to the parasite is, without microscopical examination, not very easily distinguished from the vegetative decline associated with drought or normal ripening.

As a large proportion of the leaves of the two species of Lolium are killed prematurely, it is safe to assume that the parasite interferes with the development of the plants sufficiently to cause appreciable economic loss. According to the writer's observations, the leaf disease attributable to the parasite constitutes the most destructive fungus trouble affecting the two valuable forage grasses in Maryland, Virginia, and the District of Columbia. That it has hitherto apparently escaped the attention of American pathologists is indicative more, perhaps, of neglect of diseases destructive to the graminaceous forage crops than to a possibly limited distribution.

It is interesting to note that in 1903 Diedicke (*29*) made mention of the occurrence of a species of Helminthosporium on *Lolium perenne*, causing a local infection similar to that occasioned, for example, by *Helminthosporium bromi* or *H. teres* on their respective hosts. No further description was given, and this investigator, after failing to connect the fungus with any ascigerous stage, apparently paid no further attention to it. Whether the American fungus is the same as that observed by Diedicke in Germany is a question open to conjecture. In any case, it appears not to have been described; and it evidently is distinct from the other species parasitic on grasses studied by the writer. It appears expedient to recognize it as a species, for which, because of its pathological effect on the foliage of its hosts, the specific name *siccans* is proposed.

DIAGNOSIS

Helminthosporium siccans, n. sp.

Occurring on *Lolium multiflorum* Lam. (type) and *L. perenne* L., on which hosts it causes a moderately destructive disease of the foliage, producing typically numerous dark brown longitudinally elongated spots, usually measuring 0.1 to 0.3 mm. in width by 0.2 to 1.0 mm. in length that by coalescing often appear as discolored areas several times larger. Affected leaves later dying, the withering beginning at the tip of the blade and eventually involving the sheath wholly or in part.

Conidiophores olivaceous, emerging usually singly, less frequently in pairs, and rarely in groups of three from stomata or more especially from between epidermal cells on the vascular ridges; measuring 7 to 9 μ in diameter by 50 to 300 μ in length; 1 to 9 septate, the septa inserted at intervals of 15 to 90 μ; producing first conidium at a distance of 50 to 250 μ from base; points of attachment of successive conidia at angles of geniculate irregularities occurring at intervals of 5 to 30 μ.

Conidia subhyaline or light fuliginous when newly proliferated, later becoming yellow, brownish, or brownish olivaceous, never dark olivaceous like *Helminthosporium sativum;* when fully mature provided with a moderately thick peripheral wall; typically straight or slightly curved; measuring usually 14 to 20 μ in diameter by 35 to 130 μ in length; usually subcylindrical, or tapering slightly or more markedly toward apex, the distal segment often not exceeding 10 μ in diameter, or rarely greater in diameter at the distal end than at the base. Apical and basal ends abruptly rounded off, the contours at the ends being approximately hemispherical. Hilum moderately conspicuous included within contours of peripheral wall. Germinating by the production of germ tubes from intermediate as well as end segments, the basal and distal segments both usually participating in the process by the proliferation of 1 or 2 lateral or oblique germ tubes. Of the intermediate segments one, several, or all may produce one or rarely two lateral germ tubes. Under natural conditions germination by the production of one or less frequently two sporophoric processes, each bearing usually a single conidium, is not uncommon.

HABITAT.—Collected in May and June near Annapolis, Md.; Baltimore, Md.; Rockville, Md.; Kensington, Md.; Washington, D. C.; Arlington, Va.; Mount Vernon, Va.

HELMINTHOSPORIUM STENACRUM, N. SP.

On specimens of *Agrostis stolonifera* L. collected at various times in September, October, and early November, 1920, in southwestern Connecticut, especially in the vicinity of Stamford and Norwalk, the writer found a well characterized species of Helminthosporium occurring with some regularity. The fructifications were found on dry withered leaves that formed a considerable proportion of the foliage. Owing to the relatively small size of the foliar organs concerned, and the absence of any pronounced discoloration, it was not possible to determine definitely whether the dying of the leaves was due to the presence of the fungus or to other causes. In general, the distribution of the conidiophores on the dead tissues suggested a course of events somewhat similar to that exemplified by the parasitism of *H. turcicum* in ordinary seasons—development of the fungus on leaves already reduced in vitality as a result of normal maturation, followed by the production of fructifications some time after their death.

The sporophores distributed rather sparsely over the surface of the withered leaves are moderately large structures of the same general type as those of *Helminthosporium teres,* although usually more abundantly septate and less frequently occurring in groups. (Pl. 13, Ca-d.) In width, color, character of peripheral membrane, general shape, and mode of germination, the spores show some similarity to those of *H. gramineum, H. teres,* and *H. avenae.* (Pl. 13, Aa-r, Ba-c.) In length, however, they are decidedly superior to the spores of *H. gramineum,* and in an approximately equal degree inferior to those of the other two species. While in newly proliferated spores the peripheral wall may exhibit slight constrictions at the septa, the contour of fully mature spores usually is

altogether smooth, a detail in which the species shows more similarity to *H. siccans* and *H. gramineum* than to the barley net-blotch fungus. No tendency toward the production of secondary spores, such as is manifested in *H. gramineum* and *H. catenarium*, has ever been observed. As in *H. catenarium*, however, the distal portion of the spore is frequently produced into a somewhat constricted apical prolongation. Apparently such modification is the result of development taking place subsequent to the proliferation of the spore in its original condition, and thus, in a sense, is of secondary origin. In any case, the apical extension is characterized by a conspicuous paucity of septa, such cross walls as are found present usually appearing to have developed tardily, as the delimited segments frequently have not contracted away from one another along the edge of the plane of contact.

In pure culture on artificial substrata the fungus produces abundant, although not especially characteristic, growth. Normal sporulation on the media ordinarily employed in laboratories has not been observed, although on tap water agar a relatively sparse production of somewhat small, atypical fructifications was brought about. The imbedded mycelium shows abundant anastomosis resulting in numerous complexes of inflated cells similar to those produced, for example, by *Helminthosporium teres*, *H. bromi*, and *H. tritici-repentis*; and, if the analogy is not misleading, pointing toward the existence of an ascigerous stage.

The fungus appears to be quite distinct from any graminicolous species of Helminthosporium hitherto described; as well as from several undescribed forms which the writer has collected on both wild and cultivated members of the genus Agrostis. Because of the somewhat attenuated distal prolongation characteristic of many of the spores, the specific name *stenacrum* is suggested.

DIAGNOSIS

Helminthosporium stenacrum, n. sp.

Occurring on withered leaves of *Agrostis stolonifera* L.

Sporophores dark olivaceous, emerging singly or in pairs, usually between adjacent epidermal cells; measuring 7 to 10 μ in diameter, and 80 to 250 μ in length; 3- to 10-septate, the septa occurring at intervals of 10 to 35 μ; producing the first spore usually 80 to 150 μ from the base, the points of attachment of successive spores occurring at well-defined geniculations.

Spores subhyaline to yellowish when fully mature; 15 to 23 by 53 to 135 μ; subcylindrical with hemispherical or hemi-ellipsoidal ends, or widest somewhat below the middle and tapering moderately toward the ends; the apical portion sometimes produced into a somewhat narrowed distal prolongation; 1 to 11 septate, the septa not associated with constrictions, or marked by barely perceptible constrictions. The peripheral wall thin and including the dark hilum within its contour. Germinating by the production of germ tubes from several or from all segments, the intermediate segments proliferating usually not more than one tube, the end segments occasionally giving rise to two or even three tubes.

HABITAT.—Collected near Stamford, Conn., and Norwalk, Conn., in September, October, and November, 1920.

HELMINTHOSPORIUM DEMATIOIDEUM BUBÁK & WRÓBLEWSKI

In 1916, Bubák and Wróblewski (*18*) described as *Helminthosporium dematioideum* a fungus occurring in Galicia on the glumes and paleas of sweet vernal grass, *Anthoxanthum odoratum* L.

Caespitulis minutis, dispersis, pulverulentis, atris. Mycelio dematioideo, repente, atrobrunneo. Conidiophoris cylindricis, 25–60 μ longis, 5–6 μ latis, subtorulosis, septatis, brunneis.

Conidiis cylindraceo-oblongis, 38–42 μ longis, 9–13 μ latis, maturis 3 septatis, utrinque late rotundatis, flavobrunneis, crasse tunicatis, levibus.

The description applies moderately well to a fungus found occurring in abundance on dead leaves of sweet vernal grass collected near Washington, D. C., June 20, 1920, and at various times throughout July, 1920, near Port Washington, N. Y. On the inflorescence, the fungus, it is true, also was present, but in much smaller amounts than on the foliage. It was also found on withered foliage of *Agrostis alba* L. (=*A. palustris* Huds.) and *Agrostis perennans* (Walt.) Tuckerm., collected during July and August, 1920, near Brooklyn, N. Y., and Norwalk, Conn., not infrequently on the same leaves that bore also fructifications of one or another of the two apparently undescribed larger-spored species of Helminthosporium occurring on these grasses. As the presence of the fungus was in no case associated with local discoloration of the foliar tissue that served as substratum, it was not possible to determine definitely its relationship as a saprophyte or possible parasite. It may be said, however, that the organism, although fairly common on the different species of Agrostis mentioned, was not present in sufficient quantity to justify the belief that it was the main cause of the premature withering, abundantly observed during the season of 1920. On the other hand, the manner of its occurrence on *Anthoxanthum odoratum* is not such as to exclude the possibility of a parasitic relation. For in some collections every leaf appears to bear fructifications of the fungus, sometimes, indeed, in great abundance and not infrequently to the approximate exclusion of other fungi. Owing to the small size of the leaves of sweet vernal grass, and the consequent difficulty in recognizing possibly pathological withering from the withering normally taking place during the later stages in the life of the plant, the presence or absence of a parasitic relation could be established perhaps only by well controlled infection experiments.

The principal circumstance that might suggest a possible lack of identity of the European and American forms, is the recorded occurrence of the former only on the inflorescence of *Anthoxanthum odoratum*, while the latter, although not absent from the inflorescence, is certainly much more abundant on the foliage. As sweet vernal grass is an early maturing species and as the material from which the description of the Galician fungus was drawn appears to have been collected in October, it is not improbable that the leaves of the plant were rather poorly represented in the type specimens if, indeed, not altogether missing. Thus the American fungus may at least provisionally be referred to *Helminthosporium dematioideum*, although a few remarks concerning its morphology may not be out of place, especially in view of the brevity of the diagnosis given by Bubák and Wróblewski.

The sporophores (Pl. 14, Da-c), as indicated in the original description by these authors, measure only 5 to 6 μ in width and, compared to most graminicolous congeneric species, are generally decidedly short, although not infrequently exceeding 60 μ in length (Pl. 14, Db, c), and even in some instances measuring three times as much (Pl. 14, Da). They usually are found singly or in pairs, rarely in larger groups; are septate usually at intervals of 10 to 25 μ; and often can be collected bearing 6 to 8 spores *in situ*. (Pl. 14, Db.) The spores (Pl. 14, Ba-h) are distinctly yellowish when fully mature, from 2 to 6 times septate, and measure 8.5 to 14 by 18 to 48 μ, although the range in septation and in dimensions given by Bubák and Wróblewski may be regarded as typical. In shape they are generally subcylindrical or tapering perceptibly toward the base. The proximal septum is frequently, but not constantly, associated with a

constriction; the basal, like the distal end, usually presents a hemispherical contour within which the small dark hilum marking the point of attachment may be readily recognized. Germination normally proceeds by the production of one to three germ tubes from the basal cell, at positions approximately midway between hilum and basal segment, not usually immediately adjacent to the hilum. (Pl. 14, Ca, Cc-g.) Other modes of germination, as, for example, the production of a germ tube from the apical segment (Pl. 14, Cb), may be regarded as abnormal and usually are attributable to the death of the basal segment as evidenced by the concave contour of the cross wall in contact with the adjacent living segment.

HELMINTHOSPORIUM TRISEPTATUM, N. SP.

In collections of velvet grass (*Notholcus lanatus* [L.] Nash [=*Holcus lanatus* L.]) made near Port Washington, Mineola, Douglaston, and other localities on the western end of Long Island during the months of July and August, 1920, a fungus related to the plants usually referred to the genus Helminthosporium was found occurring quite commonly on withered or withering leaves. (Pl. 14, E.) As the form is of a type somewhat different from the majority of the species of Helminthosporium on grasses, and does not appear to have been described hitherto, a brief account of it may not be out of place.

The sporophores (Pl. 14, Ha–c) are scattered relatively sparsely between the long hairs that constitute the abundant pubescense characteristic of velvet grass. They are distinguished not only by being relatively long, but also by the presence of ring-like thickenings of the peripheral well immediately below the points of attachment of the successive spores. The upper portion of the sporophore may thus present a more or less distinctly moniliform contour, within which the lumen maintains an approximately uniform diameter.

Compared to the other species of Helminthosporium discussed in this paper, the spores (Pl. 14, Fa–f) of the fungus on velvet grass show perhaps the greatest degree of constancy with regard to morphological features. Associated with the dark olivaceous color is a peripheral wall not exceeded in thickness by that of any of the related forms described in this paper. At the basal end, however, it decreases uniformly in thickness toward the hilum, where the curved inner and outer contours appear to become tangent to each other. This condition is present also in other forms as, for example, in *Helminthosporium monoceras*, where, however, the wall is similarly attenuated at the apical end, a difference reflected in mode of germination characteristic of the species. For, whereas *H. monoceras* germinates from both ends, in the form under consideration only the basal segment normally participates directly in the process. (Pl. 14, Ga–e.)

The fungus appears to be mainly, if not wholly saprophytic. It is referred to the genus Helminthosporium rather than to Brachysporium, as in well developed spores (Pl. 14, Fe) the length is somewhat greater in proportion to the diameter than generally appears to be true in fungi assigned to the latter genus. The specific name *triseptatum* is suggested because of the number of septa characteristic of typical spores.

DIAGNOSIS

Helminthosporium triseptatum, n. sp.

Fructifications scattered sparsely on withering leaves of *Notholcus lanatus* (L.) Nash (=*Holcus lanatus* L.); usually not visible macroscopically or associated with visible alterations in the substratum.

Sporophores arising singly or in pairs, dark olivaceous, 6 to 8 by 200 to 400 μ; usually 6 to 11 septate, the septa occurring at intervals 18 to 40 μ in length, averaging approximately 25 μ; producing first spore approximately 175 μ from base. Proliferation of spore associated with conspicuous local thickening of peripheral wall resembling ring or band; these thickenings occurring in series, giving upper portions of sporophore a more or less moniliform contour.

Spores dark olivaceous; ellipsoidal or short cylindrical with hemispherical ends, sometimes tapering more or less toward basal end; regularly 2 to 3 septate, the septa not associated with constrictions in the peripheral wall; the latter unusually thick, although somewhat thinner at the distal end, and at the basal end diminishing markedly in thickness toward the hilum; the hilum not projecting beyond contour of proximal end. Measuring 15 to 21 by 35 to 50 μ; germinating by the production of one or two germ tubes at positions adjacent to or in close proximity to hilum.

Habitat.—Collected during July and August, 1920, near Port Washington, Mineola, Valley Stream, Rosedale and Douglaston, N. Y.

HELMINTHOSPORIUM VAGANS, N. SP.

In July, 1919, shortly after the present studies were begun, the writer's attention was called to a leaf spot of Kentucky bluegrass (*Poa pratensis* L.) that seemed to be generally prevalent in the fields and lawns about Madison, Wis. Although almost invariably present wherever the host was found, the spots were by no means abundant. Usually only a small proportion of the leaves were found diseased, and the majority of these bore evidence of only a single infection. Although no spores were found associated with the spots during this season, the fungus that was obtained in pure culture from the diseased tissue was so similar to other species of Helminthosporium in general growth characteristics that observations were resumed the following year.

The leaf spot reappeared late in May, 1920, quite as generally and sparingly as during the preceding season. On June 7, material was collected that showed, moreover, an extension of the trouble to the leaf sheaths, especially toward the base of the plant, the lowermost ones being largely involved in diffuse brown discoloration. The condition thus brought about appeared not unlike that present in wheat plants affected with the disease attributable to *Helminthosporium sativum* that has in recent years become known as "footrot." Much more severely diseased specimens (Pl. 15, A) were collected in Brooklyn, N. Y., on August 13, 1920. The foliar lesions, which are of a bluish-black color quite intense in the center and fading out gradually at the margins, here measured up to 8 mm. in length and 3 mm. in width, although usually not exceeding a half of these dimensions. Some of the leaves had withered prematurely, the withering beginning at the tip and proceeding toward the base. The bases of the lowermost leaf sheaths surrounding the "foot" or "crown" of the plant, were thoroughly permeated with a brown pigment. On microscopic examination it was found that the withered portions of the leaves, as well as the older, dead, discolored sheaths, bore in moderate profusion sporophores with spores typical of the genus Helminthosporium.

In addition to the localities already mentioned, the fungus has been collected at many stations in the western half of Long Island, as well as

at Bloomington, Ill.; Annapolis, Md.; Washington, D. C.; Hyde Park, Mass.; Meriden, Conn.; and Lisbon Falls, Me. It thus appears to be quite widely distributed through the northeastern and middle western sections of the country. Indeed, the possibility of a distribution nearly, if not quite, approaching in extent the range of the host, is not to be excluded; for although *Poa pratensis* is one of the most common and valuable of the grasses, both in this country and in Europe, and might thus be expected to be kept under observation by pathologists, the fungus is nevertheless very apt to escape detection. Not only are the foliar lesions caused by the parasite usually small and infrequent, but to a casual observer they may readily be mistaken, in spite of the unbroken epidermis and more intense dark discoloration, for old sori of *Puccinia poarum* Niels., almost always present in some abundance. It need hardly be mentioned that the economic loss caused by a disease ordinarily so lacking in severity is relatively insignificant; yet under certain conditions of moisture and of temperature such as would favor a multiplication of foliar lesions and accentuate the footrot symptoms, the damage may not be altogether unappreciable.

On microscopic examination, the fungus shows considerable similarity to *Helminthosporium sativum*, not only in its effect on the host, but to some extent also in regard to morphological features. The sporophores (Pl. 15, Da-g) found on leaves of *Poa pratensis*, to be sure, are appreciably greater in diameter and frequently greater in length (Pl. 15, Da) than the corresponding structure of the parasite causing spotblotch; and the tendency toward branching exhibited by them (Pl. 15, Db, e) is rarely to be found in *H. sativum*. On the other hand, complete agreement prevails with reference to coloration of the spores, the latter (Pl. 15, Ba-q) when fully mature, being uniformly dark olivaceous. In both species, too, the peripheral spore wall is relatively thick and the number of septa rarely exceeds the usual maximum of 10. With respect to spore shape, the form on Kentucky bluegrass, however, is noticeably different, its conidia being typically straight, never, as a rule, becoming distinctly curved.

The most distinctive character, however, is to be found in the germination of the spore, the germ tubes being proliferated not alone from the end segments, but indiscriminately from both intermediate and end segments regardless of position; and not infrequently every segment participates in the process. (Pl. 15, Ca, b.) This behavior would seem to suggest a measure of affinity with species having straight cylindrical spores like *H. teres* and *H. gramineum*, a suggestion borne out in a measure by the cultural characters of the fungus on ordinary media. Sporulation very rarely occurs on potato dextrose agar, although an abundance of dark aerial mycelium usually is produced. The imbedded mycelium presents an unusually distinctive aspect, for instead of consisting like that of most species of Helminthosporium, of a miscellaneous growth of hyphae, it is composed largely of a relatively small number of ramifying systems, all of an intense bluish black color, and bearing hundreds of branching elements.

The literature, beyond a brief abstract published by the writer (*31*) appears to contain no reference to any species of Helminthosporium parasitic on *Poa pratensis*. Karsten (*74*), in 1884, cited *H. flexuosum* Corda (=*Brachysporium flexuosum* [Corda] Sacc.) as occurring on the leaves of a congeneric host, *Poa stricta*. However, the obvious disparity in size and septation between the spores of the species figured by Corda

(*25*) and the one found parasitic on Kentucky bluegrass is such as to make it appear altogether improbable that Karsten was dealing with the form under consideration. *Napicladium gramineum* described by Peck (*108*) as destructive to *Poa trivialis* L., is similarly a fungus of much smaller dimensions, the 1 to 3 septate, clavate spores measuring only 10 to 12 by 30 to 60 μ. In more recent years, Baudyš (*9*) has reported from Bohemia, a new species of Helminthosporium on the living leaves of *Poa trivialis*, which he named *H. poae*. Unfortunately, the writer has not been able to secure a copy of Baudyš' paper and consequently has not been able to decide definitely as to any possible identity of *H. poae* Baudyš, either with the species of Napicladium described by Peck, on the one hand, or, on the other, with the fungus attacking Kentucky bluegrass.[10] As *Poa pratensis* is presumably common in Bohemia, Baudyš' failure to record his fungus as developing on this grass would seem to have considerable significance in this connection. The species parasitic on Kentucky bluegrass is accordingly described as new; and because of its widespread occurrence in meagre quantity, the name *H. vagans* is proposed.

DIAGNOSIS

Helminthosporium vagans, n. sp.

Producing well-defined, bluish-black spots 0.5 to 3 mm. wide, 1 to 8 mm. long, on the leaf blades of *Poa pratensis* L.; on the sheaths the spots less definitely circumscribed and near the base of the plant often merging into a generally diffused brownish discoloration.

Conidiophores emerging from stomata or between epidermal cells of tissues some time after death, usually singly or less frequently in pairs; typically simple although occasionally branching; dark olivaceous; usually measuring 8 to 10 μ in diameter and 50 to 280 μ in length; 1 to 10 septate, the septa occurring at intervals of 15 to 40 μ; approximately straight up to point of attachment of first spore 40 to 150 μ from base; successive spores produced at apices of moderate or often pronounced geniculations.

Conidia dark olivaceous when fully mature; cylindrical or slightly tapering toward the hemispherical ends; measuring usually 17 to 23 μ in diameter by 25 to 130 μ in length; 1 to 10 (usually 5 to 8) septate, the septa not associated with constrictions in the peripheral wall; the latter always thick and including the dark hilum within its contour. Germinating by the production of 3 to 11 germ tubes indiscriminately from end and middle segments, a single germ tube usually being produced from several or all segments.

Habitat.—Collected on *Poa pratensis* at Madison, Wis.; Brooklyn, N. Y.; Bloomington, Ill.; Annapolis, Md.; Washington, D. C.; Hyde Park, Mass.; Meriden, Conn.; and Lisbon Falls, Me.

HELMINTHOSPORIUM RAVENELII CURTIS

Helminthosporium hoffmanni B. Mss. *or H. hoffmanni* B. & C. 1857, *in* Introduction to cryptogamic botany, p. 298.

Helminthosporium tonkinense Karst. & Roum. 1890, *in* Rev. Mycol. ann. 12, no. 46, p. 78.

Helminthosporium crustaceum P. Hennings 1902, *in* Hedwigia, Bd. 41, p. 147.

Although of very little economic importance, *Helminthosporium ravenelii*, owing to its conspicuousness and wide occurrence throughout many of the warmer regions of the globe, has become one of the best known members of the genus. It was described in 1848 by Curtis (*26*), who noted also its abundant distribution in North and South Carolina. This brief account, however, seems to have escaped the attention of mycologists generally; for when Berkeley (*11*) some years later figured

[10] Since this text was written, Baudyš' paper has become accessible. The Bohemian fungus produces spores provided with 2 to 6 cross-walls and measuring 36 to 73 μ in length. The foliar spots occasioned by it are described as yellowish with a dark brown margin. In respect to these charateristics, the departures from the morphology of the American parasite would appear to preclude any likelihood of the two forms being identical.

the fungus in his "Introduction to Cryptogamic Botany," he designated it as *H. hoffmanni* B. and C., without any further comment beyond the words, "From specimens on *Sporobolus indicus*. Sent by Rev. M. A. Curtis." Later in a brief descriptive discussion in the "Notices of North American Fungi" Berkeley (*12*) cited *H. hoffmanni* B. Mss. as a synonym of *H. ravenelii*, which binomial he correctly credited to Curtis alone. Nevertheless, as the publication of the original specific diagnosis in an American journal has apparently remained relatively unknown both in this country and abroad, the name frequently has been improperly attributed to the joint authorship of Berkeley and Curtis.

The earliest collections of the fungus made by Curtis were from North and South Carolina. Material from the rest of the South Atlantic States and from the Gulf States, Georgia, Florida, Alabama, Mississippi, Louisiana, and Texas, as well as from Mexico and China, is included in numerous collections in the herbarium of the Office of Pathological Collections. Specimens of the parasite collected in Costa Rica were distributed by Sydow.[11] Kabát and Bubák[12] distributed material from Uraguay and Theissen [13] material from Brazil. The fungus, moreover, has been reported from Cuba by Berkeley (*12*), from Bermuda by Seaver (*132*), from New Zealand by Kirk (*78*), from New South Wales by Cobb (*22*), and from the Philippines by Hennings (*60*), Baker (*5*), H. and P. Sydow (*148*), and Yates (*160*). There is good reason to believe that its distribution is practically coterminous with that of its host, *Sporobolus indicus* R. Whether other grasses also are subject to attack is not altogether certain, although Yates (*160*) records the occurrence of the fungus on the inflorescence of *Panicum auritum* in the Philippines, and Fimbristylis is given as the host on the covers of Theissen's Brazilian specimens. More information concerning the identity of the host material on which these records are based would be desirable. It may be stated that *Sporobolus angustus* Buckl. and *S. elongatus* R., sometimes mentioned as hosts, are listed in the Index Kewensis as synonyms of *S. indicus*.

Helminthosporium ravenelii attacks the inflorescence of *Sporobolus indicus* in our southeastern states with such regularity that, as has been observed by Curtis (*26*), Jennings (*67*), and others, it is often quite difficult to obtain a specimen of this species of grass entirely free from the fungus. Indeed, there is reason to believe that the distinctive color and texture of the diseased panicle is popularly regarded as commonplace attributes of the host, as is evidenced by the common names applied to it in the United States, namely, "black seed grass" (*26*) and "smut grass" (*47*). These terms are fairly accurately descriptive of the later stages, when the infected inflorescence has a black crusted appearance; but much less accurately descriptive of the earlier stages (Pl. 16, A) when the fungus is present as a velvety or spongy layer of a brownish olive color, that only later becomes increasingly dark.

This velvety layer, under the microscope, is seen to consist of crowded sporophores (Pl. 16, B) arising from a mat of interwoven colorless mycelial hyphae that occupy the superficial layers of the affected floral parts. Unlike the homologous structures of nearly all congeneric forms parasitic on grasses, the sporophores of *H. ravenelii* exhibit a constant

[11] SYDOW, H. FUNGI EXOTICA EXSICCATI. *Helminthosporium ravenelii* B. and C. No. 442. 1912.
[12] KABÁT ET BUBÁK. FUNGI IMPERFECTI EXSICCATI. No. 540. *Helminthosporium ravenelii* Curt. et Burk. 1907.
[13] THEISSEN, F. DECADES FUNGORUM BRASILIENSIUM. No. 277. 1905.

tendency toward branching. They are light fuliginous to light yellowish in color; very noticeably torulose, hence decidedly variable in diameter, this dimension ranging from 5 to 10 μ, while the length often exceeds 500 μ; and usually septate at intervals of from 20 to 40 μ.

The spores (Pl. 16, Ca-q), borne in great abundance at the apices and geniculations of the distal portions of the fructifications, are straight or show a slight crescentic or sigmoid curve; rounded at both ends, the apical end often more broadly than the basal end, owing to a tendency toward tapering in the basal and juxtabasal segments; 12 to 19 μ in diameter by 22 to 78 μ in length; and 1 to 5 (usually 3 or 4) septate, the septa rarely associated with perceptible constrictions in the contour of the thin peripheral wall within which, at the point of attachment, the small dark hilum is readily observed. The conidia germinate readily in water, sending out a germ tube from one, or more usually from both, end cells. (Pl. 16, Da-e.)

Karsten and Roumeguére (*75*) have described a fungus from Tonking growing apparently on the same host (*Sporobolus tenacissimus*, the host name given, being listed as a synonym of *S. indicus* in the Index Kewensis) and corresponding to *Helminthosporium ravenelii* in all details relating to habit, color, and structure of conidiophores, as well as to color, structure, and dimensions of conidia. This form, which they designated as a new species, *H. tonkinense*, and regarded as being related to *H. ravenelii*, is undoubtedly altogether identical with the latter. Nor can there be much question that the same holds true also of *H. crustaceum* described from Java by Hennings (*58*) as forming dark crustaceous effuse tufts on the inflorescence of a species of Sporobolus. According to this botanist, *H. crustaceum* is related to *H. ravenelii* but distinct on account of its conidia. Inasmuch as the latter are characterized as—

> Oblonge clavatis vel fusoidois, utrinque obtusis, rectis vel curvulis, 40–60×12–16 μ, 3–5 septatis, haud constrictis, fuscis—

in all of which particulars the agreement with *H. ravenelii* is at least reasonably close, the writer is inclined to believe that Hennings's binomial should be regarded as a synonym until some evidence for the justification of a new species has been adduced.

HELMINTHOSPORIUM SATIVUM P. K. & B.

Helminthosporium acrothecioides Lindfors 1918, *in* Svensk Bot. Tidskr. v. 12, p. 227.
Helminthosporium gramineum of E. C. Johnson, Massee, Palm, Bassi, not Rabenhorst.
Helminthosporium inconspicuum of Peck, Atkinson, not Cooke & Ellis.
Helminthosporium Sorokinianum Sacc. 1891, *in* Ztschr. Pflanzenkr., Bd. 1, p. 236–239.
Helminthosporium teres of Bakke, not Saccardo.
Helminthosporium sp. of Beckwith, Bolley, Evans, Hamblin, Hungerford, McKinney, Stakman, Stevens, Waterhouse.

Although the fungus to which it has appeared advisable to apply the binomial given by Pammel, King, and Bakke (*104*), probably is the species most frequently encountered by plant pathologists, it has been the subject of much confusion in the literature. This condition is largely attributable to the fact not hitherto generally recognized that it occurs on a number of graminaceous hosts, several of which, moreover, are affected by one or more congeneric parasites. As the specific characteristics of the latter have not always been clearly distinguished, and as the fungus under consideration shows a tendency toward variation in response to varied environmental conditions, occasion for erroneous identification has not been lacking.

NOMENCLATURE

In 1891, a brief anonymous account (*136*) appeared concerning *Helminthosporium Sorokinianum*, Sacc. (in litt), a fungus which Sorokin (*135*) had found occurring on the spikes of wheat and rye in the South Ussurian region in Russia. The spores were later described in Saccardo's (*128, v. 10, p. 415–416*) diagnosis of the species as—

acrogenis, ovato-fusoideus, majusculis, 80–100 x 30, rufobrunneis, 5–10 septatis, passim lenissime constrictis, rectis curvulisce.

This characterization applies fairly well to the spores found occurring on wheat spikes in the United States with reference to shape and septation. The dimensions given also are not outside of the ranges in dimensions found in this country, the figures given for length corresponding closely enough, although 30 μ constitutes a maximum width (pl. 18, Fe) not attained by a large proportion of conidia. It appears quite probable, therefore, that the Russian fungus is identical with the American form. However, because the spores of the latter, when fully matured and in a living condition, are dark brown or olivaceous rather than reddish brown, the identity of the two forms can not be regarded as firmly established. Therefore, in spite of the priority of Saccardo's binomial, the writer believes it advisable to treat it as a probable synonym.

In 1909, Pammel (*103*) recorded the occurrence during the preceding season of a barley disease in Iowa differing from the stripe disease. A more complete account of this trouble was published in 1910 by Pammel, King, and Bakke (*104*), in which the casual organism was described as having fascicled fuscous brown septate conidiophores, 8 to 10 μ wide, bearing a large cylindrical dark brown spore, with 7 to 12 divisions, and measuring 15 to 20 by 105 to 130 μ. The fungus was regarded as closely related morphologically to *Helminthosporium teres*, but in the absence of comparative cultural studies was provisionally given a new specific name, *sativum*. Later, however, one of the authors, Bakke (*6*), presumably as a result of cultural experiments and in conformity with opinion secured from Saccardo and Ravn, definitely referred the disease to *H. teres* Sacc. Although indications are not wanting that Bakke in this later work was dealing to some extent with the latter organism, the figures as well as the text leave no room for doubt that he was in the main concerned with the same disease and the same parasite that had been discussed in the preceding Iowa publication. Inasmuch as *H. teres* and the fungus causing "late blight" of barley are not identical, representing, indeed, two quite distinct congeneric types, it would seem that Bakke was in error in repudiating *H. sativum* as an independent binomial.

In 1918, Lindfors (*84*) described from Sweden as *Helminthosporium acrothecioides* a fungus he had discovered on barley seed that had been germinated on filter paper. Its morphological features correspond completely with those of the American fungus developing under the warm, damp conditions obtaining in germination apparatus, when, as the writer has observed in hundreds of instances, discolored barley seeds or wheat seeds affected with "black point" are incubated on moist filter paper. The figures and the characterization of the conidia as "narrow ellipsoid to spindle-shaped, with blunt ends, 60 to 95 by 20 to 24 μ, with 7 to 9 septa, and a thick, dark olive brown epispore," apply so well to the American form that Lindfors's binomial may very safely be regarded as a synonym.

OCCURRENCE OF THE FUNGUS ON BARLEY

Of the graminicolous species of Helminthosporium thriving in our northern latitudes, *H. sativum* shows, perhaps, the strongest omnivorous tendency, being vigorously parasitic on a number of grasses and occurring on others apparently in a manner more nearly suggesting a saprophytic relation. Its greatest luxuriance is attained, nevertheless, on the foliage of barley, from which it originally was described. According to the records of the Plant Disease Survey, the fungus has been reported on barley in 24 states, including all of the more important barley-growing sections of the country. Güssow (*48*) early recorded its occurrence on the same crop in Canada.

Although the host is susceptible at any stage of development, the use of affected seed often resulting in the appearance of severe lesions in the basal portions of the young seedlings, the disease usually does not begin to show up in quantity until the plants are heading out. It is manifested by the appearance of spots varying from 0.5 to 3 mm. in width and from 2 to 15 mm. in length, usually dark brown in the center and fading gradually at the margins into the green of the surrounding tissue. (Pl. 17, B.) The lower leaf blades are first affected, the discolored areas multiplying until scores of them may be present on one foliar organ and a considerable portion of the leaf tissue is involved (pl. 17, A). As a result the leaf soon withers and dies, the discolored areas fading slightly and becoming vaguer in outline, while the parts not visibly altered in appearance take on a grayish hue. The foliar spots never become bleached in the center as those caused by *H. leersii* on *Leersia virginica;* nor exhibit a reticulate pattern, like those induced by *H. teres* on barley; nor are they surrounded by a zone of leaf tissue from which the chlorophyll has disappeared like those produced by *H. bromi* on *Bromus inermis.* At the same time the destruction of the lower leaves takes place, the disease makes progress in the younger foliage, which then succumbs in the same way, until the uppermost leaf is affected. The effect of this virtual defoliation is to hasten the ripening processes. In a season favorable for the development of the disease, the grain may be ready for harvesting perhaps two weeks earlier than when the trouble is absent. As might be expected, the yield is decreased in a measure approximately corresponding to the shortening of the growing period.

Although the foliage of barley is most severely attacked the inflorescence, as Pammel, King, and Bakke (*104*) pointed out, is not immune. The fungus may be found on the glumes and not infrequently on the seed. The diseased kernels usually are readily distinguished because of the dark-brown discoloration at the germ ends, a feature that has, indeed, been utilized by Atanasoff and Johnson (*3*) in selecting infected material for experiments on the dry-heat treatment. When such discolored kernels are placed in a germinator the fungus becomes visible usually within 24 hours as a delicate white velvety outgrowth, that soon spreads on the filter paper or other material as an effused arachnoid mycelium and produces, if the conditions are not too moist, an abundance of fructifications. A reduction in viability, usually apparent in slightly discolored seed, may become very considerable when the seed is more severely affected. After the basal sheath has been developed this organ often is attacked by the fungus and as a result takes on a yellow or light yellowish-brown discoloration. A considerable proportion of the rootlets

may be invaded in the same way, often stunting their development and softening the discolored cortical tissue. Undoubtedly, quite similar pathological processes take place when the seedlings are planted in soil. The effect of such early infection, moreover, then becomes apparent in the development of the seedling, for, in addition to the lesions on seed, sheath, and root system, conspicuous dark brown spots may occur on the first few leaves, thus giving rise to the thoroughly diseased condition described and illustrated by Atanasoff and Johnson.

It may be mentioned in this connection that a discoloration of barley seed evidently quite similar to that frequently observed in the United States was noted by a number of European writers. Zöbl (*163*, *164*), in 1892, published some papers on "brown-pointed" (*braunspitzige*) barley, in which the discoloration is described as being most intense at the base of the seed and decreasing toward the apex. Although various fungi belonging to the genera Sporodesmium, Cladosporium, Helminthosporium, and Dematium were found associated with discolored seeds, Zöbl attributed the brown-point condition primarily to *Cladosporium herbarum*. Puchner (*113*), in some studies on "black-pointed" (*schwarzspitzige*) barley seed, found that these germinated abnormally, often producing plants the leaves of which bore brown spots. However, when barley seed similarly affected was treated with copper sulphate preliminary to sowing the foliar lesions failed to appear in the seedling, although during the later stages in development the foliage and inflorescence of the originally healthy plant were as subject to attack as those of a diseased specimen. Ravn (*115*) found a fairly close correlation existing between the prevalence of net-blotch and "brown point" (*brune Spidsers*) in the seed. Without regarding a causal relation as firmly established, he nevertheless appears to have been inclined to see in *Helminthosporium teres* the most probable cause of seed discoloration.

While the evidence adduced by Ravn would appear altogether sufficient to justify his view, as far as conditions in Denmark at the time his investigations were carried on were concerned, the fact remains that in our North Central States *H. teres* is not generally associated with black-pointed barley seeds. On the other hand, as has been mentioned before, the association of *H. sativum* with this condition appears unusually constant. For example, when discolored barley seeds from stock grown in Wisconsin are plated on agar, after proper surface sterilization, only a very small proportion of seeds will fail to give rise to mycelia and conidiophores of the parasite causing spot-blotch. Presumably *H. sativum* is of less frequent occurrence on barley in Europe than in the United States. That it is more common than the absence of references from European literature might lead one to infer is suggested, for example, by Massee's (*90*) account of *H. gramineum*. The ambiguity with regard to the host range of the fungus, and the longevity, color, and size of the conidia, fortunately is explained by figures of these bodies (fig. 132–6), which show beyond any doubt that this author was dealing not with the stripe fungus but with the parasite causing spotblotch. In Lindfors' (*84*) description of *H. acrothecioides* the existence of a species of Helminthosporium on barley other than *H. gramineum* and *H. teres* eventually was recorded, but its relation to pathological lesions in the growing plants of either barley or of wheat or rye has apparently not yet been recognized.

OCCURRENCE ON WHEAT AND RYE

Helminthosporium disease of wheat affecting seedlings as well as older plants was reported, according to the files of the Plant-Disease Survey, as doing considerable damage in several seasons during the past decades, especially in North Dakota and Minnesota. In the former State, Bolley (*14*) found one or more species of Helminthosporium responsible in large measure for the deterioration of wheat production. Isolations made from various parts of diseased wheat plants revealed the presence, in addition to representatives of the genera Alternaria, Colletotrichum, Fusarium, and Macrosporium, of strains of Helminthosporium in the nodes and internodes of the stem, as well as on the surface and in the interior of the kernels (*15*). Of special interest is the account given by the same author of a type of infection designated as "brown spot" and manifested by brown discoloration of the lower portion of the wheat stems near the soil line. Such attack was found to occasion reduction in tillering, as affected stools usually consisted of only one, or more frequently, two tillers, the other tillers being represented by abortive shoots or intercepted buds. Beckwith's (*10*) study of the occurrence of soil fungi in North Dakota showed that strains of Helminthosporium were found occurring considerably more abundantly on the nodes and internodes of wheat in the wet season of 1909 than in the dry season of 1910.

Later, E. C. Johnson (*71*) published the results of experiments on certain cereals with a fungus he designated as *Helminthosporium gramineum* Rabh. Inoculation of young seedlings of wheat, barley, oats, and rye with spores from pure cultures originally isolated from the lower parts of the culms of wheat, as well as from wheat leaves and barley leaves, resulted in the prompt appearances of leaf spot on all the four graminaceous species. When wheat seeds inoculated with spores were sown, their germination was considerably reduced, and the resulting plants were stunted in comparison with uninoculated controls. Indeed, some of the inoculated seeds were attacked so promptly that they had no opportunity to germinate; in other cases, the young plants were killed before they were an inch high. The attack on the surviving seedlings was manifested by a brown discoloration at the base of the culms, usually occurring in the basal leaf sheath, and subsequently extending to the root crown, as well as by the partial brownish discoloration and reduced development of the root system. Barley and oat seed similarly treated were not perceptibly reduced in viability, although the resulting barley seedlings were somewhat retarded in growth and exhibited in smaller degree the same type of discoloration as the diseased wheat seedlings.

In spite of E. C. Johnson's statement that the strain of Helminthosporium discussed in his paper corresponded in cultural and morphological characteristics to the descriptions of Ravn (*115*), there are strong reasons for suspecting that his fungus was not identical with the parasite causing barley stripe. The latter, in the writer's experience, can not be made to sporulate in pure culture except in meager quantity, any profuse sporulation, such as was presumably induced in all the imperfect fungi employed by E. C. Johnson, certainly never having been observed in *H. gramineum*. Moreover, the pathological lesions produced in his experimental plants obviously bore little resemblance to those of the systemic trouble described by Ravn. On the other hand, the symptoms

correspond accurately with those noted later by Atanasoff and Johnson (*3*) on seedlings grown from seed infected with *H. sativum*.

In 1918, Palm (*101*) reported from Java the occurrence of a species of Helminthosporium on wheat which he designated as *Helminthosporium gramineum* ([Rabh.] Eriks.?). The fungus was found present on the glumes as well as on the perceptibly shrunken kernels, through the agency of which the author believed the infection to be transmitted. The statements relating to the long worm-shaped, usually curved, 6–to 10–septate spores measuring 65 to 110 μ in length, and 15 to 20 μ in width, and more especially the accompanying figures, indicate that the Javan fungus differs conspicuously from the parasite causing stripe but shows complete similarity to *H. sativum*, as it occurs on the same host in this country.

Considerable attention has been given during recent years to a diseased condition found prevalent on wheat in the vicinity of Granite City, Ill. Stevens (*140*) recognized the trouble as a typical footrot which he attributed to a species of Helminthosporium found constantly associated with it and producing luxuriant growth on a variety of substrata. The conidia were described as structures approaching a narrow or broadly elliptical shape measuring 24 to 122 μ, usually 80 to 90μ, in length; containing from 0 to 13, usually 5 to 10, septa or false septa; possessing an outer thin dark wall and an inner colorless thick wall; and germinating by the production of one or two polar germ tubes. The causal relation of the fungus with the disease presumably was established, moreover, by successful inoculation of the unwounded internodes of wheat seedlings. When wheat seeds were inoculated with spores of the fungus and germinated in a seed tester, the host tissue was quickly invaded, leading to the production of brownish spots, and under favorable conditions to general rotting and death of the innermost leaves (*141*). Inoculation of the roots was followed by invasion and discoloration of the cortex.

McKinney (*86*), who also carried on studies near Granite City, Ill., similarly found a species of Helminthosporium present on wheat, associated with lesions often developing during the later stages of the disease. Apparently, however, he did not regard the latter primarily as a foot rot, but rather as a trouble having somewhat different symptoms which, at least during the early spring stages, was not found constantly associated either with any perceptible lesions or with any specific organisms whatever. In a later note, McKinney (*87*) states that all the strains of Helminthosporium isolated from wheat appear to be similar, if not identical, not only to one another, but also to *H. sativum* derived from barley leaves affected with spotblotch. And this similarity was found to obtain with reference to the morphology of the fungi from the two sources as well as to their pathological properties as evidenced in cross inoculation experiments.

A critical study of a Helminthosporium disease of wheat and rye was published recently by L. J. Stakman (*138*). The malady, which was unusually common in Minnesota in 1919, was manifested early in the season as a seedling blight, characterized by brown discoloration of the roots, either in extensive patches or in numerous small irregular lesions; by the presence of rust-brown streaks or blotches on the "foot" or base of the stem, later progressing to the inner sheaths; and by a general dwarfing of the plants, the leaves of which were conspicuously stunted, very narrow and pale reddish tan in color. Many of the affected plants

died in the seedling stage. Others, however, recovered and grew to maturity, although the older plants frequently suffered not only from the persisting foot lesions, but also from secondary infections. As a result of the latter, numerous dark brown spots, about 1 mm. long, appeared on leaves, nodes, neck, and glumes, and brownish streaks were found present on the internodes. The discolored areas later became paler, and developed a coating of Helminthosporium fructifications. On potato dextrose agar the fungus produced an abundance of spores, which were described as being straight or curved, dark blue to brown in color, 3- to 8-septate, and measuring on the average 41 by 20 μ. Cross inoculations carried out with cultures of the Helminthosporium species isolated from wheat and rye, indicated that the fungi from these two different hosts were as indistinguishable in respect to pathogenic properties as in respect to morphology. It is especially significant that the strains from rye infected barley, and that a form identified as *Helminthosporium sativum* produced infection on wheat.

A form of disease strikingly similar to that described by L. J. Stakman was observed on wheat by Bassi (*8*) during the season of 1921 near Piacenze in northern Italy. Two types of the malady, "nerume," were recognized, one affecting the tender shoots of wheat or rye in autumn, the other present the following summer on more mature plants. In the former type, growth is conspicuously stunted, the roots and nodes are involved in decay, and the leaves, besides being greatly reduced in size, assume a light reddish, olivaceous color and eventually wither and die. Characteristic lesions are found on the base of the culms either as local dark reddish streaks or as a more entensive discoloration. The roots are attacked also, the cortical tissue often being injured so severely that when the plants are pulled up the vascular elements are extracted from the cortex which remains behind. The second type of infection is manifested by the appearance on the leaves of numerous small dark brown spots, but more particularly by an attack on the nodes ("marciume dei nodi") so that the latter frequently show on their margins dark brown horizontal lines composed of spores of the fungus. Brown lesions occur on the internodes, as well as on the glumes, which in the final stages, after sporulating commences, assume a dark tinge. The kernels also often are attacked, then becoming discolored and failing to attain full development. The fungus on specimens of diseased plants sent to Padua for examination was identified as *Helminthosporium gramineum*.

In a recent paper Hamblin (*49*) reports the prevalence of a footrot disease of wheat due to a species of Helminthosporium in widely separated regions of New South Wales. The damage occasioned by the fungus in 1921 is believed to have been greater than the loss due to "take-all," varying from 2 to 3 up to 85 and 90 per cent. Apparently the symptoms of the Australian trouble are very similar to those noted in the United States and Italy. Tillering is greatly reduced, the tillers being usually not in excess of two or three. As the root system is poorly developed and affected by decay, diseased plants are easily pulled up. At and below the ground level the base of the plant shows brownish discoloration, either uniformly diffused through the culm and sheaths or present in the form of spots or streaks. The inflorescence of affected plants fails to attain normal development, some of the heads failing to set any seed, others setting seed only in a portion of the spikelets, and still others setting seed in all the spikelets, but the seeds remaining pinched and undersized. Although Hamblin gives no textual descrip-

tion of the parasite, the figures of the sporophore and conidia show an unmistakable resemblance to those of *H. sativum* developing in artificial culture or on natural substrata under moist conditions.

Another reference to the relation of an unidentified species of Helminthosporium to root rot of wheat is given by Raeder (*114*) who observed considerable damage caused by this trouble in Idaho. His description of the symptoms—stunting of the heads, shriveling of the grains, discoloration of the sheath at the base, and occasionally also of the lower nodes—certainly suggest the spotblotch fungus. Snowden's (*135*) report of *H. sorokinianum* on wheat in Uganda may plausibly be interpreted as referring to the same parasite.

The accounts briefly summarized in the preceding paragraphs concerning the occurrence of a species of Helminthosporium on wheat in association with well-defined lesions raise the questions as to whether one or several congeneric forms are concerned. The writer has examined scores of strains of Helminthosporium isolated from black-pointed wheat seeds (Pl. 18, Aa–Cb), from discolored portions of the stems and leaves of both young (Pl. 18, E) and more mature wheat plants, and from conidial fructifications occurring on the glumes of wheat heads (Pl. 18, D). Although the material was obtained from a considerable number of localities in the middle western section of the United States, no constant morphological differences such as distinguish the different species discussed in this paper, could be detected between the various strains. Nor was it possible to recognize any significant differences between the forms isolated from wheat and various strains of *H. sativum* isolated from barley leaves heavily affected by spot blotch. In some preliminary cross inoculation experiments undertaken by the writer, barley and wheat seedlings proved equally susceptible to attack by strains derived from the same and from the reciprocal host; and the lesions produced were indistinguishable regardless of the source of the inoculum. Such identity of morphological and physiological characteristics, altogether in harmony with the findings of L. J. Stakman (*138*) and of McKinney (*87*) have convinced the writer that for the most part a single species of Helminthosporium, namely, *H. sativum*, is involved in the widespread infection of wheat manifested variously by such symptoms as stunting of growth, seedling blight, basal browning, root rot, foot rot, node decay, leaf spot, stem discoloration, and black point. And as the reports of Sorokin, L. J. Stakman, and Bassi indicate, the same fungus evidently is associated with similar pathological conditions in rye.

In another connection, it is true, attention has been called to the fact that the type of injury occasioned by *Helminthosporium sativum* on the wheat plant is duplicated by the attack of several congeneric species on other hosts, as, for example, *H. vagans* on *Poa pratensis* and *H. monoceras* on *Echinochloa crus-galli*. The occurrence of one or more species of Helminthosporium other than *H. sativum*, associated with similar lesions is consequently not to be excluded as a possibility. Nevertheless, there seems to be little reason to believe that *H. gramineum* has ever been found parasitic on wheat in spite of the papers of E. C. Johnson, Palm, and Bassi, indicating such parasitic relation. The reports of Johnson and of Palm, as has been pointed out, appear to have been based on obviously erroneous identification of the fungus concerned. And the form of disease described by Bassi corresponds so well to the trouble investigated by L. J. Stakman that it is hardly probable that two separate species

are involved. While the Minnesota fungus was not definitely identified as *H. sativum*, its similarity to the latter fungus with regard to morphological and cultural characters is certainly very close. To be sure, L. J. Stakman states that the spores of the wheat parasite, while resembling those of *H. sativum* in shape, "contain few septations and are shorter than those described by Pammel, King, and Bakke." However, as the Minnesota fungus appears to have been studied chiefly in pure culture, where the spores of *H. sativum* ordinarily become greatly reduced in length and number of septa as compared with those developed on the host in the field, on which the original diagnosis undoubtedly was based, the difference is very readily explained.

It may not be superfluous to mention that several species of Helminthosporium, morphologically altogether different from *H. sativum*, have been reported as occurring on various parts of wheat and rye plants. Thus Palm (*101*) records the presence on the spikes of this host in Java of a relatively innocuous form with small geniculate spores which he identified as *H. geniculatum* T. & E. (*152*). Hennings (*59*) earlier described as a new species, *H. tritici*, another small-spored but apparently different fungus collected by Zimmerman in the region formerly included in German East Africa. The African form, described as being very injurious, is evidently similar in general habit to *H. ravenelii*, developing a dense crusty coating on the culms, leaves, and heads of the host. Stevens (*141*) makes mention of a geniculate-spored form evidently different from the one usually encountered on diseased wheat in Illinois; while L. J. Stakman records the isolation from the same host of strains of Helminthosporium producing smaller spores than the species generally found associated with the Minnesota disease. A species of Helminthosporium obviously different from *H. sativum* was described from Alabama growing apparently as a saprophyte on decaying culms of rye by Atkinson (*4*) under the name of *H. tuberosum*. The same author also reported *H. inconspicuum* "on living and languid leaves of *Secale cereale*," evidently in a parasitic relation. It appears at least not improbable that Atkinson, following Peck (*107*), applied the binomial of Cooke and Ellis not to *H. turcicum*, but to a form morphologically very similar if not altogether identical to *H. sativum*.

As has been indicated in other connections, when the inflorescence of wheat is attacked (Pl. 18, D), the kernels often are affected, resulting in dark brown, bluish brown, or nearly black discolorations involving more or less extensive and irregular patches at the germ end or extending along the ventral furrow (Pl. 18, Ba-Cb). Hungerford (*64*) and Waterhouse (*156*) noted the presence of Helminthosporium fructifications on wheat grains. A special type of such pathological effect in which the discoloration is relatively intense and limited to the region occupied by the embryo (Pl. 18, Aa, b) has become known as "black point." It is characteristic especially of durum wheat, having been reported by Bolley (*14*) in 1910 as being on the increase on this species, and more recently made the subject of investigation by Evans (*38*) as well as by Weniger (*158*) and by Henry (*61*). Greenhouse experiments, now in progress, the results of which will be published later, have shown that, compared to healthy seeds, badly discolored seeds show a much smaller degree of viability, and that the plants resulting from them frequently develop lesions varying in number and severity. (Pl. 18, E.) It may be mentioned that other fungi, notably one or several species of Alternaria, also have been found associated with discoloration of wheat grains, which, however,

while somewhat similar to the type of discoloration attributable to *H. sativum*, can usually be distinguished by its lesser intensity, being diffused brownish rather than dark brown.

Especially pertinent in this connection is Henry's (*61*) recent report of the isolation from black-pointed wheat kernels, of certain forms he designated as "*Helminthosporium* sp." and "*Brachysporium*," which, while of much less frequent occurrence than the spot-blotch fungus, were found to be efficient causes of black point. The writer, who was kindly permitted to examine transfers of these fungi, can only confirm the correctness of Henry's judgment in regarding the "*Helminthosporium* sp." as distinct from *H. sativum*. It appears to be different from any species figured in this paper, though, perhaps, most suggestive of *H. monoceras*. The strains designated as "*Brachysporium*" appear to represent one or several smaller-spored forms that might not improperly also have been referred to the genus Helminthosporium, bearing a good deal of resemblance to a species frequently observed by the writer on withered leaves of *Danthonia spicata* (L.) Beauv.

It may not be amiss to refer here to a fungus described under the name *Podosporiella verticillata* by O'Gara (*98*) who found it on the kernels of germinating wheat in the Salt Lake Valley, causing stunting of the resulting seedlings, and an uneven stand. The conidia of this form show a very obvious general resemblance to those of the larger species of Helminthosporium. The synnemata figured by O'Gara are evidently not dissimilar from the threadlike or columnar structures observable, for example, in cultures of *H. inequalis* Shear, or of *H. cyclops*. Indeed, except for the arrangement of the conidia on the sporophore, which according to O'Gara is verticillate, the fungus shows considerable similarity especially to the last named of these species.

While *Helminthosporium sativum* has not been reported as attacking maize in the field, the writer has found it of more or less frequent occurrence on the dead remains of mature plants. Cultures derived from fructifications on old culms and leaves were not observed to differ in any important detail from cultures of the spot-blotch fungus isolated from barley. In a few instances, sporophores and spores of a form similar to *H. sativum*, at least in respect to morphological characteristics, were found on dead areas of leaves of sweet corn collected on Long Island. However, as the fructifications of *H. turcicum* were also present in excessive abundance, it was not possible to draw any definite conclusions concering the biological relations of the form under consideration. In general, its distribution on corn suggests a saprophytic existence on this host, modified, perhaps, by a capacity to establish itself on moribund foliage. It may be mentioned in this connection that while Stevens found his strains of the Illinois foot-rot organism capable of infecting corn and corn-fodder, L. J. Stakman secured negative results from the inoculation of corn plants with strains of Helminthosporium isolated from a node of affected wheat and from rye seed. In some preliminary infection experiments the writer applied spores of *H. sativum* from barley to corn seedlings about 5 inches high, very liberally stroking them in water suspension on the moistened leaves with a spatula, and then confined the seedlings in a saturated atmosphere for 48 hours. At the end of this time the leaves had lost their mechanical rigidity and the entire plants looked as if they had been steamed, although the controls, treated in the same way except that no inoculum was applied, were nearly normal. That much significance attaches to such injury, unlike any naturally

produced by the parasite on any host, and, indeed, unlike any injury produced under conditions obtaining in nature by any congeneric form with the exception of *H. micropus*, seems altogether improbable. The results obtained by exposing experimental plants to the rigorous treatment incident to the application of relatively large quantities of inoculum and incubation in a saturated atmosphere during long periods, should at all events be interpreted with caution. And the necessity of caution would seem especially evident in dealing with a fungus, more or less promiscuous in its parasitism, the experimental host range of which would tend to be out of all proportion to its actual range in nature.

The occurence of *Helminthosporium sativum* on corn has brought about a measure of confusion regarding the indentity of *H. turcicum*. It is interesting to note that in Peck's account (*107*) of *H. inconspicuum*, the spores of this fungus are described as "nearly black, septate up to 8 to 9 times;" and figured as decidedly dark, curved cylindrical, with rounded ends, attached to sporophores emerging singly from the substratum. In all details of habit and morphology the correspondence with *H. sativum* is much closer than with *H. turcicum*; and it appears probable that although Peck observed the corn disease correctly, he inadvertently based his account of the fungus not on the well characterized parasite, but on the adventitious form. Specimens deposited in the herbarium of the Office of Pathological Collections of old corn leaves from various localities in the United States, labeled "*Helminthosporium inconspicuum*," on examination, were found to bear dark olivaceous spores with non-protuding hila, not dissimilar from those of *H. sativum*, although their collapsed condition precluded the possibility of reliable identification.

OCCURRENCE OF FUNGUS ON GRASSES OTHER THAN CEREALS

Bolley (*15*) reported the occurrence of a species of Helminthosporium on quack grass collected in North Dakota and Wisconsin. As the fructifications developing on this host were held responsible in certain instances for the infection of wheat plants in the field, it is apparent that he regarded the species as identical to the parasite causing root rot and brown spot. In any case, the writer found *H. sativum* quite commonly present on quack grass in the northern tier of Middle Western States, being associated with a leaf-spot disease, affecting the foliage of plants of all ages. (Pl. 18, H.) The discolored leaf areas, although quite numerous, are decidedly smaller than those characteristic of spot blotch of barley, occurring usually in the form of linear streaks, dark chocolate brown in color, 0.3 to 0.4 mm. in width and 0.5 to 3 mm. in length. During the season of 1919 the writer collected in the vicinity of Madison, Wis., diseased green leaves as early as May 6, and as late as October 24. The injury to the host appeared to be greater than that resulting from the parasitism of *H. tritici-repentis*, although neither of the two congeneric parasites could be said to have been especially destructive. In the region about New York City, during the season of 1920, the leaf spot due to *H. sativum* was, however, relatively rare, being considerably less abundant than the blight due to *H. tritici-repentis*.

A fungus quite similar to the parasite causing "late blight" of barley was reported by Pammel, King, and Bakke (*104*) as occurring on *Festuca elatior* L. (=*Festuca pratensis* Hud.) in Iowa. Examination of material deposited in the herbarium of the Office of Pathological Collections only confirms the opinion of these authors concerning the similarity of the lesions to those characteristic of spotblotch of barley, and the very

probable identity of the parasite to *H. sativum*. It is interesting to note, however, that in the vicinity of New York City and Washington, D. C., during the seasons of 1920 and 1921, meadow fescue was not observed affected with the spot blotch described from Iowa, although very commonly showing the symptoms of the net blotch disease discussed elsewhere in this paper.

In addition to the graminaceous species already discussed, the literature contains references to successful infection of other species, by artificial inoculation with strains of Helminthosporium that, as has been pointed out, may be referred to *H. sativum*. Thus Massee (*90*) secured growth and abundant sporulation by transferring spores of the fungus he regarded as *H. gramineum* to leaves of *Festuca ovina*, *Briza media*, *Dactylis glomerata*, *Poa annua*, and *Arrhenatherum avenaceum* that had been cut off and incubated in a damp chamber. Stevens (*141*) secured infection of Sudan grass and millet as a result of inoculation with the footrot organism. E. C. Johnson (*71*) found that the strain of Helminthosporium isolated by him from the node of a wheat plant seriously attacked the foliage of young oat seedlings. L. J. Stakman (*138*) reports positive results from the inoculation of over a dozen new hosts with strains originally isolated from wheat and from rye. Taken altogether the results are not in complete harmony, a fact which may probably in part be explained by differences in the conditions under which the experiments were carried on. The method employed by Massee certainly would seem to make for an inordinately extensive experimental host range. On the other hand, the existence of biological races, corresponding to those of stem rust (*Puccinia graminis*) for example, is not outside the realm of possibility. Indeed, absolute agreement in regard to host interrelations between different strains of a fungus attacking so many allied graminaceous species as *H. sativum* could, perhaps, scarcely be expected.

MORPHOLOGY OF THE FUNGUS

The fructifications of *Helminthosporium sativum* make their appearance after the death of the affected tissue, emerging from the stomata or more frequently between the epidermal cells singly or in fascicles of 2 or 3. (Pl. 17, Ea–e.) According to Pammel, King, and Bakke (*104*), the sporophores vary from 8 to 10 μ in width, but these figures appear somewhat too high, the measurements for this dimension varying usually from 6 to 7 μ, and rarely exceeding 8 μ. The first spore is produced generally at a distance of 50 to 90 μ from the base. The scars marking the points of attachment of successive spores at well-defined geniculations occur at very variable intervals, approaching 5 μ as a minimum limit, and occasionally exceeding 60 μ. As found in nature, the sporophores rarely show more than 5 or 6 scars or more than 8 septa, the distances between the latter usually varying from 5 to 40 μ. The measurements for *H. teres* given by Bakke (*6*), 150 to 180 μ by 60 to 80 μ, while obviously incorrect for the width of the sporophores of the causal organism of spotblotch or "late blight" of barley, probably as a result of a typographical error, are also sufficiently in excess of the usual range in length of these structures, 110 to 150 μ, to indicate that this author was, indeed, in this instance referring to the parasite causing netblotch.

Such indication is strengthened by the portions of his description of the spores referring to their length and color, "150 to 130 μ," and "pale

greenish gray," respectively. Plate 18, Fg, represents a 12-septate spore of *Helminthosporium sativum* that was scraped from a wheat inflorescence and found to measure 134 μ in length, which may be regarded as approximating the maximum for this dimension. The maximum width represented, for example, in the spore shown in Plate 18, Fe, is approximately 30 μ. The minimum for width of spore, as found in material occurring in nature, is about 14 μ (Pl. 17, Ca), that for length in the region of 25 μ (Pl. 18, Fc). When collected on diseased barley (Pl. 17, Ca–i) or quack grass leaves (Pl. 18, Ga–k) in midsummer, the spores are typically slightly or distinctly curved; 3 to 10 septate, widest near the middle, tapering slightly or sometimes quite considerably toward the ends which are rounded off abruptly, and show a hemispherical or hemiellipsoidal contour; and measuring usually 15 to 20 by 60 to 120 μ. On wheat heads, (Pl. 18, Fa–q), or on the bases of wheat or barley plants, apparently in response to more moist conditions, the spores are more apt to be atypical, being either straight or, if curved, curved irregularly (Pl. 18, Fd, f, j); showing unusual variability in width, which fluctuates not only with respect to different individuals but also in respect to different portions of the same spore (Pl. 18, Fi); exhibiting often marked irregularity in regard to septation, the septa occurring at unequal intervals, often at planes decidedly oblique to a plane perpendicular to the longitudinal axes of the spore (Pl. 18, Fk, n), and occasionally quite approximating a longitudinal position, thus bringing about a muriformly septate condition (Pl. 18, Ff, h, i). The same departures from the curved, long-ellipsoidal type is exhibited also by spores developed on plants in the greenhouse and perhaps to an even greater extent by those produced in pure culture on artificial media. In the latter case the diminution in size is unusually great, the spores there (Pl. 19, C, Da–c) generally not exceeding in length more than one-half the length of the typical ones to which, however, they are not markedly inferior in width. Very frequently, indeed, they become reduced to subspherical bodies, not appreciably greater in length than in diameter, often nonseptate or with a single cross wall (Pl. 19, C). A straight, short ellipsoidal shape is thus characteristic of the spores developed in the greenhouse or in artificial culture (Pl. 19, E), a shape which may be modified by irregular curvatures or distentions, or by the flattening or even incipient bifurcation of the apical end (Pl. 19, Dc).

But, however variable in shape, the spores of *Helminthosporium sativum*, when fully matured, are uniformly of a dark olivaceous color, and always exhibit a thick peripheral wall and a conspicuous hilum that is situated within the contour of the rounded basal end. As long as the peripheral wall is uninjured, germination regularly proceeds by the proliferation of two terminal germ tubes, one at the apex and the other immediately adjacent to the hilum (Pl. 17, Da, b; Pl. 18, Gc; Pl. 19, Da, b, d). Atypical spores, with the distal end flattened or bilobed, may produce three germ tubes, one arising from each of the lateral apices as well as from the proximal end. (Pl. 19, Dc.) Germ tubes apparently are never produced normally from the intermediate segments. Viability is retained for a considerable period of time, spores from material stored in the laboratory a whole year having been germinated by the writer without much difficulty.

Helminthosporium sativum is very readily cultivated on the substrata ordinarily employed in laboratories. On hard potato glucose agar containing an abundance of organic food material the aerial growth is

represented usually by a compact velvety layer of a black olivaceous color, composed entirely of short crowded sporophores. If less agar-agar is used, leaving a slight excess of free water, a fluffy, dark greenish brown aerial mycelium, interspersed often with matted masses of white mycelium, is produced. Often the vegetative growth is largely present as a firm coriaceous bluish-black crust. In older cultures masses of compacted white hyphae of secondary origin may be thrust through the layer of sporophores giving rise to an appearance suggesting contamination by another fungus. On corn-meal agar or tap-water agar, containing only small amounts of organic food material, the growth below the surface of the substratum is relatively sparse, being limited to moderately remote hyphae branching and anastomosing freely but without the production of lobulate segments. (Pl. 19.) Scattering sporophores arise at intervals as branches of these hyphae, and in the course of 4 to 6 weeks develop into long structures, intricately contorted, often bearing one or several branches, and scores of successively proliferated spores. (Pl. 19, A, B, C.) *Helminthosporium sativum* is thus readily distinguished by its macroscopic cultural characteristics from congeneric species with subhyaline spores even when occurring on the same hosts, as *H. teres* and *H. gramineum* on barley and *H. tritici-repentis* on quack grass. Cultural characters, however, are of little assistance in distinguishing it from a considerable number of forms like *H. monoceras*, that exhibit great similarity in development and in general habit of growth.

The responsiveness of *Helminthosporium sativum* to obvious differences in environmental conditions, illustrated, for example, in the results obtained by Dosdall and Christensen (*30*) in their study of variations in length of spores, makes it less easy to define this, as well as many other species, with as great precision as might be desired. The difficulty is further accentuated by evidence of lack of uniformity under apparently similar conditions of growth. In a recent extensive paper, Stevens (*142*) has called attention to morphological differences existing between strains of Helminthosporium isolated from wheat affected with footrot. These strains this author assigned to the "*H. sativum* group, which consists of a large number of elementary species." The mode of origin of presumably new strains as aberrant sectors in plate cultures was designated as "saltation," and found in some races to be of frequent occurrence. What the significance of such phenomena may be, and to what extent they correspond to realities in nature, constitute questions open to conjecture. In any case, the fungus under consideration can hardly be regarded as especially extraordinary. Variability of the sort represented here, is probably more nearly the rule among species of fungi than the exception. The writer is inclined to believe that the amount of morphological versatility observed in a species is often contingent in a larger measure on the possibilities its distinctive structures possess for expressing readily demonstrable differences than on the degree in which it may be lacking in genetic unity. Thus, for example, the relatively short conidia of *H. triseptatum* with a maximum of three cross walls, could never exhibit the wide fluctuations in length and septation possible in spores of species like *H. oryzae* or *H. rostratum*. This obvious limitation in the possibility of variation, may very largely account for the uniformity of the small-spored species of Helminthosporium as compared with the larger-spored forms, of which *H. sativum* is an example.

CONTROL

As the disease of barley and wheat due to *Helminthosporium sativum* is attributable on the one hand to primary infection of the seedling, resulting from the use of infected seed, and on the other to secondary infection of the growing plant, two lines of procedure in attempting their control are indicated. To prevent primary seedling infection, the use of none but clean seed naturally suggests itself. If affected seed stock must be used, the hot air treatment devised by Atanasoff and Johnson (*3*) constitutes a very effective means of obviating the primary manifestations of the disease in barley. Although these authors did not include wheat seed infected with *H. sativum* in their tests, there is good reason to believe that the hot air treatment will prevent the appearance of primary lesions in the resulting seedlings. Some preliminary trials with badly discolored wheat seed carried out in Washington indicate that treatment with certain organic mercury compounds controls seedling blight quite completely.

Although the elimination of primary infection, by bringing about a reduction of the amount of inoculum available early in the season, may well be expected to moderate subsequent infection, it is scarcely probable that injury to individual fields of barley, wheat, and rye can be prevented except by controlling the fungus on a more comprehensive scale. Such control, barring the likelihood of strictly specialized races, would appear to involve measures like the widespread use of clean or disinfected seed by all growers within the community, and the suppression of uncultivated grasses that serve as congenial hosts, as, for example, quack grass. Generally approved agricultural practices, like judicious rotation of crops, clean cultivation, and the use of manure containing diseased straw only on fields that are to be planted to immune crops—all tending toward making the inoculum of one season ineffective in reestablishing the fungus in the next—also ought to prove beneficial.

As methods of disease control contemplating the approximate elimination of a fungus as generally distributed as *Helminthosporium sativum* are necessarily rather uncertain, the possible development of resistant varieties of the various cereals affected would seem to hold forth most promise. The work of Hayes and Stakman (*53*) revealed pronounced differences in susceptibility to spotblotch between various varieties of barley, and showed that resistance is inherited. Thus, on crossing a rough-awned resistant type with a smooth-awned susceptible one, these authors demonstrated the possibility of obtaining a variety combining the desirable smooth-awned character of one parent with the resistance of the other. A study of commercial varieties suggested that resistant barleys of any botanical group could be produced. While in wheat and rye the organs most severely attacked are the roots and stem rather than the leaves, it is hardly possible that this fact in itself would constitute an obstacle to successful breeding work with these cereals.

HELMINTHOSPORIUM MONOCERAS, N. SP.

In September, 1920, the writer observed near Port Washington, N. Y., on Long Island, a stand of barnyard grass (*Echinochloa crus-galli* [L.] Beauv.) that appeared to be quite severely affected by a type of spotblotch. (Pl. 20, A.) The upper leaf blades, otherwise entirely green and healthy, bore dark brown or chocolate-colored spots (pl. 20, B), measuring 0.3

to 1.0 mm. in width by 1 to 3 mm. in length, without any indication of a zone of etiolated tissue, as is present on leaves of *Bromus inermis* attacked by *Helminthosporium bromi*. The lower leaves were largely withered, apparently as a result of the same pathological conditions present in the younger foliage, and exhibited an abundance of similar elliptical spots, which here, however, were somewhat larger, often attaining a width of 1.5 and a length of 5 mm. The older spots, moreover, showed less distinct outlines, having faded to a dull medium brown. On the leaf sheaths the discoloration was less intense than on the blades and extended over larger, less well-defined areas, especially near the base of the plant, the lower portions of the basal sheaths being often quite completely tinged with a diffused brown. In short, the symptoms were very similar to those previously observed on *Poa pratensis* attacked by *H. vagans*, representing a combination of foliar lesions like those caused by *H. sativum* on barley, and a footrot condition such as is caused by the latter fungus on wheat; and a cursory examination of the material might readily have led one to attribute the injury to either one or the other of these two parasitic species.

The microscope did, indeed, reveal an abundance of fructifications of Helminthosporium on the older withered leaf blades and dead basal sheaths of the host, which by their distribution left no doubt as to their relation to the foliar lesions observed. Somewhat to the writer's surprise the fungus, however, was markedly different in a number of details from both *H. sativum* and *H. vagans*. The dark brown sporophores (Pl. 20, Ca–Cc), which usually emerge from the stomata singly or in groups of two or three, and are only slightly greater in diameter than those of *H. sativum*, attain a height frequently more than twice as great as those of the latter species and in a corresponding measure are more remotely septate. The spores (Pl. 20, Da–n), the points of attachment of which are marked by geniculations occurring at moderately long intervals, are the most characteristic structures, however, being of a form so distinctive that they would readily be recognized in a mixture with spores of *H. sativum* or *H. vagans*, or indeed of any species discussed in the present paper. Usually widest near the middle, they taper gradually and quite uniformly toward the distal end until, at a point where the diameter scarcely exceeds one-third of the maximum diameter, they are abruptly rounded off to form the tip. The proximal portion of the spore tapers toward the base, usually somewhat more sharply, and the tapering is here prolonged uniformly until the diameter often represents less than one-sixth of the maximum diameter. A short distance above the hilum the contours usually exhibit a slight convexity. The hilum protrudes quite conspicuously, the fungus in this respect resembling *H. turcicum* and *H. rostratum*.

The conidia on being mounted in water germinate readily by two polar germ tubes, one from each end cell. (Pl. 20, Ea–c.) As has been mentioned in another connection, the spore wall is perceptibly thinner in the regions from which the germ tubes always are produced; that is, at the apex and in the narrow contracted zone adjacent to the hilum. Owing to the absence of olivaceous coloration from the modified regions, the portion of the spore near the hilum appears conspicuously subhyaline.

On the types of artificial media ordinarily employed in laboratories the fungus grows luxuriantly, though without manifesting any cultural characters which would enable one to distinguish it readily from other dark, abundantly sporulating congeneric species, as for example, *Hel-*

minthosporium sativum, *H. halodes*, or even *H. turcicum*. On media containing much organic food material, like potato glucose agar, a black velvety mat of sporophores results; on less concentrated media, like tap-water agar or cornmeal agar, growth is much less profuse and the individual fructifications are scattered sparsely over the surface. A comparison of Plate 21 with Plates 19, 23, and 25 reveals the similarity of the fungus to the other species mentioned in respect to general habit, mode of development, and relationship of conidiophore and imbedded mycelium. The conidia, which are produced in abundance (Pl. 21), although in general smaller than those developed in nature, are otherwise quite similar to the latter in shape and septation, and show very clearly the characteristic tapering of the acuminate basal portion toward the protruding hilum (Pl. 21, F).

Although observed thus far only at one station, perhaps largely because the host is not especially abundant in the region in which the writer carried on his studies, the fungus appears to be a parasite of more than moderate virulence. It would not be surprising to find it fairly common in agricultural regions where barnyard grass occurs to some extent.[14] In this connection it may be mentioned that Schweinfurth and de Thuemen (*131*) reported *Helminthosporium flexuosum* Corda (= *Brachysporium flexuosum* [Cda] Sacc.) on the culm and inflorescence of *Echinochloa crus-galli* in Egypt. While there is some reason to doubt that the fungi from various sources that have been referred by different writers to Corda's species are all identical with each other, such identification may safely be interpreted to indicate spores of a relatively small size, measuring approximately 8 to 16 μ in length, and divided by 2 or 3 septa. Manifestly the fungus in question is altogether different from the Egyptian form. It is apparently new to science and, owing to the characteristic shape of the proximal portion of its spores crudely suggesting that of a horn, is designated here as *Helminthosporium monoceras*.

DIAGNOSIS

Helminthosporium monoceras, n. sp.

Producing dark brown or chocolate-colored spots on leaf blades of *Echinochloa crusgalli* (L.) Beauv., at first measuring 0.3 by 1 mm., later increasing in size and eventually attaining linear dimensions five times as large. On the sheaths the spots are larger, less intensely colored, and tending to become confluent, especially toward the base of the plant, where they frequently merge into a generally diffused light brown discoloration; the foliar structures attacked dying prematurely, the withering proceeding from the tip toward the base.

Conidiophores appearing after the death of the affected tissues, thick-walled, emerging usually from stomata, singly or in groups of 2 or 3; measuring 6 to 9 μ in diameter and 120 to 325 μ in length; dark brown or olivaceous except at the extreme tip which is nearly hyaline; usually 3- to 7-septate, the septa occurring at intervals of 30 to 60 μ; producing the first spore 120 to 200 μ from the base, its position, as well as that of subquent spores, indicated by scars at moderate geniculations separated at intervals varying from 5 to 50 μ.

Spores yellowish when young, becoming dark olivaceous when fully matured, exactly similar in color to *Helminthosporium sativum*, *H. vagans*, and *H. rostratum;* measuring usually 15 to 22 μ in diameter and 40 to 150 μ in length; typically straight or showing a slight crescentic or sigmoid curve, yet not infrequently exhibiting more pronounced irregular or geniculate bends; widest at the middle segment except where modified by an irregular median constriction, typically tapering gradually toward the tip to one-third or one-half of its maximum width, then bluntly rounded off; tapering toward the base to approximately one-sixth of the median diameter, the contours then

[14] Since this text was written the fungus has been collected on barnyard grass at Bladensburg, Md., and Takoma Park, Md., both of these stations being located in the immediate vicinity of Washington, D. C.

curving gently into the protruding hilum; 3 to 10 septate, the septa not usually associated with perceptible constrictions in the peripheral wall. The peripheral wall at maturity, thick as in *H. sativum* or *H. vagans*, except at the apex and about a subhyaline narrow zone at the proximal end immediately adjacent to the hilum, where it remains thin. Germinating by the production of two polar germ tubes, one from each of the thin-walled regions.

On artificial media, at ordinary temperatures, producing conidia smaller than those produced under natural conditions but of the same characteristic shape. Vegetative mycelium light fuliginous, 2 to 4 mm. in diameter, anastomosing abundantly by smaller subhyaline branches without the production of lobulate segments. Sporophores fuligenous, thin-walled, often exhibiting a tendency toward branching; approximately 5 μ in diameter, arising abruptly as branches from vegetative hyphae; or narrow at the point of origin and expanding more gradually; provided with septa generally at intervals of from 30 to 50 μ and proliferating spores at considerably shorter intervals than in nature, thus producing a moderately compact racemose cluster.

HABITAT.—Collected at Port Washington, N. Y., September 20, 1920, in a moderately moist situation near the sea.

HELMINTHOSPORIUM HALODES, N. SP.

During the latter part of the growing season of 1920 the writer kept under observation a stand of *Distichlis spicata* (L.) Greene growing on a salt marsh near Douglaston, N. Y., on the northern coast of Long Island. Collections of the grass made on different dates, September 10, September 26, and October 5, revealed indications of injury immediately suggesting similarity to the symptoms of spotblotch and footrot produced on other graminaceous hosts by various species of Helminthosporium, like *H. sativum*, *H. monoceras*, and *H. vagans*. The lesions were present on the otherwise healthy foliar parts as dark, discolored areas, with a bluish cast, not definitely circumscribed, the margins fading insensibly into the green of the surrounding tissue. (Pl. 22, A.) On the leaf blades the discolored spots were generally relatively infrequent; they were found more commonly on the sheath, especially on the upper portion immediately below the attachment of the blade. After the death of the affected tissue, the discoloration usually lost some of its intensity, the spots then appearing as rather vague blotches not readily distinguishable from similar blotches commonly found on the dead plants but attributable to other agencies.

Microscopical examination of dead affected foliar parts revealed a species of Helminthosporium present in meager quantity as the probable cause of the disease. Sporophores (pl. 22, Ef–g) occurring on the leaf blades were always found entirely denuded; and whatever spores could be discovered were as frequently found adhering to obviously unaffected tissues or lodged under the upper edge of the leaf sheaths as scattered on the epidermis in proximity to the sporophores. In view of the fact that the host is occasionally inundated by tidal water, an explanation for such a condition is manifestly not difficult to find. To determine whether the disease observed bore any relation to the Helminthosporium fructifications, freshly affected green parts were dissected out and incubated in a damp chamber. After 15 days the discolored areas were covered with a dense growth of conidiosphores bearing conidia corresponding quite closely to those found in nature. Pure cultures made from spores obtained directly from the material as it was collected and from spores developed in the damp chamber further demonstrated the specific identity of the two lots of material and consequently also the parasitic nature of the fungus originally observed.

As might be expected from the similarity of pathological symptoms, the fungus shows a fairly close resemblance to *Helminthosporium sativum*.

The conidiophores, as occurring in nature (Pl. 22, Ef, g) or developed on the natural substratum in a damp chamber (Pl. 22, Ea–e), are somewhat inferior in diameter to those found on barley leaves affected with spotblotch and generally noticeably shorter. The conidia are of the same type as those of *H. sativum*. As found on material collected in the field (Pl. 22, Ba–f), however, they are usually more narrow and not as regularly crescentic, being more frequently straight or irregularly curved. In color they are usually brownish yellow instead of olivaceous. In a certain proportion of the spores, moreover, the end cells are less deeply colored and the basal and distal septa appear conspicuously darker or heavier than the intermediate cross-walls. (Pl. 22, Bd–f.) When developed in a damp chamber on diseased host material the conidia approach those of *H. sativum* in depth of coloration; but the distinction between dark intermediate segments and subhyaline or fuliginous end segments set off by conspicuously accentuated septa becomes a constant characteristic. (Pl. 22, Da–f.) And while the apical end is rounded off quite abruptly as in *H. sativum*, the proximal end shows a more perceptible tendency to taper, recalling in some instances the decidedly acuminate contour of the proximal portion of the spores of *H. monoceras*. As in the latter species, this gradual tapering is associated with a hilum that protrudes from the basal contour of the conidium.

In the species parasitic on *Distichlis spicata* the peripheral wall of the conidium varies more or less in thickness, depending apparently on the conditions under which the fructifications are developed. The darker, more mature conidia usually possess a peripheral wall which, if not as massive as in *Helminthosporium sativum*, nevertheless is of at least moderate thickness. As in *H. monoceras*, the wall is very thin at the apical end and over a narrow zone at the proximal end immediately adjacent to the hilum. Germination regularly occurs by the production of two polar germ tubes, one from each of these thin-walled regions. (Pl. 22, Ca, e, i.) In the case of immature spores and often in the brownish yellow ones found in nature the peripheral wall is thinner, frequently collapsing when the contents of the segments inclosed degenerate. Such spores may germinate by the production of lateral germ tubes from one or more of the intermediate segments as well as from the end segments (Pl. 22, Cb, c, d, f, g). This mode of germination ,however, can scarcely be regarded as typical for the species.

The fungus is cultivated readily on artificial media, producing a luxuriant dark olivaceous aerial growth, consisting of a variable quantity of mycelium bearing an abundance of sporophores. When grown on tap-water agar, the fructifications (Pl. 23, A–C) resemble those developed on parts of the diseased host after incubation in a damp chamber. The spores (Pl. 23, B, D–G), which are attached at short intervals on the very irregular sporophore at wide angles and in moderately compact racemose arrangement, however, are usually perceptibly shorter. As in *Helminthosporium sativum*, the conidia frequently exhibit irregularities in shape, including flattening or bifurcation of the apical portion. (Pl. 23, D, F.)

Besides resembling in some details the different species which have already been mentioned, the fungus suggests comparison with *Helminthosporium leersii*, and perhaps more especially with *H. oryzae* and *H. rostratum*. From *H. leersii* it may be distinguished readily by the subhyaline end segments, the accentuated end septa, and the protruding hila characteristic of its spores. While *H. leersii* on artificial media

produces a greyish aerial mycelium, the growth of the parasite on *Distichlis spicata*, as has been noted previously, has a very dark if not almost black appearance. *Helminthosporium oryzae*, on the other hand, could be confused with the fungus under consideration only when cultivated on a substratum on which its spores develop dark pigment, as, for example, potato dextrose agar. The greater dimensions of the conidiophores and conidia produced by the rice parasite and the position of the hilum within the contour of the peripheral spore wall constitute, however, features by which the two species can be distinguished in spite of some similarity due to the occurrence of subhyaline end segments in both. Moreover, while the spores of *H. oryzae* tend to taper strongly toward the tip, and to a smaller extent toward the base, those of the fungus on *Distichlis spicata* show relatively slight tapering toward the abruptly rounded apex, while the proximal portion usually tapers markedly toward the base. The resemblance between the latter fungus and *H. rostratum* is attributable to similarity in color of the conidia as well as to the presence in the spores of both species, of protruding hila, and of accentuated proximal and distal septa. The parasite on *Distichlis spicata*, however, is inferior in the dimensions of both sporophores and spores and never exhibits the rostrate modification of the apical end characteristic of the conidia of *H. rostratum*. It appears not to have been described hitherto. As it is the first member of the genus, as far as the writer is aware, to be found occurring as a parasite on a host more or less frequently flooded with sea water, the specific name *halodes* is suggested.

DIAGNOSIS

Helminthosporium halodes, n. sp.

Occurring on the foliar organs of *Distichlis spicata* (L.) Greene, on which it causes a disease not usually destructive, manifested by the appearance of poorly defined bluish discolored areas especially on the leaf sheath immediately below its juncture with the blade.

Conidiophores arising from discolored spots after death of host, singly or in groups of two; measuring generally 4 to 7 μ in diameter by 60 to 150 μ in length; producing first spore usually 60 to 100 μ from base, successive spores at intervals of 5 to 15 μ at apices of geniculate irregularities; 1 to 5 septate, the length of segments highly variable, typically 15 to 30 μ.

Conidia as produced under natural conditions straight or curved; measuring 10 to 14 μ in width by 20 to 105 μ in length; 1 to 12 septate, the septa in immature spores associated with barely perceptible constrictions of the peripheral wall, in mature spores not associated with constrictions; brownish yellow, the end segments sometimes lighter in color or subhyaline, and delimited by accentuated septa; tapering slightly toward the broadly rounded apex, and more markedly toward the more narrowly rounded or somewhat acuminate basal end, which is uniformly distinguished by the protruding hilum. Mature spores germinating typically by the production of two polar germ tubes, produced at thin-walled regions at tip and immediately adjacent to hilum; germination of immature thin-walled conidia often atypical, owing to the production of additional germ tubes from one or more intermediate segments.

When cultivated on natural substratum in damp chamber, fructifications similar but capable of more extensive development; spores somewhat shorter, usually thicker, and tapering more perceptibly toward both ends; dark olivaceous, the end segments subhyaline or light fuliginous and set off by accentuated septa. On artificial media not rich in organic food material conidiophores arising as lateral branches from prostrate hyphae, bearing conidia in moderately dense racemose arrangement at geniculate irregularities occurring at short intervals; conidia as on natural substratum dark olivaceous provided with subhyaline end segments and with moderately thick peripheral wall but shorter, often being nearly ellipsoidal in shape.

HABITAT.—Collected at Douglaston, N. Y., near New York City, in September and October, 1920.

HEMINTHOSPORIUM LEUCOSTYLUM, N. SP.

In October, 1921, the writer collected specimens of *Eleusine indica* (L.) Gaertn. near Washington, D. C., some of the leaves of which were partially or wholly withered. Most frequently the tip alone was affected, but in other instances the blade was involved down to its juncture with the sheath. The dying portions of the leaf were characterized by the presence of small, irregular, poorly defined, reddish-brown spots, somewhat suggestive of the discoloration produced by *Helminthosporium avenae* on the foliage of oats.

On examining thoroughly withered portions of leaf which, like barley leaves affected with stripe, showed a pronounced tendency to split into numerous longitudinal shreds, fructifications of a fungus apparently referable to the genus Helminthosporium were found in moderate abundance. As these portions frequently bore also a considerable growth of undoubtedly saprophytic forms, mainly Alternaria spp., the spots previously mentioned were largely obliterated by miscellaneous local and diffused discolorations. It was consequently not possible to decide definitely as to the existence of any causal relation of the species of Helminthosporium, either to the withering observed or more particularly to the foliar lesions that appeared to be associated with it. To distinguish the fungus from the congeneric forms, *H. giganteum* and *H. cynodontis*, occurring on the same host, as well as from *H. nodulosum* B. & C., an apparently widely distributed parasite attacking the inflorescence of goose grass, it may not be superfluous to include a brief account of its morphological features.

The conidiophores (Pl. 23, Ha-e, Ia-d) exhibit a particularly striking departure of the fungus from the type that appears to be common to most graminicolous species of Helminthosporium. Instead of growing to a considerable length before beginning to proliferate conidia, they develop the first spore while still relatively short. Owing to this initial economy and to the moderately short intervals between successive spores, the ultimate length of the sporophore is conspicuously inferior to that of most congeneric species. Perhaps quite as remarkable is the absence of the usual deep olivaceous color from these structures, which are, generally, nearly colorless or of a light fuliginous tinge. It is interesting to note, besides, that the sporophores, like those of *H. turcicum*, appear to emerge only from the stomata, and in relatively large groups.

The conidia (Pl. 23, Ja-r), which resemble those of *Helminthosporium sativum* and many other species in being of a deep olivaceous color, in the possession of a thick peripheral wall, and in mode of germination, frequently show a pronounced tendency to taper toward the apex (Pl. 23, Jg, i). Indeed, the writer was at first inclined to refer the species to the genus Napicladium because of this tendency. However, as in numerous instances a subcylindrical shape is approximated (Pl. 23, Jb, p.), it appears better to refer it to the genus Helminthosporium, which, moreover, contains other species characterized by spores frequently tapering very perceptibly toward the apex, as, for example, *H. gramineum*. The attenuation of the peripheral wall at the places from which the germ tubes are destined to appear is very marked, being evidenced as in *H. halodes* and *H. monoceras*, for example, by small, altogether hyaline regions, one at the apex and another immediately surrounding the hilum. The germ tubes are relatively narrow, and, in elongating, more

inclined to make irregular turns and unusual angles than those of most congeneric forms. (Pl. 23, Ka-c.)

The specific name *leucostylum*, descriptive of the conidiophores of the fungus, is suggested.

DIAGNOSIS

Helminthosporium leucostylum, n. sp.

Occurring on withering or withered leaves of *Eleusine indica* (L.) Gaertn.

Conidiophores subhyaline to light fuliginous, emerging from stomata singly or in groups of 2 to 6: measuring usually 5 to 6 μ in diameter by 35 to 100 μ in length; 2 to 8 septate, the cross walls usually inserted at intervals of 10 to 30 μ; producing the first spore 20 to 50 μ from base, and successive spores at intervals of 5 to 25 μ, the points of attachment being marked by circular scars at the apices of usually very pronounced geniculations or more rarely at the tips of short lateral branches.

Spores dark olivaceous; measuring 11 to 17 by 15 to 67 μ, typically approximately 15 by 50 μ; 1 to 6 septate, typically 3 to 5 septate, the septa never associated with constrictions; usually narrowly ovoid, widest well below middle, near a point approximately one-third of distance from base to apex, the proximal portion exhibiting a paraboloid contour, the distal portion tapering uniformly to the narrow apex, then abruptly rounded off; more rarely and atypically subcylindrical, and, when short, ellipsoidal, ovoid or obovoid; exospore thick except at a small subhyaline spot at apex and in narrow subhyaline zone immediately surrounding the hilum which is contained within basal contour. Spore germinating by production of 2 polar germ tubes, one from each of the small hyaline regions.

HABITAT.—Collected near Washington, D. C., October 9, 1921.

During August and September, 1922, after this paper had been submitted for publication, the fungus was collected repeatedly on *Eragrostis major* in several localities in Virginia and Maryland near Washington, D. C., generally occurring together with *Helminthosporium rostratum* and, more rarely, also with *H. giganteum*. It was found to be present in abundance on the same host near Seaford, Delaware, where collections were made August 29, 1922. The relation of *H. leucostylum* to *Eragrostis major* is an obviously parasitic one, as on green and otherwise healthy leaves the fungus gives rise to dead regions that appear on the upper (adaxial) side, typically as sharply delimited medium-gray linear streaks measuring 0.3 to 0.5 mm. in width and 5 to 20 mm. or more in length. The sporophores arise on the killed areas usually in dense clusters which often are found in longitudinal alignment. Apparently they develop earlier and often in greater abundance on the upper side than on the lower side of the leaf, probably emerging from between epidermal cells as well as from the stomata. On being dried, the diseased leaves, like those of similarly affected goose grass, split readily into longitudinal shreds along the streaks representing the lesions.

The occurrence of the fungus on *Eragrostis major* suggests its comparison with *Helminthosporium hadotrichoides*, described on the same host from Delaware by Ellis and Everhart (*36*) in 1888. According to the diagnosis given by these authors, *H. hadotrichoides* would appear to produce conidiophores not greatly unlike those of the fungus under consideration in size and septation, but differing from them in being of a "smoky brown" color, and in having "the apex swollen so as to form a knob like the head of a pestle, 8 to 12 μ in diameter." A modification so striking and unusual could scarcely fail to arrest attention, yet was certainly never observed in any material examined by the writer. The conidia, which are rather inadequately described as "clavate-obovate, or clavate cylindrical or yellowish brown" without any mention being made either of their dimensions or septation, would seem to differ somewhat, at least in coloration, from the corresponding dark brown or dark olivaceous structures of the parasite discussed in the present account.

HELMINTHOSPORIUM TURCICUM PASSERINI

Helminthosporium inconspicuum Cooke & Ellis 1878, *in* Grevillea, v. 6, p. 88–89.

The disease of maize commonly known as "leaf blight" and less frequently as "white blast" is found in many regions of the globe in which this important cereal is cultivated. It appears to have been first observed by Passerini (*105*) in 1876, who noted its occurrence in Italy under the term "nebbia" and attributed it to a species of Helminthosporium, which he named *H. turcicum*. Two years later, Cooke and Ellis (*24*) described a form from New Jersey as *H. inconspicuum*, similarly thriving on maize. Although the descriptions of the American and Italian forms are not especially similar, there appears to be good reason to believe that they were based on material belonging to the same species.

In the account given by Cooke and Ellis, the American fungus is very briefly described:

> Tenuissime effusum. Hyphis elongatis, septatis, nodulosis, pallidebrunneis. Sporis lanceolatis, 3–5 septatis; episporio tenui.
>
> On *Zea mays*.
>
> Effused, but so thinly as not to be visible to the naked eye. Spores 0.08 to 0.12 by 0.02 mm., at first with the endochrome divided, at length septate.

The parts of the statement regarding the number of septa, the visibility of the fructifications, and the alleged division of the endochrome previous to the division of the spore can scarcely be regarded as altogether correct, either for the parasite causing leaf blight or for *Helminthosporium sativum*, which, as has been pointed out in another connection, is known to occur on the organs of mature corn plants. The parts regarding the description of the sporophore, the shape and measurements of the spore, as well as the figure of the latter that accompanies the text, on the other hand, might apply about equally well to both species. Indeed the chief reason for regarding the description given by Cooke and Ellis as applying to the leaf blight fungus is not found in the single distinctive characterization—that concerning the thin epispore—but rather in the abundance of the parasite in the general region in which Ellis made his collections. In 1881, Peck (*107*) recorded the leaf blight disease in New York, although, as has been stated in another connection, his description of the fungus is less applicable to the parasite under consideration than to *H. sativum*. The trouble again was found in New York by Stewart (*144*) in 1896. In 1889 Thaxter (*149*) observed a serious outbreak of the disease in Connecticut, where 14 years later Clinton (*21*) found it again doing considerable damage. In 1903 the malady was quite destructive also in Delaware, according to the account given by C. A. Smith (*133*). During the same season Stone and Monahan (*145*) noted its abundance in Massachusetts; and Orton (*99*) found it very general also in eastern Pennsylvania and New Jersey. Since then it has been reported repeatedly not only from the Middle Atlantic and New England States, but from other sections as well, the records of the Plant Disease Survey indicating its presence in nearly every State east and in some of the States immediately west of the Mississippi River.

Stevenson and Rose (*143*) reported leaf blight of corn as occurring in Porto Rico. Robinson (*122*) in 1911 observed the disease in the Philippines, where it has been noted also since then by Baker (*5*) as well as by Reinking (*117*). Tryon (*153*) records an outbreak of the trouble in New South Wales in the season of 1886 so destructive as to attract

widespread attention; and in recent years Darnell-Smith (*27*) lists "leaf stripe" among the diseases affecting maize in the same Province. Yoshino (*161*) reported *Helminthosporium turcicum* as occurring on *Zea mays* in the Province of Higo, Japan, in 1905. According to Butler (*19*) the disease is fairly common in India and occurs in South Africa. The European literature concerning the disease does not appear to be extensive. Ducomet (*34*), in 1903, recorded its appearance under the name of "brulure" in the southwestern part of France. More recently the publication of a paper by Zhavoronkov (*162*) would seem to imply its distribution in Russia.

In general, leaf blight is a disease characteristic of the later stages in the development of the host. During the season of 1920, when the writer had occasion to follow its development in the sweetcorn fields of the western part of Long Island, it made its appearance toward the end of August. On plants examined on August 20, elongated straw-colored spots were present especially on the lower leaves, varying from 2 to 4 mm. in width, and from 5 to 15 mm. in length. The tissues involved in these spots were altogether dry. Usually at this stage the dry areas were delimited from the surrounding healthy parts by a brownish margin, quite conspicuous and distinct in some instances and barely distinguishable or absent in others. As the season progressed the affected areas rapidly increased in size, individual lesions frequently measuring more than 4 cm. in width and 10 cm. in length by September 16. (Pl. 24, A.) By the coalescences of the enlarging spots, extensive areas embracing often considerable portions of the leaf were found to be involved. Toward the end of September the foliage was withered to such an extent that some of the plants appeared, as some writers have suggested, as if affected by a frost.

When leaf blight appears as late in the season as in 1920, which probably was very nearly typical in regard to the development of the trouble at least for the northeastern section of the United States, the resultant economic damage is not likely to be serious. In most ordinary seasons Peck's (*107*) observation that *Helminthosporium inconspicuum* "seems to attack on lower leaves with vitality already impaired; not very noxious as it only hastens death of leaves by a few days or weeks," perhaps is not without a good deal of justification. Nevertheless, in other seasons, apparently as a result of weather conditions favorable to the fungus, the disease may make its appearance while the plant is still relatively young, and thus cause very considerable destruction. Thus Ducomet (*34*) records a severe outbreak in France in 1900 during an exceptionally wet season. According to this writer the foliar lesions appear when the plants are only 0.5 to 0.6 meters high. The destructiveness of the disease in 1903 in the States of Connecticut and Delaware apparently was associated also with its early appearance, Clinton (*21*) finding "many fields looking in August and September as if struck by early frost; heavy attack due to unfavorable season." In New South Wales the blight is reported (*118*) to appear invariably in all the late plantings of maize when the heavy autumn rains set in, particularly on low-lying situations. In the season of 1915 (*118*), however—

> it appeared very much earlier owing to the phenomenally wet spring and early summer. Many areas were completely destroyed on account of the leaves being killed off long before the plants were half grown. Where cobbing had been well advanced the effects were not so serious.

Equally severe epiphytotics apparently are not uncommon in the Philippines, where Reinking (*117*) found the disease sometimes extremely destructive, entire plots of field corn and sweetcorn having been ruined by it.

Several weeks after the initiation of a foliar lesion, when the affected tract usually exceeds 5 cm. in length, a grayish, greenish efflorescence makes its appearance in the center of the withered area, becoming gradually more extensive with the continued enlargement of the latter. This efflorescence consists of the numerous fructifications of the fungus, which, in spite of the statement of Cooke and Ellis (*24*), are more readily perceived with the naked eye than the fructifications of the majority of the species of Helminthosporium developing on the foliage of grasses. When such material is examined under the microscope the fructifications can be seen emerging in groups of 2 to 6, always from the stomata. The olivaceous conidiophores (Pl. 24, Ca–g) usually measure from 7.5 to 9.0 μ in width, although Saccardo (*128, v. 4, p. 420–421*) gives 6 μ for this dimension. His characterization of these structures as "3-pauciseptatis" is more nearly correct, as the number of septa varies usually from 2 to 4. As the sporophores have been found to attain a length of 260 μ (Pl. 24, Cf) or more, Saccardo's (*128*) figure, 150 μ, being apparently a decided understatement of this dimension, the intervals between the septa, compared to those of most other species, are relatively large.

The spores of the fungus, which are quite characteristic, have been described and figured in a great variety of ways, but rarely altogether correctly. As shown in Plate 24, Ba–p, drawn from material derived from diseased leaves of sweet corn collected on Long Island, they vary considerably in size and shape. The measurements for length and width, ranging from 45 to 132 μ (Pl. 24, Bl, o) and from 15 to 25 μ (pl. 24, Bi, o), respectively, agree fairly well with those given by other authors: Saccardo (*128*) 85 to 92 by 20 to 24 μ; Ducomet (*34*) 65 to 95 by 20 to 25 μ; Cooke and Ellis (*24*), and Schwarze (*130*) 80 to 120 by 20 μ; Butler (*19*) 80 to 120 by 20 to 24 μ; and Massee (*90*) 80 to 140 by 20 to 26 μ. In shape the spores are typically straight or slightly curved, widest near the middle and tapering decidedly toward the ends. The proximal portion of the spore may taper toward the hilum somewhat in the manner of a cone (Pl. 24, Bf, h, k) although a tendency toward the basal end being rounded off usually is discernible (Pl. 24, Bb, i) and not infrequently quite pronounced (Pl. 24, Bm, n). In any case, however, the shape defined by Saccardo as "perfecte fusoideis utrinque acutis" is never realized, because the apical end of the spore is always rounded, even where the distal segment is very considerably inferior in width to the middle segment. Certainly, conidia like those figured by Saccardo (*126, pl. 824*) with the basal and apical ends drawn out into attenuated beaks, have never been observed by the writer. Nor have any been observed entirely comparable with those figured by Massee, with the acumination of the ends considerably exaggerated and the septation so close that the segments appear more than twice as wide as they are long. On the other hand, Schwarze's (*130*) figures represent them as rather too blunt, a fact for which the evident use of dead herbarium material may, perhaps, largely be held responsible.

The number of septa in the spores was found to vary from 1 to 8 in the material collected by the writer. Inasmuch as the 1 or 2 septate individual spores are manifestly undersized, the correspondence with the numbers given by most authors is, on the whole, satisfactory—Saccardo

(*128*), 5 to 8; Ducomet (*34*), 3 to 8; Butler (*19*), 3 to 7; although the range 3 to 5 given by Cooke and Ellis (*24*), while perhaps expressing an average condition, would not seem to be large enough. It may be noted that the septa are somewhat tardy in making their appearance and, as a result, many of the spores when newly proliferated show segments of a length not usual in species of similar dimensions. (Pl. 24, Ba, g, h.) The peripheral spore wall always is relatively thin, the accounts of Saccardo (*128*) and Schwarze (*130*), for example, as well as figures like those of Massee (*90*) and of Smith (*133*), describing or illustrating a thick membrane, being apparently based upon dead material. As usual in species of Helminthosporium, the thin peripheral wall is associated with a relatively light color of the conidia. This color varies from a subhyaline light fuliginous tint when the spores are newly proliferated to a moderate fuliginous, greenish yellow, yellowish brown, or pale olive when they are fully mature. The dark olivaceous color, characteristic of the conidia of *Helminthosporium sativum*, *H. monoceras*, or *H. vagans*, is never approached, descriptive phrases like that given, for example, by Saccardo (*128*), "olivaceofuscis," indicating such approximation, being evidently quite erroneous and misleading.

A morphological feature that the writer feels inclined to emphasize, particularly as a diagnostic character useful in separating *Helminthosporium turcicum* from most of the congeneric forms with which the student of economic botany has to deal, is the protruding hilum. (Pl. 25, C.) This apiculate basal protuberance, while rather minute, is uniformly present, regardless of whether the proximal portion of the spore is distinctly tapering or more nearly rounded. It must, therefore, be considered apart from the basal contour of the conidium. That it has not been mentioned in the writings of previous workers is somewhat surprising. Ideta (*65*), it is true, figured the proximal cell as being conspicuously constricted at the base. If this attenuated portion was intended to represent the part of the conidium by which the latter is inserted on the conidiophore, its proportions would seem, in view of the condition obtaining in American material, greatly exaggerated.

As has been pointed out or figured in the publications of Ducomet (*34*), Smith (*133*), Butler (*19*), Reinking (*117*), and Zhavoronkov (*162*), the spores of *Helminthosporium turcicum* germinate regularly by the production of two polar germ tubes, one from each end. (Pl. 15, Db, c.) Occasionally an intermediate segment may proliferate one or several lateral germ tubes. Such atypical germination apparently is more likely to occur with newly proliferated subhyaline conidia (pl. 25, Da, d), or with abnormally curved spores than with fully mature spores of typical shape; and sometimes occurs as the result of injury and death to one of the end cells.

The fungus develops well on the media usually employed in laboratories. On substrata rich in organic food materials, as for example, potato glucose agar, a luxuriant growth of grayish black aerial mycelium is produced. On substrata poorer in organic substances, like tap-water agar, growth is less luxuriant, but may be studied to better advantage. The embedded mycelium anastomoses abundantly with the resultant production of numerous complexes consisting of dark brown lobulate segments. The sporophores arise from the prostrate fuliginous hyphae that compose a large portion of the aerial growth. They are somewhat inferior in diameter to those developing in nature, measuring approximately 6 μ in width, but, at the same time, are considerably longer. As the first spore

is developed 200 μ or more from the point of attachment of the sporophore (Pl. 25, Bi) and the successive spores are proliferated at relatively long intervals, varying usually from 20 to 40 μ, the number of spores produced on a single fructification measuring from 300 to 600 μ is less than might be expected (Pl. 25, Bh). Although of the same general type as those produced in nature, the conidia produced, at least under certain conditions, on tap-water agar are often more slender, measuring up to 140 μ in length (Pl. 25, Ba-g) and varying usually between 15 and 20 μ in diameter. A tendency toward curvature also becomes apparent, and in some instances quite pronounced. (Pl. 25, Bb.) Further details concerning the conspicuous variation in the dimensions of hyphae, conidiophores and conida resulting from the use of different substrata and different temperatures come outside the scope of the present paper. Some information concerning the cultural characters of the fungus is accessible in Zhavoronkov's (*162*) paper.

In the foregoing text the writer has intentionally confined his discussion to the parasite associated with the leaf blight of maize, as he has had opportunity to examine the fungus in a living condition only on diseased foliage collected on Long Island. It is interesting to note that Reinking (*117*) reports the parasite as attacking also the tassels of corn in the Philippines. Through the courtesy of Dr. W. H. Weston, jr., the writer was able to examine material of diseased corn plants collected on these islands, showing an abundance of Helminthosporium fructifications on both leaves and tassels. The spores scraped from the leaves did not appear to differ materially from those produced on these organs in the United States. Preparations made from the fructifications on the tassel, however, showed conidia which, while of the same color and approximate maximum length, were perceptibly inferior in diameter, measuring approximately 11 to 14 μ in this dimension; more abundantly septate, 12 transverse walls being not uncommon; usually quite distinctly curved; and evidently similar to those figured by Reinking (*117, Pl. 20, B, C*). However, as none of these conidia were any longer viable, their collapsed condition and incapacity for use in starting cultures made it impossible to determine definitely whether the two types belong to the same or to different species. The behavior of the leaf-blight parasite in culture makes the former alternative appear less improbable than a comparison of material collected in the field might suggest. If the forms on the leaves and on the tassels should indeed prove to be identical, the morphology of *Helminthosporium turcicum* as generally understood would stand in considerable need of revision.

Helminthosporium turcicum was reported as occurring on *Sorghum vulgare* by Saccardo (*128*). Butler (*19*) also recorded its presence on cultivated sorghum in India, Egypt, and China, with the statement that it was not very common or destructive on this host. It has been reported on sorghum (*Andropogon sorghum* [L.] Brot.) from Texas by Heald and Wolf (*55, p. 54*); and by these authors, as well as by Atkinson (*4*) on Johnson grass (*Sorghum halepense* [L.] Pers.) in Texas and Alabama, respectively. According to Atkinson, it occurs also on Elymus sp. and on *Cinna arundinacea* L. in Alabama. There is at least a possibility that the fungus which this author found on the latter host may have been identical with the one described in this paper as *H. catenarium*. Certainly, a critical comparative study of the forms of Helminthosporium found on Johnson grass and on various types of sorghum will be necessary

before their identity with the corn leaf-blight fungus can be regarded as definitely established.

Very little experimentation seems to have been done on methods of controlling leaf blight. Most writers, apparently on general grounds, have recommended rotation of crops and sanitary measures like the destruction of stubble and other refuse of diseased plants, the avoidance of manure containing diseased material, and even the roguing of infected growing plants. Ducomet (*34*) believed that spacing the plants at wider intervals might have a beneficial effect by encouraging aeration; while Ferraris (*40*) advised the treatment of seed with fungicidal solutions. Until definite information on the overwintering of the parasite, and, more particularly, information concerning the possible existence of an asciger-ous or sclerotial stage is available, no additional methods of combating the disease can well be suggested.

Since this paper was submitted for publication, a lengthy account of the Helminthrosporium diseases of maize and sorghum in India has been published by Mitra (*91a*). *Helminthosporium turcicum* is reported to be of widespread occurrence on both these hosts, although certain facts regarding its distribution were held to indicate that the parasite on sorghum represents a strain biologically distinct from that on maize, with which, however, it is morphologically identical. It is interesting to note that Mitra observed that spores derived from the glumes of male spikelets are longer and more distinctly curved than those found on diseased foliage. In cultural characters as well as in pathogenesis, this author failed to discover any differences between the two types, and consequently regarded both as belonging to the same species.

Although it is evident that, for the most part, Mitra undoubtedly was dealing with the same parasite as that commonly attacking sweet corn in the northeastern States, in order to forestall possible confusion, it may not be superfluous to call attention to certain discrepancies in morphological detail, even if these can not be very satisfactorily explained. Mitra states that "typical spores of *H. turcicum* Pass. are spindle-shaped and pointed," and in some of his figures (*91a, Pl. 2, fig. 12, 16, Pl. 3, fig. 7, 8, 9*) these structures are represented with both ends acutely pointed. As has been suggested in another connection, the writer has never observed conidia of any species of Helminthosporium thriving on maize, or, indeed, on any graminaeceous host whatsoever, in which such a condition is realized. While in American material of the maize blight fungus, the conidia with strongly tapering proximal portion and protruding hilum, might not improperly be described as pointed at the basal end, the apical end is never pointed, but is always rounded off either bluntly or more narrowly, depending on the degree of tapering exhibited by the distal segments. Perhaps Mitra may have been influenced to some extent by the erroneous drawings of Saccardo and Massee. No mention is made of the presence of the hilum, a number of figures (*91a, Pl. 2, fig. 6, 22 to 25, Pl. 3, fig. 11*) clearly showing no indication of this protruding modification.

According to the account given by Mitra, germination is often associated with a breaking down of the internal partitions of the spore. Judging from his figures of conidia, in which the peripheral wall is frequently represented as a thick envelope (*91a, Pl. 2, fig. 6 to 10, 14 to 17, Pl. 3, fig. 7 to 11*), it is evident that a not inconsiderable proportion of the spores employed by him were entirely dead, while others must have contained one or more dead segments. In the writer's experience, only

the spores of the latter type have exhibited the irregularity in question. When such conidia germinate, the surviving segments collectively produce 1 or 2 polar germ tubes, which traverse the neighboring dead segments and their degenerate swollen envelopes like so much inert material, to emerge usually, though not always, from the ends of the spore. In conidia of which all segments are in a living condition, the juxtaposed portions of segment membranes that constitute the septa normally undergo no degeneration; at least until the germ tubes have attained extensive development.

HELMINTHOSPORIUM LEERSII ATKINSON

In 1897, Atkinson (*4*) described as *Helminthosporium leersii*, a fungus collected on leaves of *Leersia virginica* Willd. (=*Homolocenchrus virginicus* [Willd.] Britton) at Auburn, Ala., September 13, 1891:

> Spots irregularly oblong, amphigenous, at first dark brown, then dirty white with dark brown border. Hyphae amphigenous, brown, irregularly nodulose or flexuous, 200 to 350 by 4 to 6 μ. Conidia slightly curved, 5 to 9 septate, elliptical, faintly fuliginous.

Although the form was later included in Earle's list of Alabama fungi (*92*), it seems to have escaped the attention of botanists in other states. Nevertheless, the parasite appears to be widely distributed, as the writer found it of frequent occurrence on the leaf blades of *L. virginica* near Meriden, Conn., in September, 1920, as well as in the vicinity of Washington, D. C., during the summer of 1921.

The first indication of attack becomes evident as a minute brown spot often not exceeding 1 mm. in length. (Pl. 26, A, C.) As this spot enlarges, the central area for some time remains dark brown, while the discoloration at the margin merges insensibly into the light green of the surrounding healthy tissue. With further increase in size, the tissues in the center succumb, their dark brown in the meantime fading to a dirty straw color. As the line of demarcation between the gray central region and the peripheral brown zone is sharply defined, a foliar lesion of the eye spot type (Pl. 26, A, C) results. The parasite seems to hasten, if not to cause, the death of the older leaves. When the latter have withered, either in whole or in part, the fructifications make their appearance, scattered sparsely, first near the center of the spot but later also beyond the margin.

According to Atkinson (*4*) *Helminthosporium leersii* is "near *H. turcicum* but hyphae and conidia more slender." It seems questionable with which one of a number of congeneric organisms this author intended to compare the fungus. Certainly, no striking similarity to the parasite causing leaf blight of maize is discernible with respect either to the dimensions or to the shape of spores and sporophores. In the material collected by the writer, the latter were found to arise singly or more rarely in pairs from between the epidermal cells of the host. (Pl. 26, Fa, b.) The moderately fuliginous conidia (Pl. 26, Ba–1), measuring from 11 to 14 μ in diameter by 50 to 95 μ in length, were found to contain from 3 to 12 cross walls, never associated with perceptible constrictions in the unusually thin peripheral wall. Irregularities in the insertion of cross walls, resulting in muriformly septate conditions are present as in the spores of *H. sativum*. (Pl. 26, Bk.) As in *H. sativum* also, germination normally takes place by the production of two polar germ tubes. (Pl. 26, Da–c.) However, owing to the fragility of the

external wall, the intermediate segments frequently are exposed and sometimes entirely liberated in the course of manipulation, with the result that abnormal germination is much more common than in most congeneric forms. The germ tubes usually attain a considerable length before branching, thus differing in this respect from those of *H. cynodontis*, which frequently proliferate a branch at the very point of origin. The hilum in the spores of *H. leersii* is represented by a scar generally contained within the contour of the basal end, although occasionally it may be seen to protrude slightly. In any case, a basal modification like that characteristic of the conidia of *H. monoceras* is never present; nor is the hilum a distinctly protruding structure as, for example, in *H. turcicum*.

When grown on artificial media the fungus, unlike the other species similar to *Helminthosporium sativum*, develops a dense gray aerial mycelium, the rate of enlargement being relatively slow. This slow development is associated with a peculiarity in manner of growth at the margins that is more or less characteristic and has not been observed in other species. The imbedded hyphae, although ramifying profusely, remain short, thus giving rise to an intricate system of short, rather swollen elements of which Plate 26, E, represents merely an incipient stage. Here and there a relatively delicate hypha (Pl. 26, Ea–b) grows out into the air and by curving downward brings its tip in contact with the substratum. From the tip numerous short branches are soon proliferated which, by continued ramification, again yield an intricate system of hyphae.

HELMINTHOSPORIUM CYNODONTIS MARIGNONI

In 1909, Marignoni (*89*) described as *Helminthosporium cynodontis* a fungus occurring on dry leaves of *Cynodon dactylon* L. (=*Capriola dactylon* [L.] Kuntze) near Schio in northern Italy. In the brief diagnosis given by Saccardo (*128, v. 22, p. 1394*), the species is thus characterized:

> Effusum, atro-olivaceum v. fuligineum; conidiophoris aggregatis, laxis, simplicibus, parce septatis, tortuosis, 80–150×6–7; conidiis elongatis, utrinque rotundatis plerumque octo-septatis, fuligineis, 60–75×12–14.

Apparently the fungus has not been reported again, as the subsequent literature appears to contain no reference either to Marignoni's binomial, or to any species of Helminthosporium occurring on Bermuda grass and answering the description given above. The writer nevertheless is convinced that the parasite is exceedingly common throughout the southeastern section of the United States where the host is everywhere present in the fields and on the roadsides as a noxious weed. In Florida, near Wauchula, Fort Myers, Tampa, and Gainesville, during the months of February, March, and April, 1921, it was found difficult to collect specimens of *Cynodon dactylon*, not bearing fructifications of the fungus in considerable abundance. (Pl. 27, A.)

Although the writer has not been able to consult Marignoni's (*89*) illustrated publication, the American form answers sufficiently well in morphological detail to Saccardo's (*128*) account of *Helminthosporium cynodontis* that, in view of its abundant occurrence on the same host, it can at least provisionally be regarded as belonging to this species. In the Florida material, the conidiophores (Pl. 27, Da-g) which are dark brown in color, emerge singly or in pairs from stomata or between

epidermal cells, and thus show less tendency to occur in groups than those of most congeneric species. They measure from 50 to 150 μ in length, and from 4 to 6 μ, typically 5 μ, in diameter, the range in length agreeing well with that given in Saccardo's diagnosis. On the other hand, the measure of agreement in diameter of sporophore is somewhat less satisfactory than might be expected in a dimension exhibiting relatively little variability. The number of septa in the conidiophores range from 2 to 5, depending largely on the length of these structures.

The conidia (Pl. 27, Ba-u) of the American form, measuring 11 to 14 by 27 to 80 μ, are straight or more frequently somewhat curved; widest near the middle from which they taper slightly toward the abruptly rounded ends; subhyaline to fuliginous in color, never brown or dark olivaceous; and 3 to 9 septate, the septa not being associated with constrictions in the relatively thin peripheral wall. On being mounted in water they germinate promptly by the production of two polar germ tubes approximately 3 μ in thickness, one from the apex and the other in immediate proximity to the hilum that can be distinguished within the rounded contour of the basal cell. (Pl. 27, Ca-i.) Very frequently a lateral branch is produced near the origin of the germ tube, thus often simulating the appearance of two germ tubes. (Pl. 27, Cc, e.) The spores are obviously of the same type as those of *Helminthosporium sativum*, from which they differ markedly however, in size, thickness of peripheral wall, and depth of coloration. They are inferior also to those of *H. leersii* in length and number of septa; and to those of *H. micropus* in width, besides lacking altogether the peculiar modification of the basal segment characteristic of the latter species. The fungus grows on artificial media, producing a moderate quantity of light gray, fluffy mycelium, especially at some distance from the point at which the inoculum was planted. It has not been observed to sporulate in culture on media ordinarily employed in laboratories, although the production of spores could probably be induced by providing more suitable substrata.

It may be mentioned that while the fructifications of *Helminthosporium cynodontis* are found occurring most abundantly on moribund or withered leaves of *Cynodon dactylon*, the fungus also has been collected repeatedly on *Eleusine indica* in Florida in the spring of 1921. In the vicinity of Washington, where the parasite was present in moderate quantity on Bermuda grass from August to October, 1921, it was not found on goose grass, indicating that the latter host is somewhat less favorable for its development. A form morphologically very similar and probably identical has been found to occur very consistently on withered leaves of *Muhlenbergia mexicana* (L.) Trin. collected in the vicinity of New York City and Washington, D. C. Although further investigation of the host range is necessary, indications are not wanting that the species will eventually be found on more than a few members of the Gramineae.

HELMINTHOSPORIUM MICROPUS, N. SP.

About the middle of April, 1921, the writer observed a peculiar disease affecting young plants belonging to a species of Paspalum, provisionally identified as *Paspalum boscianum* Flügge, that was found common in moist, poorly cultivated fields near Wauchula, Fla. The trouble occurred on seedlings from 3 to 6 inches in height, first becoming apparent on the tender young foliage as a localized wilting. (Pl. 28, A.) Portions of foliar tissue from a few millimeters to several centimeters in length, and

from 2 to 4 mm. in width, were found in an entirely collapsed condition. (Pl. 28, B.) The absence of any indication of discoloration and the entire loss of mechanical stiffness combined to present an appearance such as might be brought about, for example, by scalding with boiling water. Soon after the wilting became visible, the portions of leaf involved dried out completely, becoming somewhat shrunken, dark in color and crisp in texture. Usually the death of the entire leaf blade ensued within a week, as much apparently because of the interruption of the vascular elements by the enlargement and multiplication of infected regions as because of the extension of the trouble to healthy parts. On the older leaves the disease was found less destructive, the injury tending to be restricted to more definitely circumscribed elliptical spots, varying from 2 to 10 mm. in length, and not infrequently delimited by a brownish margin. Nevertheless, these older leaves likewise slowly succumbed, the withering beginning at the tip and gradually progressing toward the base.

On examining the dead foliage under the microscope, it was found that the regions involved in the lesions bore numerous fructifications of a well defined species of Helminthosporium. The first conidiophores to appear after the death of the tissue seemed usually to emerge from the stomata (Pl. 28, Ea–g), although later they could be found emerging between epidermal cells as well. Except that the sporophores are smaller in diameter than might be expected from the size of the spores, they exhibit no distinctive characteristic.

The conidia (Pl. 28, Ca–m) on the other hand are decidedly characteristic and can be readily distinguished from those of any congeneric species which the writer has examined. Generally subhyaline or slightly fuliginous, they resemble in respect to coloration the spores of *Helminthosporium teres*, *H. bromi*, *H. giganteum*, and *H. tritici-repentis*. Unlike the conidia of any of these fungi, however, they are typically more or less curved, a fact which together with the mode of germination by the production of two polar germ tubes (Pl. 28, Da-c) suggests comparison with *H. leersii*, *H. cynodontis*, and *H. turcicum*. From the spores of the fungus causing leaf blight of maize, those of the parasite on *Paspalum boscianum* are readily distinguished by their smaller dimensions, a very perceptible difference obtaining in respect to length and diameter. As the number of septa in the conidia are approximately equal, or even somewhat greater in the fungus thriving on *P. boscianum*, a very pronounced difference in massiveness between the spore segments of the two species is readily apparent. In general shape, the conidia of the parasite on *Paspalum boscianum* are less inclined to taper toward the ends than those of the *H. turcicum*, frequently all the segments except the terminal ones being of nearly the same diameter. The most characteristic feature, however, is found in the shape of the proximal end of the spore, which tapers quite abruptly and uniformly from the basal septum into a nearly cylindrical short prolongation, terminating abruptly in the flat hilum. Because of this curious modification, the contour of the peripheral wall usually exhibits a slight, barely perceptible, reentrant curve.

Among the species of Helminthosporium discussed in this paper the form on *Paspalum boscianum* is altogether unique in causing under natural conditions a violent wilting effect on the tissues of its host. The only instance known to the writer of similar pathological symptoms attributable to a congeneric species is the wilting produced experi-

mentally by applying large quantities of conidia of *Helminthosporium sativum* to the foliage of corn seedlings and incubating in a damp chamber for several days. The parasite has not been found on other hosts. It is undoubtedly distinct from *H. penicillosum*, a saprophyte described from Argentina by Speggazini (*137*) on the decaying culms of *P. platensis* and of an unidentified species of Andropogon. The measurements given for the sporophores of the South American fungus, 150 to 600 by 10 to 15 μ, as well as for the diameter of the spores, 10 to 12 μ, indicate a great difference in the dimensions of the two forms. Nor does the parasite on *P. boscianum* bear any close resemblance to *H. mayaguezense* described by Miles (*91*) as occurring on the culms and leaves of P. *conjugatum* in Porto Rico, for the measurements of the conidiophores and conidia, 300 to 500 by 18 to 22 μ and 135 to 155 by 35 to 45 μ, respectively, point toward an order of dimensions not approached by the fungus under consideration. Furthermore, the spores of the Porto Rican fungus, in spite of their relatively large size, are described as being only 3 to 4 septate; and are figured as having the septa associated with constrictions, a condition altogether absent from those of the Florida form. As the latter appears not to have been described, the specific name *micropus* is suggested to signalize the characteristic modification of the basal end of its spores.

DIAGNOSIS

Helminthosporium micropus, n. sp.

Attacking the leaf blades of young plants of a species of Paspalum, provisionally identified as *Paspalum boscianum* Flügge, killing the foliar tissues in elongated regions usually varying in length from 5 to 30 mm., the affected parts having first a scalded appearance and later becoming dry and shriveled. On the foliage of older plants, affected regions more restricted in extent, elliptical usually with brownish margins; bringing about death of leaf by more gradual progressive withering.

Conidiophores appearing on portions of withered leaves previously involved in lesions; emerging singly or in groups of 2 or 3, from stomata or between epidermal cells; dark brown; 4 to 6 μ in diameter; septate at intervals of 5 to 40 μ; producing first spore 65 to 140 μ from base; successive spores produced at intervals of 5 to 15 μ marked by pronounced geniculations.

Spores subhyaline to light fuliginous; straight or more typically somewhat curved; of nearly uniform diameter between end segments or slightly tapering from middle; longer ones usually of cylindrical type, shorter one more nearly elliptical. Apex usually rounded off by hemispherical or hemiellipsoidal contour; basal segment approximately obconical, tapering uniformly to a width of 2 to 3 μ, then produced as a very short prolongation terminating in hilum. Peripheral wall moderately thin, but visibly double-contoured except in small circular spot at apex and about basal modification immediately adjacent to hilum, where it becomes very thin and appears single-contoured. Germinating by two polar germ tubes, one being proliferated from each of the thin-walled regions. Measuring 10 to 18 by 28 to 92 μ; 3 to 9 septate, the septa not marked by constrictions.

HABITAT.—Collected near Wauchula, Fla., April 18, 1921, and May 2, 1921.

HELMINTHOSPORIUM ROSTRATUM, N. SP.

In an effort to collect material of a fungus reported by Ellis and Everhart (*36*) on *Eragrostis major* Host, in Delaware, more than three decades ago and described by them as *Helminthosporium hadotrichoides*, the writer took occasion to examine specimens of stink grass gathered in the vicinity of Washington, D. C., at various times in September and October, 1921. As the grass matures relatively early, it was represented exclusively during these months by entirely dry mature plants—a fact that could hardly be supposed to facilitate search for a parasite such as the fungus described by Ellis and Everhart presumably represents. At any

rate, no species of Helminthosporium having canidiophores with the swollen pestle-like tip mentioned in the diagnosis of *H. hadotrichoides* was found. However, a fungus obviously belonging to the genus was observed with considerable frequency on the dry leaf blades. As the form has never been found occurring on other grasses, it would appear to be more or less closely restricted to *Eragrostis major*. The mature condition of the grass precluded any inquiry into the biological relation of the fungus to its substratum; so that the present account is necessarily confined to a discussion of morphological facts.

The conidiphores (Pl. 29, Ca–h), which are of moderate dimensions, differ in no important detail from those of many other congeneric graminicolous species. They show usually a pronounced tendency to fuse at the base, the whole cluster often appearing to arise from a single superficial basal portion communicating with the mycelium within the leaf structures by hyphal connections passing through stomata or between adjacent epidermal cells. A similar tendency has been observed not infrequently in *Helminthosporium gramineum*. The conidia (Pl. 29, Aa–q) resemble those of *H. sativum* in possessing, when mature, a dark olivaceous color and a thick peripheral wall. An even greater degree of similarity to *H. monoceras* is manifested in the conspicuously protruding hilum, as well as in the attenuation of the peripheral wall in the small subhyaline regions at the apex and immediately adjacent to the hilum, from which the two polar germ tubes are destined to emerge. (Pl. 29, Ba–c.) Although most of the spores taper moderately toward the base, the pronounced acuminate tapering of the proximal portion characteristic of the spores of the parasite on barnyard grass is not frequently approximated. Many, although certainly not all, of the spores exhibit the same accentuation of basal and distal septa as *H. halodes* (Pl. 29, Ag, i, o). The basal septum is more frequently found modified than the distal one, mainly because in the longer, rostrate individual conidia the distal septum apparently never differs perceptibly from the intermediate septa. (Pl. 29, Ah, k, q.) Where a septum is conspicuously thickened, the delimited proximal or distal segment usually is noticeably paler in color, being grayish brown rather than dark olivaceous (Pl. 29, Aa, g, o), although in other cases such differentiation is exceedingly slight or altogether absent (Pl. 29, Ai, k, p, q). Another point of similarity to *H. halodes* is evident in the germination of immature spores, the peripheral wall of which has not undergone the processes of thickening and induration incident to maturation, by the production of germ tubes from one or several intermediate segments as well as from the end segments (Pl. 29, Bd). On artificial media the fungus produces abundant growth, with numerous conidiophores bearing conidia altogether similar to those found in nature.

The species is readily distinguished from the other graminicolous forms discussed in this paper by the production of the distal portion of many of the conidia into a more or less attenuated apical prolongation (pl. 29, Ad, e, h, p, q), which in extreme cases may appear as a pronounced rostrate termination (Pl. 29, Ak, Bd). A very similar condition was illustrated by Saccardo (126), in his figures of *Helminthosporium leptosporium* Sacc. and *H. tiliae* Fr. And the same author illustrated a greatly exaggerated modification of the same type in some figures of *H. hormisciodes* (Corda) Sacc., a fungus which he later referred (*128*) to the genus Clasterosporium. From these three forms the fungus on *Eragrostis major* differs markedly, being considerably superior, for example, in the

diameter of its spores. Nor does it appear possible on morphological grounds to identify it with other species of Helminthosporium, including the numerous saprophytic types that have been referred to the genus. The specific name *rostratum* is suggested as descriptive of a conspicuous feature characteristic of many of the conidia.

DIAGNOSIS

Helminthosporium rostratum, n. sp.

Occurring on the dry leaves of *Eragrostis major,* Host.

Conidiophores dark olivaceous, emerging singly or in groups of 2 to 5 from stomata or between epidermal cells, the swollen bases often more or less united; measuring 6 to 8 by 40 to 180 μ; 1 to 6 septate, the septa separated by intervals of 15 to 40 μ; proliferating the first spore 40 to 140 μ from the base, and successive spores at intervals of 10 to 30 μ, at the apices of well-defined geniculations.

Conidia, when mature, dark olivaceous; straight or less frequently somewhat curved; often short, widest at or somewhat below the middle, tapering moderately or more markedly toward both ends, the hemispherical apex abruptly rounded off, the basal end somewhat more acute, often exhibiting a rounded conical contour; or less frequently produced at the tip into a more or less elongated rostrate prolongation. The elliptical spores 3 to 9 septate, the rostrate types usually 8 to 15 septate, the proximal cross wall occasionally associated with a perceptible constriction in the peripheral wall. The basal septum often, and the distal septum less frequently, appearing darker and thicker than the intermediate cross walls, such modification not unusually associated with a more dilute coloration of the delimited basal or distal segments. Peripheral wall thick except in two small subhyaline regions, one at apex, the other surrounding the conspicuously protruding hilum at the base. Mature spores germinating by the production of two polar germ tubes, one from each of the subhyaline thin-walled regions; immature spores often producing germ tubes also from intermediate segments. Measuring 14 to 22 μ in diameter by 32 to 184 μ in length.

HABITAT.—Collected near Washington, D. C., September and October, 1921.

HELMINTHOSPORIUM ORYZAE B. DE H.

Helminthosporium macrocarpum of von Thümen not Greville.
Helminthosporium oryzae Miyabe & Hori 1901, *in* Nôji Shikenjo Hôkoku, no. 18, p. 67–81.

Probably the earliest record of the occurrence on rice of a species of Helminthosporium resembling the form now recognized as a widely distributed parasite on this cereal may be credited to von Thümen (*150*). This writer in a paper published in 1889 reported *Helminthosporium macrocarpum* Grev. as not infrequently appearing indiscriminately on dead parts of rice plants immediately after the tissues involved have ceased to live. To the presence of the fungus was attributed a discolored appearance of the crop that had occasioned popular discussion of "attack" and "sooty mould." Von Thümen believed that the fungus nevertheless is not the cause of any disease, but that it makes its appearance rather as the result of disease or as a saphropyte accompanying entirely normal maturation.

While the various saprophytic organisms that have been referred to *Helminthosporium macrocarpum* by different authors in all probability are not specifically identical, it may be assumed that such reference implies a moderately close correspondence to the diagnosis of Greville's species. In most details, indeed, this diagnosis is not widely at variance with descriptions of the fungus causing leaf spot of rice. A significant departure is evident, however, in the width of the sporophore, which in *H. macrocarpum* measures 15 to 20 μ, thus equaling or slightly exceeding the diameter of the conidia. The identity of the fungus observed by von Thümen thus is rendered somewhat doubtful. In any event it seems clear that no useful purpose could be served by associating Greville's binomial with the parasite on rice.

In 1900 van Breda de Haan (*16*) described from Java as *Helminthosporium oryzae*, a fungus producing spots on living rice leaves, the affected areas being entirely dry in the center and surrounded by a brown margin. The brown conidiophores arising from the under side of the leaves, according to his characterization, bear large, fuliginous fusiform acrogenous 6- to 9-celled conidia, measuring 16 by 90 μ and germinating from both end cells. The fungus which had also been found on the fruits of rice, the author, evidently influenced by von Thümen's paper, regarded as probably identical with *H. macrocarpum* Grev.

The next year (1901) Hori (*62*) gave an account of the same disease in Japan, and apparently without knowing of van Breda de Haan's paper, named the parasite *Helminthosporium oryzae* Miyabe and Hori. The fungus has since been reported from Japan by Yoshino (*161*), Kurosawa (*80*), and others, while in more recent years Suematsu (*146*) has investigated its cultural characters, and Nishikado and Miyake (*94*) have studied methods for its control. An illustrated account of the disease and the parasite is given in Ideta's large handbook (*65*). An unidentified species of Helminthosporium on rice was reported from the Straits and Federated Malay States by Gallagher (*46*), from Madras by Sundararaman (*147*), from Uganda by Snowden (*135*), from Ceylon by Bryce (*17*), from Cochin-China by Vincens (*155*), as well as from the Philippines by Reinking (*117*). In a recent note, Ocfemia (*96*) states that in 1918 he observed a seedling and leaf blight attributable to *H. oryzae* doing considerable damage to rice in the Philippines. Farneti (*39*) ascribed the "brusone" disease of rice in Italy to a fungus highly variable in its morphological characteristics and pathological manifestations; presumably appearing as either *Piricularia grisea*, *P. oryzae*, *H. turcicum*, or *H. oryzae*, its form in any particular case being contingent on the host plant, the organ attacked, and the environment.

Beyond a statement in Ocfemia's (*96*) note that the "sesame spot disease" of rice caused by *Helminthosporium oryzae* was observed by W. H. Tisdale in Louisana in 1920, the American literature does not seem to contain any reference to the occurrence of the parasite in this country. Dr. Tisdale has advised the writer that the Helminthosporium leaf spot is of not uncommon occurrence in the rice fields of Louisiana and Texas, and has kindly supplied specimens of affected mature rice inflorescences collected in this locality on September 15, 1920. An examination of these specimens showed that the fungus occurs on the glumes at first as a grayish efflorescence, and later, because of continued development, as a black velvety mat, somewhat similar in texture to the crustose growth of *H. ravenelii* on *Sporobolus indica* but much less extensive. (Pl. 30, A.) Through the courtesy of Mr. Ocfemia, pure cultures of the fungus originally isolated from Louisiana material were obtained, as well as specimens of rice leaves from experimental plants artificially inoculated. The leaves bore an abundance of dark brown or reddish brown spots, longitudinally elongated, the larger ones measuring up to 0.5 by 3.0 mm., and showing a small straw-colored area in the center. (Pl. 30, B.) No indication of an etiolated zone surrounding the foliar spot like that characteristic, for example, of the blotch caused by *H. bromi*, was present, the discoloration caused by the fungus manifestly resembling that produced by *H. leersii* on *Leersia virginica*, and belonging to the type that in other instances has suggested the term "eye spot." In pathological symptoms the American parasite thus resembles the fungi described from Java (*16*) and from Japan (*62*).

The correspondence in morphological features between the three forms is sufficiently close to warrant regarding them, at least provisionally, as specifically identical. To be sure, in respect to the dimensions of conidiophore and conidium as well as to numerical range in spore septation, Breda de Haan's (*16*) account is not altogether in perfect agreement with Hori's description; and either account reveals shortcomings when considered in relation to the American fungus. However, if the more extreme and relatively infrequent expressions of length and septation are disregarded, the differences are not especially large. Perhaps the most serious discrepancy is found in diameter of condium, the measurements given in Hori's account, 16 to 22 μ, exceeding the measurement given by Breda de Haan and also the measurements obtained from American material, by somewhat more than can readily be referred to ordinary variability, in view of the comparative constancy generally characteristic of this dimension within a particular species. Although Ideta's (*65*) figures indicate that the Japanese fungus is at least of the same general type as the American form, a brief morphological account based on material from Louisiana nevertheless may not be superfluous.

The black, velvety, mycelial mats on the glumes of affected spikelets, which are found distributed irregularly and usually rather sparsely through otherwise healthy panicles (Pl. 30, A), are composed of prostrate hyphae and more or less erect sporophores. The former, which communicate directly with the mycelium in the tissues of the host, when well developed, show abundant branching and anastomosis and are composed of short segments, dark brown or olivaceous in color, more or less inflated or lobulate, and measuring from 8 to 15 μ or more in diameter (Pl. 30, D). The sporophores arise as lateral branches from these hyphae, which indeed they resemble toward the base, in possessing a dark olivaceous color, and in showing a tendency toward ramification. (Pl. 30, D.) Some distance from their base, the sporophores gradually change from an olivaceous color to a light fuliginous hue, and at the tip may even be subhyaline. They vary in width from 4 to 8 μ, and in length from 150 to 600 μ or more, depending on the age of the growth. The scar marking the point of attachment of the first spore is found above the olivaceous proximal portion of the sporophore, usually not less than 200 μ from the base; successive scars occur at relatively long intervals (10 to 90 μ) at geniculations not always well defined or conspicuous.

As collected on rice plants naturally infected, the conidia measure 11 to 17 μ in diameter by 35 to 170 μ in length. The larger ones like those shown in Plate 30, Ca, b, and containing as many as 13 septa, appear to be produced for the most part on well developed mats of sporophores occurring on the glumes; while the less extreme sizes (Pl. 30, Cc, m) are associated with the scattered fructifications on the glumes or leaves. Apparently because of the absence of the longer spores from diseased leaves, those of more moderate length have been regarded as characteristic of the parasite, the one figured in Plate 30, Cf, for example, fitting almost exactly the description given by van Breda de Haan. Typically the spores are slightly curved, widest at the middle or somewhat below the middle; the distal portion tapering toward the hemispherical apex where its width approximates half the median width; the proximal portion tapering toward the base, which is similarly rounded off, although the diminution in diameter is usually perceptibly less. When fully mature they are fuliginous or brownish and provided with a moderately thin peripheral wall that is further attenuated at the apex as well as imme-

diately around the rather inconspicuous hilum visible within the contour of the base. Normal germination of mature spores proceeds by the proliferation of two polar germ tubes, one from each of the thin-walled regions (Pl. 30, Cd, e); while less mature, subhyaline spores may produce germ tubes from intermediate segments as well (Pl. 30, Ci, k).

On tap-water agar the fungus grows sparsely, producing conidiophores and conidia (Pl. 31, Aa, b; B) which, while somewhat narrower and more nearly colorless, are essentially similar to those found in nature. However, on other substrata, as, for example, potato glucose agar, growth is very profuse, and owing to the blackish olivaceous color and velvety or felt-like texture of the mycelium, somewhat similar in general appearance to the growth of *Helminthosporium sativum* (Pl. 31, D). The spores, although of a shape and size not entirely unlike those found in nature (Pl. 31, Cd, Da) in spite of many markedly irregular examples (Pl. 31, Db, c) frequently exhibit such pronounced departures in respect to coloration as to suggest the suspicion that one might be dealing with a separate species. Instead of a uniform fuliginous, brownish or dark olivaceous color, they show in the same culture all gradations from subhyaline to deep olivaceous, and in some instances are so nearly black as to appear opaque even when a moderately bright illumination is used. Associated with the dark coloration is a peripheral wall conspicuously and uniformly thick except over the apical and basal regions involved in germination. (Pl. 31, Ca.) Frequently one or both end segments are altogether subhyaline (Pl. 31, Cb, c, e, Db), in sharp contrast to the other segments, and occasionally one or more intermediate segments show similar differentiation (Pl. 30, Cb, e). But whatever its position, the subhyaline segment is always set off from the dark segments by greatly accentuated heavy septa. Manifestly, coloration, and, in a smaller measure, structure are contingent here upon conditions not usually present when the fungus grows on rice plants under natural conditions or on tap-water agar in artificial culture.

In general morphological characteristics *Helminthosporium oryzae* suggests comparison with *H. sativum*, *H. monoceras*, and *H. turcicum*. From *H. sativum* it may be distinguished by the greater length and the more pronounced tapering toward apex and base, characteristic of its conidia. On the other hand, the conidia of *H. oryzae* taper less markedly toward the basal end than the homologous structure of the parasites on barnyard grass and on corn; and the hilum is contained within the contour of the base, not protruding as in the latter two species. Certainly Farneti's (*39*) belief that *H. oryzae* and *H. turcicum* represent specifically identical fungi seems altogether incredible, in view of the well-defined morphological differences between the parasite affecting rice and the form causing leaf-blight of maize.

During recent years methods for controlling the brown-spot disease have been investigated by Nishikado and Miyake (*94*) in Japan, where it constitutes one of the most serious troubles affecting rice culture. In the province of Okayama, for example, 90 per cent of the seedlings in the seed-bed were found affected to a greater or less extent, and sometimes practically all the seedlings bore lesions, making it difficult to find entirely healthy specimens. Controlled experiments brought to light the fact that this very general seedling infection was largely attributable to infected or contaminated seed. It was found that the spores of *Helminthosporium oryzae* are killed by immersion in water at a temperature of 51° C. for 10 minutes, while air-dry rice seed is not injured by immersion for 10 to 15

minutes in water at a temperature of 54 to 55° C. As a practical method of control, treatment of rice seed in water for 10 minutes at 52° C., or for 5 minutes at 54° after preliminary soaking for 24 hours at room temperature, was recommended.

The disinfection of rice seed by other methods, including possibly the hot air method devised by Atanasoff and Johnson (*3*) and treatment with various organic mercury compounds, presents a profitable field for further research. In this connection it may be mentioned, however, that the conidia of *Helminthosporium oryzae* appear to remain viable relatively long periods, the writer having germinated, in September, 1921, spores from material collected in Louisiana on October 1, 1920, nearly 12 months earlier. (Pl. 30, Cd, i, k.) As a result the fungus undoubtedly is able to survive from season to season on stubble, straw, and other refuse. Although the prevention of primary seedling infection may reduce to some extent the number of secondary infections at later stages in the development of the plant, it can scarcely be expected to eliminate them altogether. As in the somewhat analogous disease of barley, wheat, and rye caused by *H. sativum*, generally approved agricultural practices making for soil sanitation should prove of some value.

In nature the fungus does not seem to have been found to attack plants other than rice, although on artificial inoculation Ocfemia (*97*) secured infection of 31 species of grasses belonging to 23 genera. Evidently this represents another instance in which the experimental host range is more an expression of the rigorous conditions attending the procedure followed than of significant parasitic relationships.

Since the submission of this paper for publication, a valuable account of the Helminthosporium disease of rice has been published in English by Nisikado and Miyake (*94a*), not only incorporating the results of their own comprehensive studies but also including suggestive allusions to a considerable volume of investigations, the reports of which have not hitherto become generally known among readers of the European languages. Their full account of the morphology, pathogenicity, and cultural characters of the fungus, and their abundant illustrations, leave no doubt that the parasite found destructive in Japan and presumably in many other rice-growing countries of the Orient is altogether identical with the one discussed in the foregoing paragraphs. The lack of close agreement in measurements of conidia and conidiophores given by different authors would seem to be due in large measure to the variability of the fungus under different conditions of growth both in nature and more especially in artificial culture—its behavior in this respect being again analogous to that of *Helminthosporium sativum*. In addition, the inclusion in the range of dimensions of the more extreme measurements by some authors, and their exclusion by others, have not made for any close correspondence. As a special instance, the range in diameter of the sporophore may be cited, some writers having included measurements of the inflated basal segments, or of the distended segments of the prostrate elements from which the sporophores arise, while others have excluded them. The latter course was followed in the present account, as it was believed that in a comparative treatment involving a number of species the figures thus obtained would have the greater significance.

HELMINTHOSPORIUM CYCLOPS, N. SP.

In July, 1921, the writer collected, near Lisbon Falls, Me., specimens of *Danthonia spicata* (L.) Beauv. that appeared to be slightly affected with a leaf spot of the type caused by *Helminthosporium sativum*. Although the grass had completely headed and most of the lower basal leaves were dead, the remaining foliage was still green and in an actively vegetative condition. The dark brown or black foliar lesions, usually not exceeding 0.5 by 2 mm. in length, were found distributed very sparsely over the living foliage. On the dead basal leaves corresponding spots were observed, although considerably faded; and in a number of instances microscopic examination revealed these as the foci of fructifications of a species of Helminthosporium differing markedly from a small-spored congeneric form that also was present in moderate quantity. Although the fructifications of the former species were not confined to the conspicuously discolored areas, their distribution on the dead leaves was such as suggest a causal relation between the fungus and the brown foliar lesions. Owing to the small size of the leaves of *Danthonia spicata* and the tendency of the foliage to begin withering early in the course of the growing season, the matter of referring such type of foliar discoloration to a fungus not appearing on the surface until after the death of the tissues involved, and then not in great abundance, is attended with some uncertainty, as has been noted in another connection. The possible damage resulting to the host from the leaf spot in any case would appear to be quite insignificant.

The conidiophores (Pl. 32, Ea-c) of the fungus are not especially characteristic, and show little to distinguish them from those of *Helminthosporium sativum* or *H. vagans*, in size, color, or general appearance. The conidia (Pl. 32, ca-m) also resemble the analogous structures of these two species in possessing, when mature, a conspicuously thick peripheral wall, and, associated with this thick wall, a dark olivaceous color. In length they are approximately equal to the conidia of *H. sativum* and not greatly inferior to those of *H. vagans;* while in diameter they fall slightly below the spores of the former species and more considerably below those of the latter. Uniformly straight, and either nearly cylindrical or tapering more perceptibly toward the ends, they perhaps resemble most closely the spores of *H. vagans* in general shape. A specific difference readily distinguishing the spores of the form on *Danthonia spicata* from those of *H. sativum* and *H. vagans* is found in the more pronounced attenuation of the peripheral wall at the base and apex. The basal end, moreover, is distinguished by a conspicuous hilum, the largest observed in any species reported in this paper, which, because of a somewhat fanciful resemblance to an eye, has suggested the specific name *cyclops*.

The conidia (Pl. 32, Da, b) germinate readily in water, producing two polar germ tubes, which emerge, as might be anticipated, from the hyaline, thin-walled regions at the tip and immediately surrounding the hilum. In mode of germination the fungus thus again resembles *Helminthosporium sativum*. That the relationship with the latter species nevertheless is not a very close one is indicated in agar cultures by the development from the imbedded mycelium of an abundance of inflated elements altogether analogous to those observed, for example, in cultures of *H. tritici-repentis*, *H. bromi*, *H. teres*, and *H. turcicum*. On potato-dextrose agar a moderately profuse gray aerial mycelium is

produced, interspersed with a considerable number of subspherical, dark, superficial sclerotia, not unlike those produced under similar conditions by *H. bromi* and probably representing also in this instance, immature perithecia. If analogy is not entirely misleading, it would seem that search for an ascigerous condition of this fungus might perhaps not be without success.

When cultivated on substrata containing little organic food material, as, for example, Beijerinck's agar, the fungus usually develops a variable number of sclerotia below the surface of the medium resembling the superficial ones described above, but occasionally departing from the subspherical type by growing into elongated, rather irregular bodies. (Pl. 32, A.) Microscopically, the aerial growth is relatively scant, the loose mycelium being limited to a small quantity near the point of inoculation. The larger portion of the surface is peppered with discrete fructifications such as are represented on Plate 33, Aa, Bb, C, D. Although generally noticeably smaller, the conidia developed in culture resemble in essential details those found on the host in nature. The discrete conidial fructification is, in general, of the type exemplified in *H. sativum*, but a few marked differences frequently occur. After a spore has been proliferated, it frequently grows out into an apical prolongation (Pl. 33, Ba, Bb, Da) having the same diameter as the sporophore and proliferating spores in the same way (Pl. 33, Baa, Bba, Daa-ab). Or, without any special modification it (Pl. 33, Dc) may produce a secondary spore by apical proliferation (Pl. 33, Dca). The sporophore occasionally also, shows an aberrant tendency by continuing apical growth not in the usual manner, but by a process of budding exactly similar to that occurring in the production of a conidium. (Pl. 33, Ab-Abc.) In such cases the distal part of the sporophore is similarly marked at the base by a conspicuous hilum. Obviously the fungus shares in some measure the Alternaria-like habit and tendency toward the obliteration of spore and sporophore manifested in *H. catenarium*.

A compound type of fructification also is produced by the fungus. In tube cultures of Beijerinck's agar, these occur in the form of erect, stiff, thread-like black structures, measuring from 0.3 to 0.5 mm. in diameter and arising usually from a slightly expanded base in the center of the loose aerial mycelium. (Pl. 32, A B.) The lower portion for a distance of about 1 mm. usually is sterile. (Pl. 32, Bd.) Above this sterile portion the fructification bristles with a dense array of sporophores resembling the discrete conidiophores and bearing similar spores. (Pl. 32, Bc.) The axial column is hard in texture and when broken and examined microscopically appears to be composed of dense white pseudoparenchyma of which the surface layer is largely quite black but interspersed with numerous small lighter areas. Except at the abruptly truncated tip (Pl. 32, Ba) where further growth of the fructification occurs and where new sporophores (Pl. 32, Bb) are constantly being proliferated, it is impossible to recognize the hypal origin of the axial-column. These fructifications, which appear moreover to show some similarity to the sclerotia produced by the fungus, have been seen to attain a length exceeding 1 cm. and undoubtedly could be grown much larger.

DIAGNOSIS

Helminthosporium cyclops, n. sp.

Occurring on the leaves of *Danthonia spicata* (L.) Beauv. on which it causes small dark-brown spots not usually in abundance.

Conidiophores, olivaceous, usually 3 to 7 septate, the septa occurring at intervals of 18 to 50 μ; measuring 7 to 8 μ in diameter by 100 to 250 μ in length; producing the first spore from 80 to 160 μ from the base, and successive spores at the apices of moderately pronounced geniculations.

Conidia, dark olivaceous except in restricted subhyaline regions at apex and base; straight or rarely slightly curved, cylindrical or tapering gently toward the bluntly rounded ends, the shorter ones often ellipsoid; measuring usually 12 to 17 μ in diameter by 45 to 110 μ in length; 4 to 12 septate, the septa not usually associated with constrictions in the peripheral wall; the latter thick except in the subhyaline regions from which the two polar germ tubes are produced, one at the apex, and the other immediately surrounding the unusually large and conspicuous hilum included within the basal contour.

On agar media containing abundant organic food material, producing numbers of superficial subspherical sclerotia resembling immature perithecia of congeneric forms. On agar media containing little organic food material, developing imbedded sclerotia, discrete fructifications and compound fructifications. Conidiphores of discrete fructifications arising from more delicate subhyaline vegetative hyphae often becoming branched as a result of individual spores being produced into sporophoric prolongations and occasionally continuing growth by budding in a manner analogous to the proliferation of spores. Conidia like those produced in nature but usually shorter, ellipsoidal, rarely exceeding 18 μ in diameter and 60 μ in length.

Compound fructifications consisting of a threadlike axis 0.3 to 0.5 mm. in diameter, 10 mm. or more in length, composed of hard, white pseudoparenchyma with black mottled surface, and bearing numerous radially oriented sporophores above the somewhat expanded sterile basal portion.

HABITAT.—Collected near Norwood, Mass., November 7, 1920; and Lisbon Falls, Me., July 20, 1921.

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PLATE 1[1]

Helminthosporium gramineum

A.—Upper portion of barley plant collected near Fort Atkinson, Wis., June 25, 1921, illustrating condition soon after death. *Aa* represents abortive inflorescence with distorted awns slightly protruding laterally from the uppermost leaf sheath. The leaves show longitudinal splitting into strips as well as lack of mechanical rigidity evidenced by their drooping (*Ab*) and contorted (*Ac, d*) positions. *Ae* represents basal shreds of leaf, the remainder of which has broken off as result of brittle texture due to disease. ×¾.

Ba–h.—Normal spores showing variation in size, shape, and septation. ×500.

Ca–d.—Normal spores germinating in water mount, showing single germ tube produced from distal and middle segments, and 1 to 3 germ tubes produced from basal segment. ×500.

Da–c.—Conidia from the apical segment of which has been produced a single conidiophore with scars showing places of attachment of secondary conidia; also secondary conidia *Dca–cd*. *Dd*.—Spore with two apical secondary sporophores, one showing secondary spore attached. *De*.—Spore with two secondary sporophores, one produced from basal and one from apical segments. ×500.

Ea–e.—Group of typical sporophores emerging between distorted epidermal cells, showing enlargement of basal segment. ×500.

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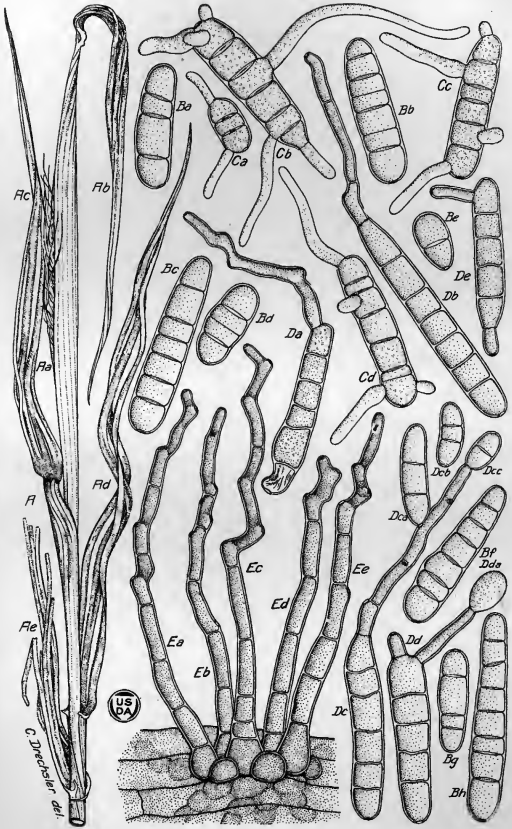
[1] All figures of conidia, germinating conidia, mycelia, conidiophores, asci, and ascospores in the plates accompanying this paper were drawn with the aid of a camera lucida to the same scale, and in reproduction, reduced in equal proportion to give a uniform magnification of 500 diameters. Host epidermis figured in connection with conidiophores was drawn in surface aspect with the aid of the camera lucida, and the resulting figures redrawn as if projected at an angle of 30 degrees, yielding in reproduction a magnification of 500 diameters in a longitudinal direction and approximately 250 diameters in transverse direction. Figures of pathological habit, of perithecia, of compound fructifications, and of test tube cultures were drawn at convenient magnifications, reduced in reproduction to the scales specifically indicated in the legends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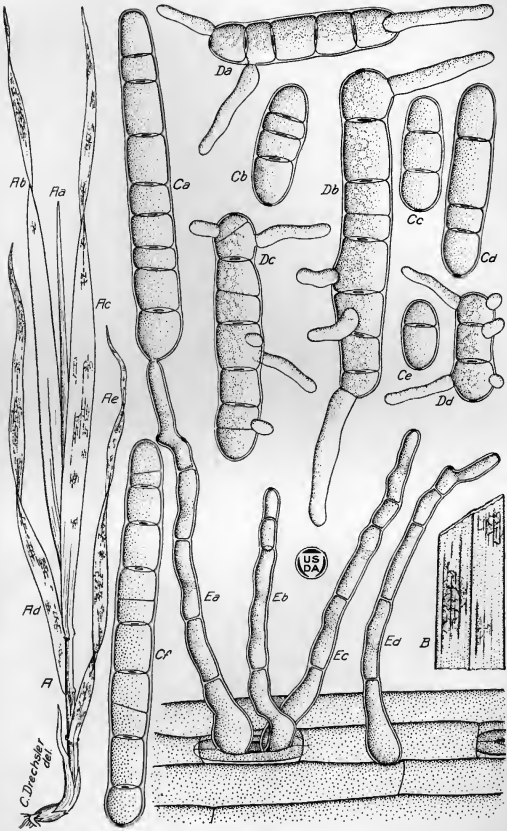
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PLATE 2

Helminthosporium teres

A.—Young volunteer barley plant showing heavy infection of net blotch as found occurring near Madison, Wis., during the earlier part of September, 1919. Youngest leaf (*Aa*) healthy; second and third leaves (*Ab*, *Ac*) showing increasing amount of infection; fourth leaf (*Ad*) beginning to wither at the tip; fifth leaf (*Ae*) entirely withered. ×¾.

B.—Portion of diseased leaf showing discoloration in characteristic irregular reticulate pattern. ×3.

Ca–f.—Conidia from diseased barley leaf showing variation in size, shape, and septation. ×500.

Da–d.—Conidia from diseased barley leaf germinating in tap water, showing proliferation of germ tubes from end and middle segments. ×500.

Ea–d.—Conidiophores emerging from stoma in group of 3, and singly from between adjacent epidermal cells. ×500.

PLATE 3

Pyrenophora teres

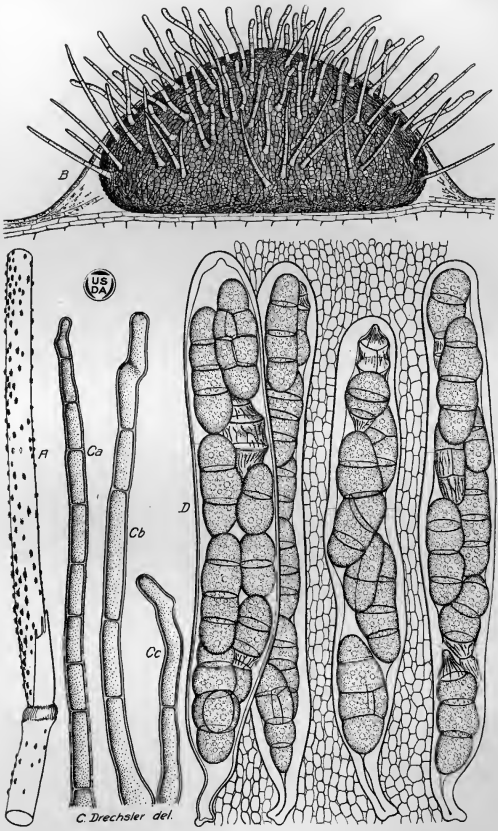
A.—Portion of barley culm as found occurring near Madison, Wis., April 15, 1919, showing numerous erumpent perithecia. ×2.

B.—Perithecium bearing pointed setae near base, and an abundance of flexuous conidiophores on upper surface. ×115.

Ca.—Seta from pe'ithecium. ×500.

Cb–c.—Conidiophores from perithecium. ×500.

D.—Four asci showing imperfect development of ascospores, and pseudoparenchyma surrounding them. ×500.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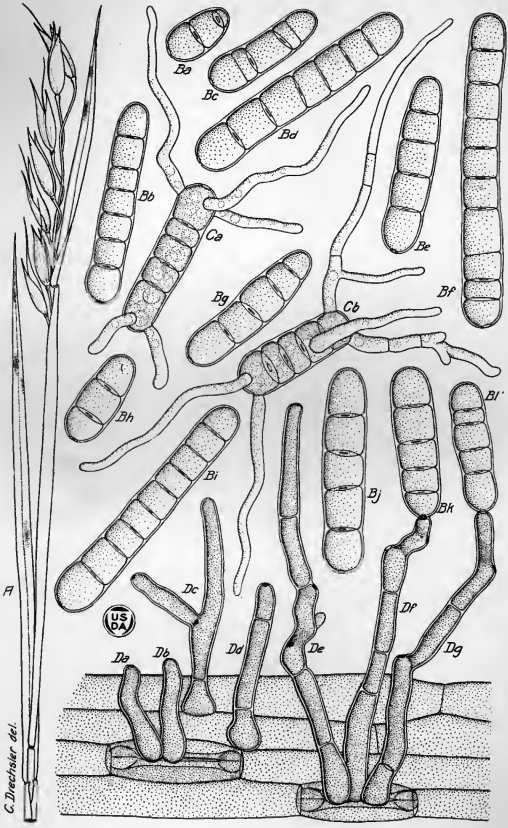
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PLATE 4

Helminthosporium avenae

A.—Portion of oat plant showing a number of discolored areas on upper leaves due to attack by *H. avenae*. ×¾.

Ba–l.—Spores of *H. avenae* from diseased oat leaf showing variation in size, shape, and septation. ×500.

Ca–b.—Spores from diseased oat leaf, germinating in water showing production of germ tubes from end and middle segments. ×500.

Da–g.—Conidiophores emerging singly or in groups of 2 or 3, from stomata or between contiguous epidermal cells. *Dc* and *De* exhibit type of branching occurring occasionally in this species. ×500.

PLATE 5

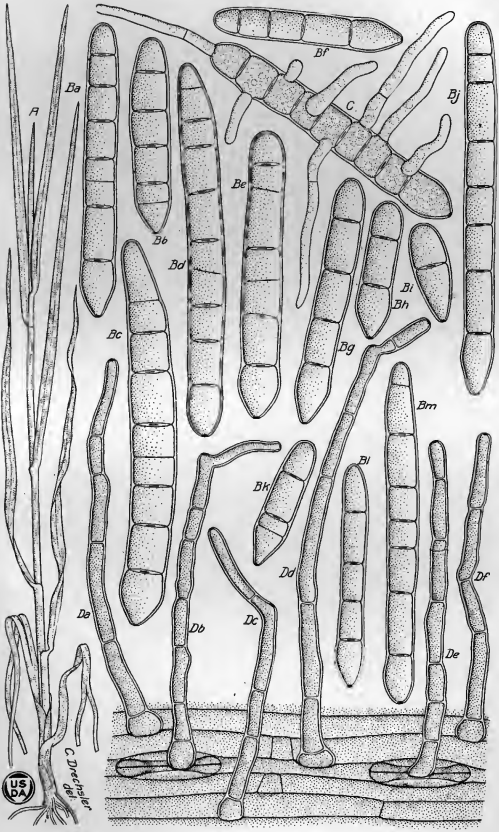
Helminthosporium tritici-repentis

A.—Small plant of *Agropyron repens*, as found near Brooklyn, N. Y., July 12, 1920, showing four lower leaves withered and killed as result of attack of fungus; discoloration absent but microscopical examination revealing abundance of fructifications of *H. tritici-repentis* on three lowermost leaves. × ¾.

Ba-m.—Conidia showing variation in size, shape, dimensions, and abundance of septation and the distinctive contour of the basal segment characteristic of the species. × 500.

C.—Conidium germinating normally in water mount by proliferation of germ tubes indiscriminately from nearly all segments. × 500.

Da-f.—Conidiophores showing mode of emergence from stomata and from between adjacent epidermal cells. × 500.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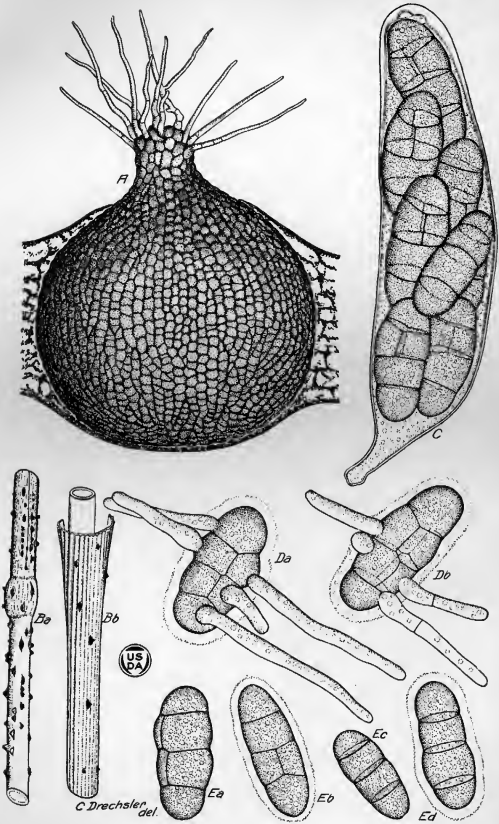
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PLATE 6

Pyrenophora tritici-repentis

A.—Perithecium of *Pyrenophora tritici-repentis* collected on decaying leaves of *Agropyron repens* near Madison, Wis., early in April, 1919, showing well-defined ostiolar beak bearing a number of setae at the tip; the latter being more or less curved, often several times septate, occasionally branching, and tapering perceptibly from base to distal end. × 175.

Ba-b.—Portions of old stem of *Agropyron repens*, showing numerous erumpent perithecia appearing on denuded stalk (Ba); and less numerous perithecia on leaf sheath (Bb). × 3.

C.—Ascus freshly dissected from perithecium before undergoing swelling preliminary to spore discharge. × 500.

Da-b.—Ascospores germinating by production of germ tubes from the greater number of their segments; showing also the enveloping gelatinous layer. × 500.

Ea-d.—Ascospores showing variation in size and septation, as well as presence and absence of enveloping gelatinous layer. × 500.

PLATE 7

Helminthosporium catenarium

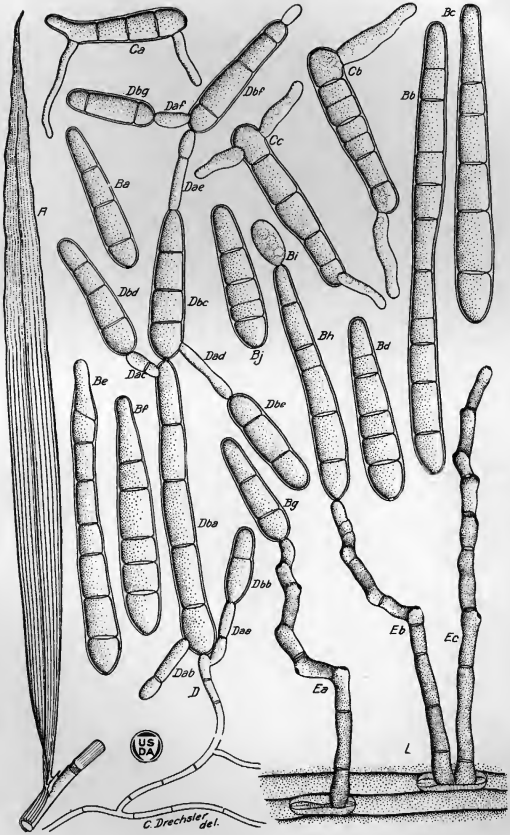
A.—Leaf of *Cinna arundinacea*, the proximal portion healthy, the distal portion entirely withered as result of attack by *H. catenarium*. × ¾.

Ba-j.—Spores of *Helminthosporium catenarium* from material collected on host, showing variation in size, shape, and septation; *Ba, d, g, j*, short spores, straight and tapering toward tip; *Bb*, long spore with median constriction; *Bc, f*, long spores showing constricted apical prolongation, with apical scar marking point of attachment of secondary spore; *Be*, long spore, irregular in diameter, growing out at apex to produce a secondary spore; *Bh*, primary spore with young secondary spore *Bi* attached at apex. × 500.

Ca-c.—Spores from material collected on host, germinating in tap water by production of germ tubes from basal and apical segments. ×500.

D.—Conidial fructification in 20-day-old corn-meal agar culture, consisting of sporophoric segments *Daa-af*, and spores *Dba-bg*. × 500.

Ea-c.—Sporophores from material collected in field, emerging from stomata (epidermis and stomata considerably distorted as result of withering). × 500.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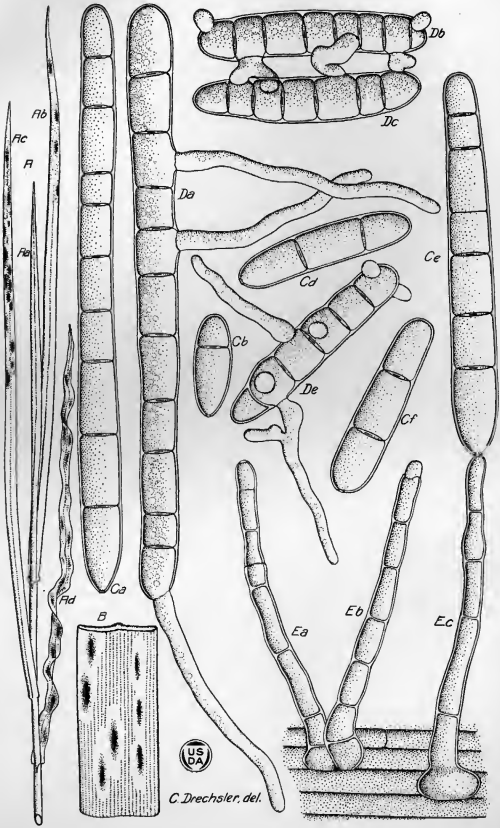
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PLATE 8

Helminthosporium bromi

A.—Portion of plant of *Bromus inermis*, showing youngest leaf (*Aa*) healthy, the second (*Ab*), and third (*Ac*) bearing a number of spots, and the fourth (*Ad*) largely withered as a result of attack by *Helminthosporium bromi*. × ¾.

B.—Portion of diseased leaf of *Bromus inermis*, showing etiolated areas surrounding dark spots, the discoloration in the latter being most intense in the center and fading out toward the periphery. × 3.

Ca-f.—Conidia from diseased leaf, showing variation in size, shape, and septation. × 500.

Da-e.—Conidia germinating in tap water by the production of germ tubes indiscriminately from end and middle segments. The spores *Db* and *Dc* germinated lying in contact, the resulting germ tubes anastomosing immediately after their proliferation. × 500.

Ea-c.—Conidiophores of *H. bromi* emerging between adjacent epidermal cells of host. × 500.

PLATE 9

Pyrenophora bromi

A.—Old leaf of *Bromus inermis* of growth of preceding season as collected near Madison, Wis., May 1, 1920, showing scattered mature perithecia of *P. bromi*. × ¾.

B.—Perithecium of *P. bromi*, showing wide ostiolar beak, with scattering setae. × 155.

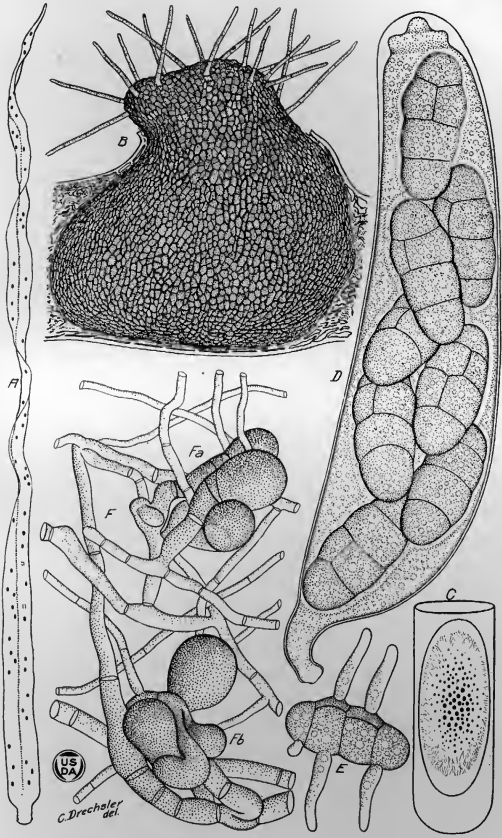
C.—Twenty-five day old potato agar culture of *P. bromi*, showing sclerotia partly covered with white mat of aerial mycelium.

D.—Ascus of *P. bromi* after some swelling due to absorption of water, preliminary to spore discharge. × 500.

E.—Ascospore of *P. bromi* germinating in water.

Pyrenophora teres

F.—Submerged mycelium of *P. teres* from 5-day old water-agar culture, showing relatively small complexes of inflated hyphal segments. × 500.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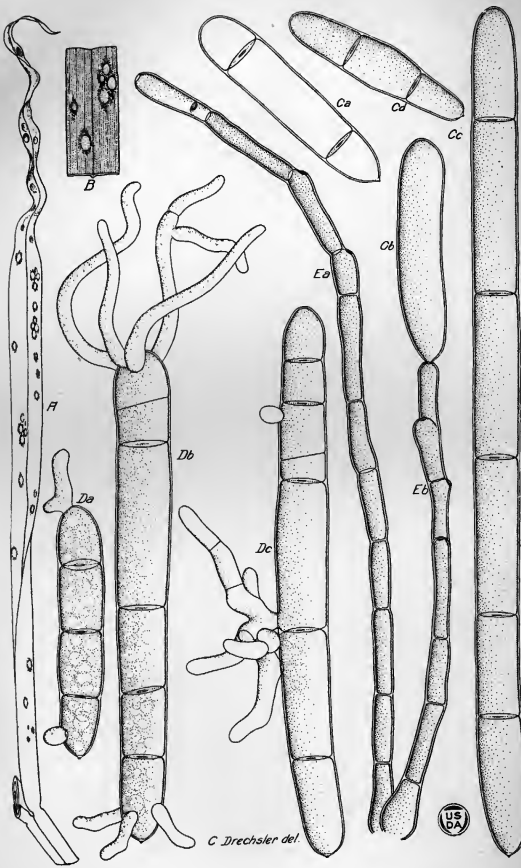
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PLATE 10

Helminthosporium giganteum

A.—Leaf of *Eleusine indica* attacked by *H. giganteum*, showing the presence of numerous "eye-spot" lesions and the withering of the tip as a result of the disease. × 1.

B.—Portion of leaf of *Eleusine indica*, showing eye-spot lesions in various stages of development. × 2.

Ca-d.—Normal conidia from leaf of *Eleusine indica*, showing variation in size and shape and presence of conical protuberance at base. The conidium *Cc* represents approximately the maximum length attained by spores of the fungus. × 500.

Da-c.—Conidia from diseased leaf germinating in water by the production of germ tubes singly (*Da*), or in groups from the end cells (*Db*), or from intermediate cells (*Dc*). × 500.

Ea-b.—Conidiophores from diseased leaf of *Eleusine indica* showing enlarged base and spacing of septa and of scars. × 500.

PLATE 11

Helminthosporium dictyoides

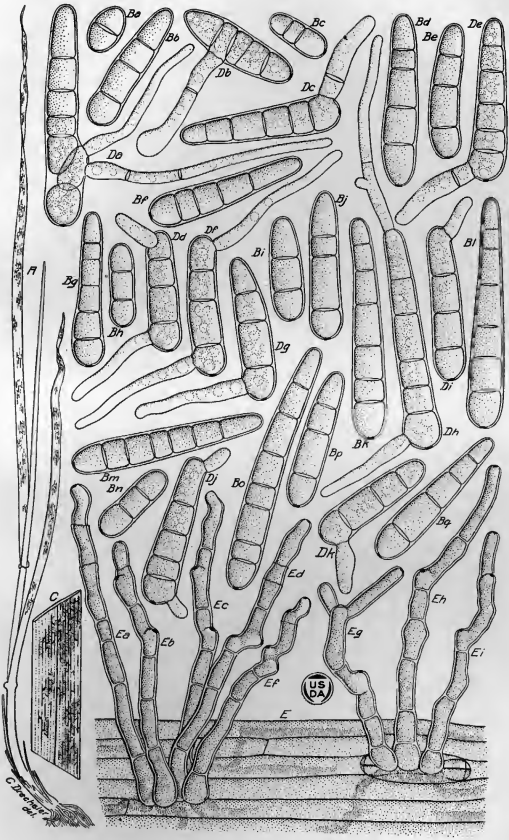
A.—Young plant of *Festuca elatior*, showing numerous net-blotch lesions on the two older leaves, the lowermost one beginning to wither at the tip. × ¾.

Ba-q.—Conidia from leaf of *Festuca elatior*, showing variation in size, shape, and septation. × 500.

C.—Part of leaf of *Festuca elatior*, showing reticulate pattern of dark longitudinal and transverse streaks within diffusely discolored areas. × 3.

Da-k.—Conidia from leaf of *Festuca elatior*, germinating in water by the production of lateral or oblique germ tubes, usually from the end segments, and less frequently from an intermediate segment (*Da, b*). × 500.

Ea-i.—Conidiophores of *H. dictyoides*, one group (*Ea-f*) emerging from between adjacent epidermal cells, the other group (*Eg-i*) emerging from a stoma. Branching like that shown in *Eg* is relatively infrequent. Hyphae within the tissue of the leaf, visible in glycerine preparations stained with eosin are indicated by heavier stippling. × 500.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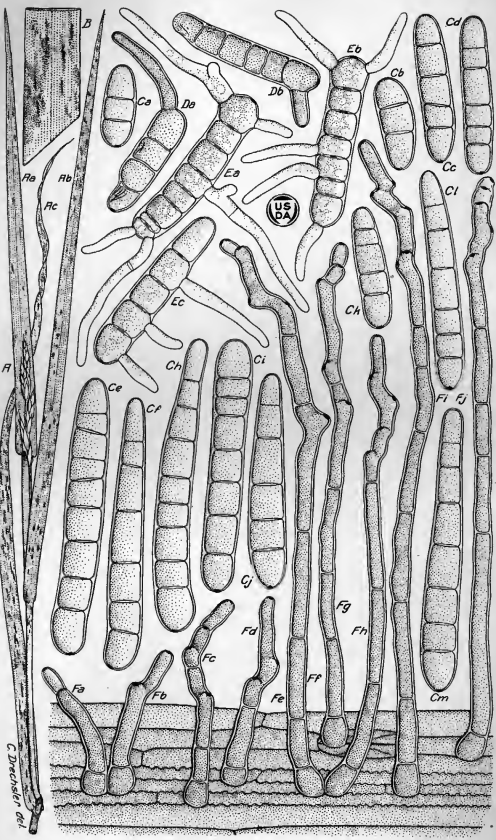
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PLATE 12

Helminthosporium siccans

A.—Portion of plant of *Lolium multiflorum* attacked by *Helminthosporium siccans*, the upper leaf *Aa* showing relatively few discolored spots, the next leaf *Ab* showing a greater number, and the third, which has begun to wither at the tip, showing a large number of spots. × 1.

B.—Portion of diseased leaf, showing spots somewhat magnified. × 4.

Ca-m.—Conidia from leaf of *Lolium multiflorum*, showing variation in size, shape, and abundance of septation. × 500.

Da-b.—Conidia from leaf of *Lolium multiflorum* that have germinated by the production of a short sporophoric process on which has been produced a single secondary conidium. × 500.

Ea-c.—Conidia from leaf of *Lolium multiflorum* germinating in water by the production of germ tubes from both end and intermediate segments. × 500.

Fa-j.—Conidiophores from leaf of *Lolium multiflorum*, showing variation in size, and mode of emergence from stoma or more abundantly from between epidermal cells on vascular ridges. × 500.

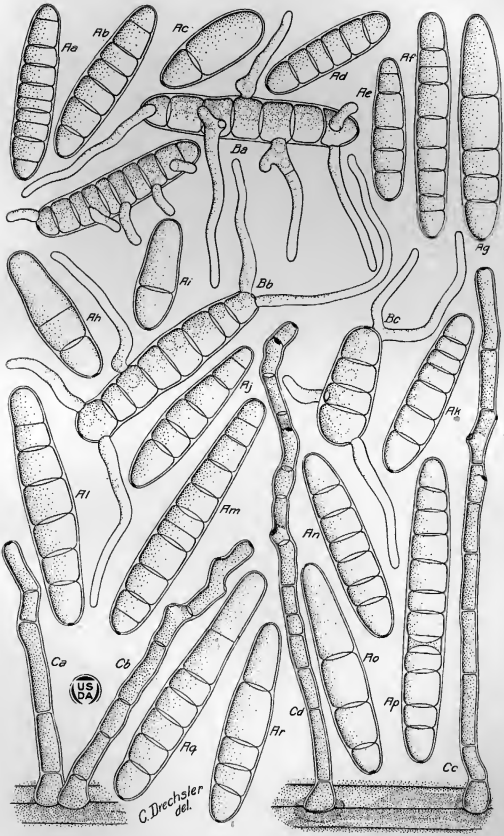
PLATE 13

Helminthosporium stenacrum

Aa–r.—Conidia from leaf of *Agrostis stolonifera* showing variation in size, shape, and septation. In *Ag*, *h*, *i*, *l*, *o*, *q*, *r*, distal portion produced as a somewhat attenuated prolongation, in which septa are absent or present in smaller numbers than in unmodified region. ×500.

Ba–c.—Conidia from leaf of *Agrostis stolonifera* germinating in water by the production of germ tubes from both middle and end segments. ×500.

Ca–b.—Conidiophores of *H. stenacrum* showing variation in size and mode of emergence singly or in pairs from between adjacent epidermal cells. ×500.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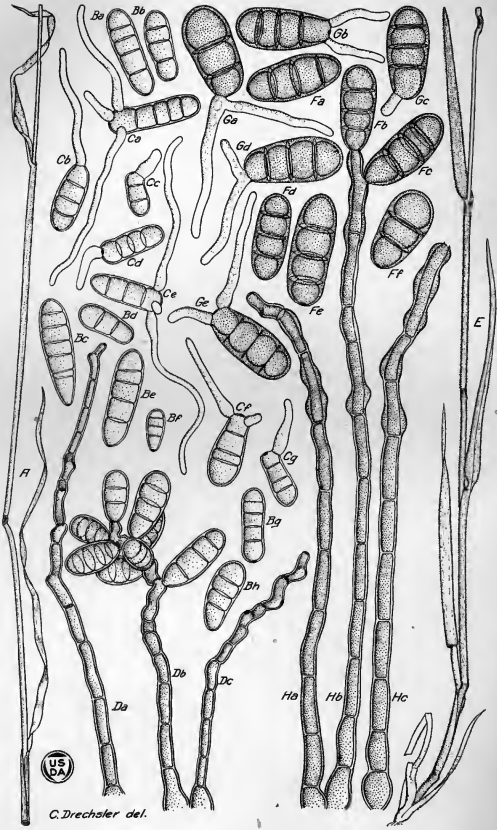
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PLATE 14

Helminthosporium dematioideum

A.—Portion of plant of *Anthoxanthum odoratum*, with leaves withered and bearing fructifications of *H. dematioideum*. ×¾.

Ba–h.—Conidia from withered leaf of *Anthoxanthum odoratum*, showing variation in size, shape, and septation. ×500.

Ca–g.—Conidia from withered leaf of *Anthoxantum odoratum* germinating in water, atypically (*Ca, c–g*) by the production of one to three germ tubes from the basal segment, and typically (*Cb*) by production of one germ tube from apex, owing to death of basal segment. ×500.

Da–c.—Conidiophores of *H. dematioideum* from leaf of *Anthoxanthum odoratum*. ×500.

Helminthosporium triseptatum

E.—Portion of plant of *Notholcus lanatus* with lower leaves withered and bearing fructifications of *H. triseptatum*. ×¾.

Fa–f.—Conidia from leaf of *Notholcus lanatus* showing variation in size, shape, and septation. ×500.

Ga–e.—Conidia from leaf of *Notholcus lanatus* germinating in water by the production of one or two germ tubes from basal end. ×500.

Ha–c.—Conidiophores from leaf of *Notholcus lanatus* showing local ring-like thickenings of wall immediately below points of attachment of successive spores. ×500.

PLATE 15

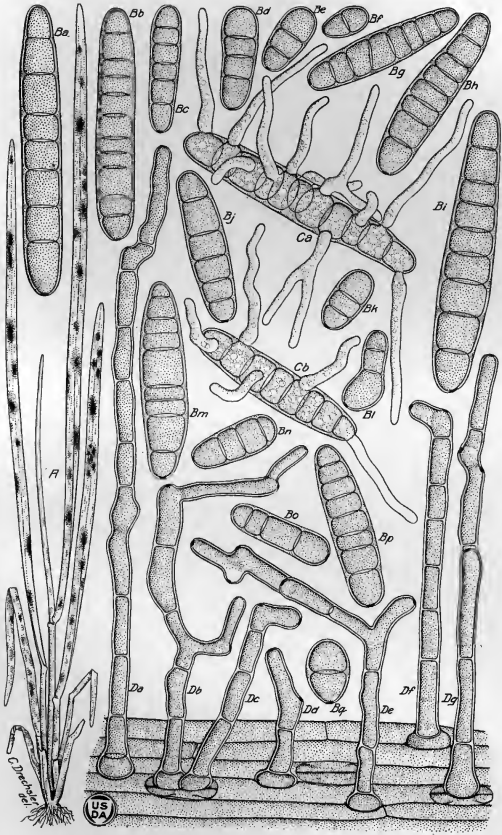
Helminthosporium vagans

A.—*Poa pratensis* with leaves abundantly spotted as result of numerous local infection by *H. vagans*. ×¾. Drawn from material collected in Brooklyn, N. Y., August, 1920.

Ba–q.—Conidia produced on leaf of *Poa pratensis*, showing variation in size, shape, and septation. ×500.

Ca, b.—Conidia produced on leaf of *Poa pratensis*, germinating in water by production of germ tubes indiscriminately from end and middle segments. ×500.

Da–g.—Conidiophores of *H. vagans*, showing variation in dimension, shape, and septation; emergence singly or in pairs from stomata or between epidermal cells; and occasional irregular branching characteristic of the species. ×500.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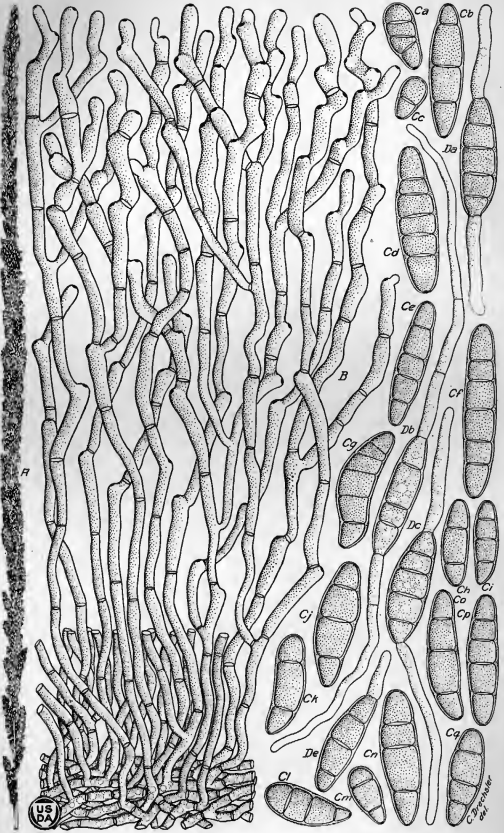
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PLATE 16

Helminthosporium ravenelii

A.—Inflorescence of *Sporobolus indicus*, the lighter portions healthy, the darker portions covered with the conidial fructifications of *H. ravenelii*. ×¾. Drawn from material collected at Wauchula, Florida, April 15, 1921.

B.—Conidiophores of *H. ravenelii*, showing origin from interwoven hyphae on superficial layers of floral parts of host, crowded condition, branching habit, irregularity in diameter, and scars marking points of attachment of spores. ×500.

Ca–q.—Conidia showing variation in shape, size, and septation. ×500.

Da–e.—Conidia germinating in water by production of one or more, typically two, polar germ tubes, one from each end cell. ×500.

PLATE 17

Helminthosporium sativum

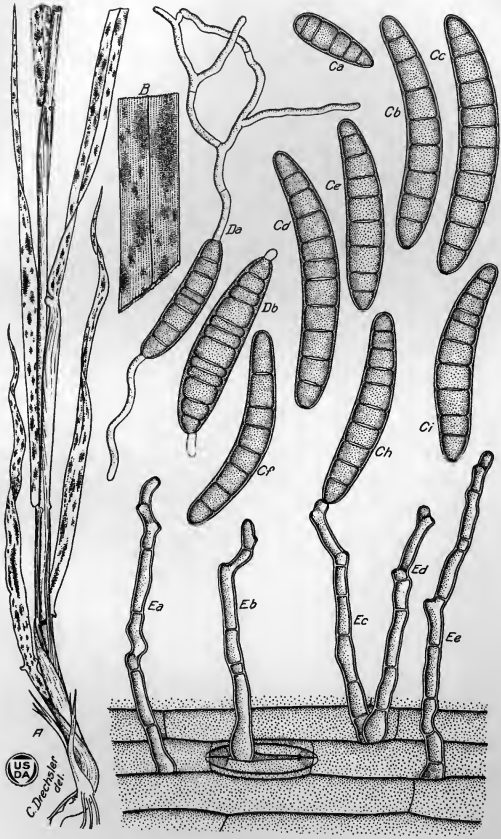
A.—Portion of barley affected with spot-blotch, showing abundance of foliar lesions and withering of lower leaves. ×¾. Drawn from material collected at Madison, Wis., July 22, 1919.

B.—Portion of barley leaf showing numerous discolored areas due to infection with *H. sativum*. ×2.

C.—Conidia produced on diseased barley, showing characteristic shape, and variation in size and septation. ×500.

D.—Conidia of *H.* sativum from diseased barley leaf germinating in water by the production of two polar germ tubes. ×500.

Eane.—Conidiophores of *H. sativum* emerging from stoma and between epidermal cells of barley leaf. ×500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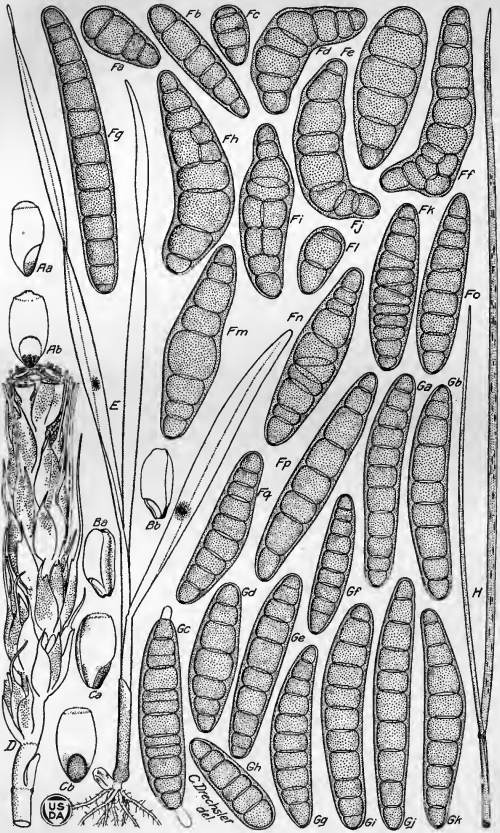
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PLATE 18

Helminthosporium sativum

Aa–b.—Kernels of wheat affected with "black point," lateral and dorsal views. ×3.

Ba–b.—Wheat kernels attacked by *H. sativum*, showing extensive discoloration of embryo and ventral suture; median longitudinal section and lateral view. ×3.

Ca–b.—Wheat kernels showing severe discoloration in region of embryo; lateral and dorsal views. ×3.

D.—Portion of mature wheat head, showing grayish efflorescence on glumes, composed of fructifications of *H. sativum*. ×3/2.

E.—Wheat seedling grown from "black-pointed" kernel showing moderately severe infection with *H. sativum;* discoloration of the basal sheath and two foliar spots. ×¾.

Fa–q.—Conidia of *H. sativum* produced on wheat head, showing atypical irregularities in shape, size, and septation. ×500.

Ga–k.—Typical conidia produced on infected leaves of *Agropyron repens*, illustrating variation in shape, size, and septation. ×500.

H.—Upper leaves of *Agropyron repens* attacked by *H. sativum*, showing abundance of small elongated foliar spots. ×¾

PLATE 19

Helminthosporium sativum

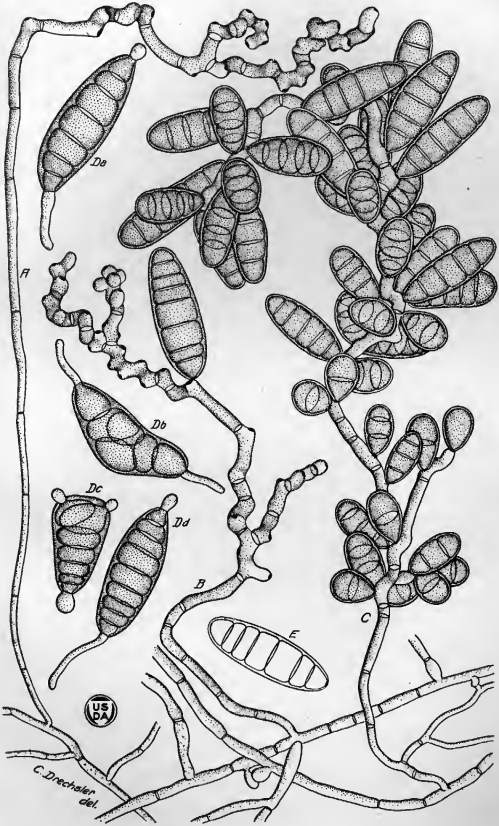
A.—Denuded conidiophore from 50-day old cornmeal agar culture originally isolated from barley leaf, showing numerous scars marking places of attachment of spores on branched, contorted, distal portion. ×500.

B.—Same as *A*, but shorter and bearing several branches. ×500.

C.—Same as *A*, but showing approximately 50 conidia attached *in situ*. Note the variation in size of the spores from small subglobose non-septate forms borne mostly on the proximal portion of the conidiophore to the larger, many septate ellipsoidal specimens on the distal portion. ×500.

Da–d.—Conidia produced in cornmeal agar culture, germinating in water. Typical germination by the production of two polar germ tubes is represented in *Da, b d;* atypical germination by the production of three germ tubes in *Dc*. In *Db* is illustrated also marked irregularity in septation frequent in conidia produced on artificial media. ×500.

E.—Conidium produced on cornmeal agar showing position of hilum, thickness of peripheral wall, and shape characteristic of spores produced on artificial media. ×500.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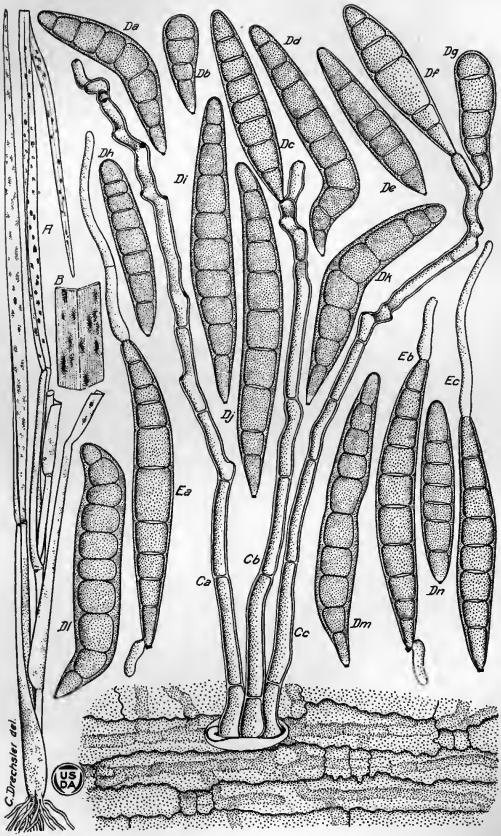
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PLATE 20

Helminthosporium monoceras

A.—Portion of plant of *Echinochloa crusgalli* attacked by *H. monoceras* showing presence of spots on leaf blades and of diffused discoloration on basal sheaths. ×¾.

B.—Portion of leaf blade of *Echinochloa crusgalli* attacked by *H. monoceras* showing variation in size of spots. ×¾.

Ca–b.—Group of conidiophores emerging from stoma of host; showing also mycelium ramifying in mesophyll as revealed in glycerine preparations stained with eosin. ×500.

Da–n.—Conidia from *Echinochloa crusgalli* collected at Port Washington, N. Y., September 20, 1920, showing variation in size, shape, and septation. ×500.

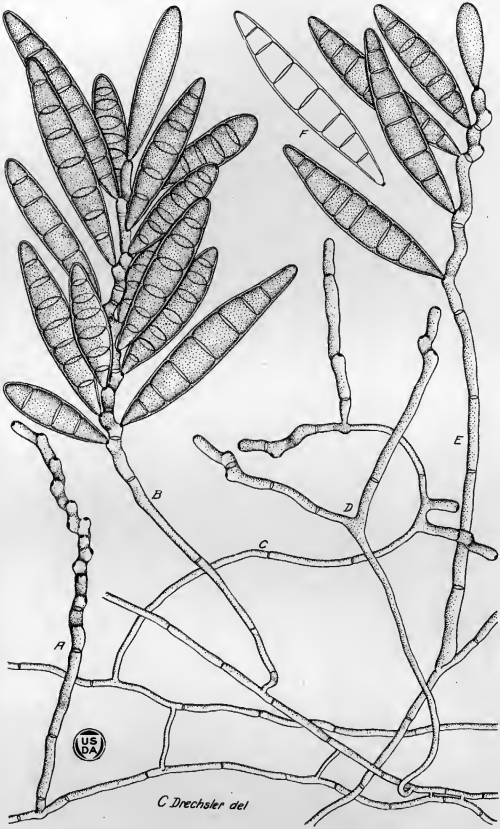
Ea–c.—Conidia from *Echinochloa crusgalli* germinating by the production of two polar germ tubes. ×500.

PLATE 21

Helminthosporium monoceras

A–E.—Conidiophores of *H. monoceras* arising from imbedded anastomosing mycelium, showing habit of growth, irregular ramifications and production of conidia in racemose arrangement. ×500. Drawn from 20-day old culture on tap-water agar.

F.—Outline of conidium from pure culture, showing attenuated regions in peripheral wall at apex and at basal end. ×500.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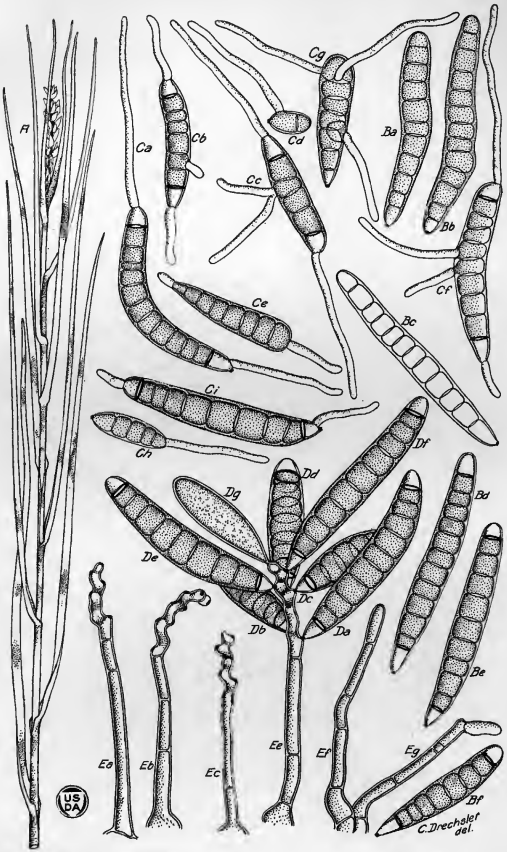
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PLATE 22

Helminthosporium halodes

A.—Portion of *Distichlis spicata* showing discolored areas on blades and sheaths of leaves, due to attack by *H. halodes*. × ¾.

Ba–f.—Conidia produced on leaf of *Distichlis spicata* in natural environment. ×500.

Ca–i.—Conidia of *H. halodes* germinating in water. *Ca–h*, conidia produced on host in natural environment; Ci, conidium produced on portion of diseased leaf after incubation in damp chamber. Germination typical in *Ca, e, h, i* by production of two polar germ tubes; atypical in *Cb, c, d, f, g* by production of lateral germ tubes alone or in addition to polar germ tubes. ×500.

Da–f.—Conidia produced on diseased part of leaf of *Distichlis spicata* incubated in damp chamber. ×500.

Ea–g.—Conidiophores of *H. halodes; Ea-e* produced on host tissue incubated in damp chamber; *Ef–g* produced on host in natural environment. ×500.

PLATE 23

Helminthosporium halodes

A.—Conidiophore of *H. halodes* from tap-water agar culture, 30 days old, showing scars marking points of attachment of conidia relatively close together. ×500.

B.—Fructification of *H. halodes* developed on 30-day old water-agar culture, showing compact racemose arrangement of conidia. ×500.

C.—Conidiophore of *H. halodes* from 30-day old water-agar culture, showing branching and relationship to attached conidia. ×500.

D.—Irregularly curved spore developed on tap-water agar. ×500.

E.—Conidium produced on tap-water agar germinating typically by production of two polar germ tubes. ×500.

F.—Conidium produced on tap-water agar, showing bifurcating apex. ×500.

G.—Conidium produced on tap-water agar, illustrating attenuation of peripheral wall at apex and immediately adjacent to hilum. ×500.

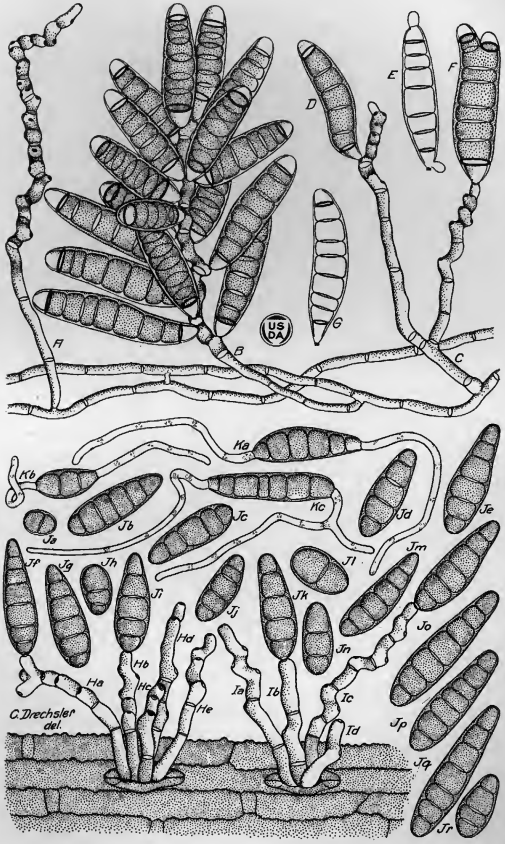
Helminthosporium leucostylum

Ha–e.—Group of five conidiophores of *H. leucostylum* emerging from stoma of *Eleusine indica*. ×500.

Ia–d.—Group of four conidiophores of *H. leucostylum* emerging from stoma of *Eleusine indica*. ×500.

Ja–r.—Conidia from leaf of *Eleusine indica* showing variation in size, shape, and septation. ×500.

Ka–c.—Conidia from leaf of *Eleusine indica* showing typical germination by the production of two polar germ tubes. ×500.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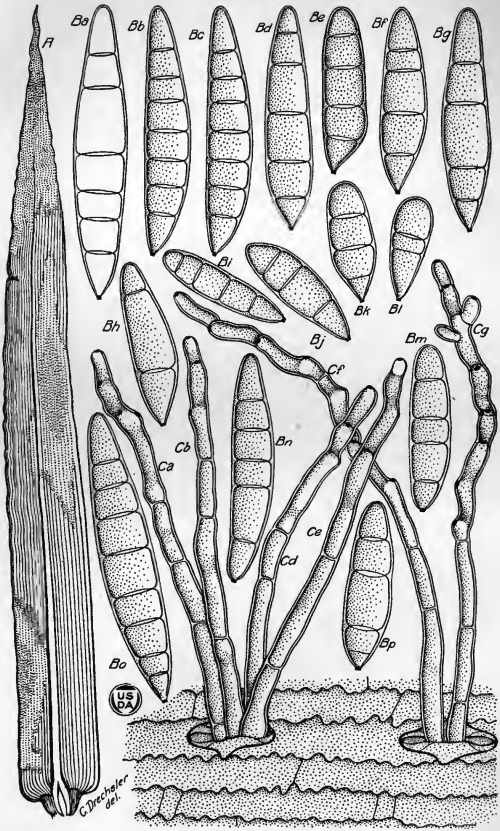
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PLATE 24

Helminthosporium turcicum

A.—Leaf of sweet corn attacked by *H. turcicum*, showing extensive dry areas bearing fructifications near the center, and surrounded by slightly discolored margin. ×⅓. Drawn from material collected near Valley Stream, N. Y., September 16, 1920.

Ba–p.—Conidia of *H. turcicum* produced on leaves of sweet corn, showing variation in size, shape, and septation. ×500.

Ca–g.—Conidiophores emerging in groups from stomata of sweet corn. ×500.

PLATE 25

Helminthosporium turcicum

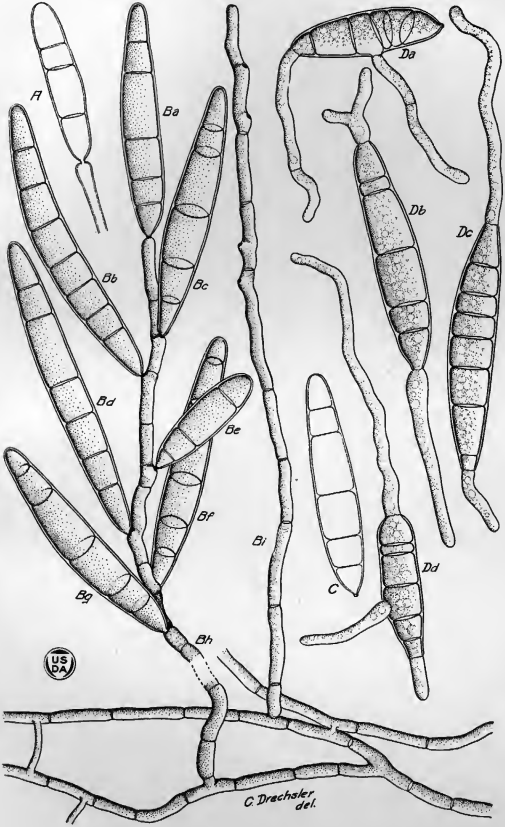
A.—Outline of conidium of *H. turcicum*, showing attachment to conidiophore. × 500. From material grown in 20-day old tap-water agar culture.

Ba–Bg.—Conidia of *H. turcicum* developed in pure culture on tap-water agar. × 500.

Bh–i.—Conidiophores from 20-day old tap-water agar culture, showing relation to mycelium. ×500.

C.—Outline of conidium from diseased corn leaf, showing relation of hilum to basal segment. ×500.

Da–d.—Conidia germinating in water, either typically (*Db*, *c*) by the production of two polar germ tubes; or atypically (*Da*, *d*) by the proliferation of a lateral germ tube in addition to one or two polar tubes. ×500.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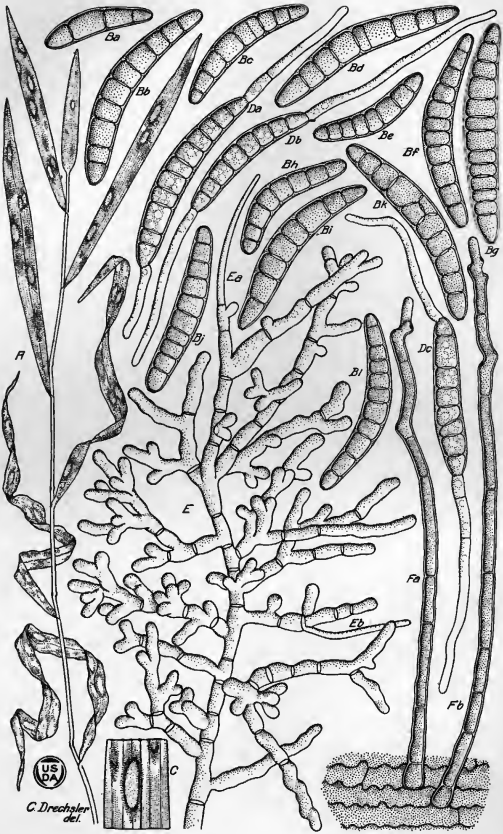
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PLATE 26

Helminthosporium leersii

A.—Portion of plant of *Leersia virginica* attacked by *H. leersii* showing foliar spots in various stages of development and resultant withering of successively younger leaves. ×1. Drawn from material collected near Meriden, Conn., September 7, 1920.

Ba–l.—Conidia of *H. leersii* produced on *Leersia virginica* under natural conditions, showing variations in size, shape, and septation. ×500.

C.—Portion of leaf of, *Leersia virginica* attacked by *H. leersii*, showing foliar "eye spot" in detail. × 3.

Da–c.—Conidia from diseased leaf of *Leersia virginica* germinating by the production of two polar germ tubes. ×500.

E.—Ramifying system of short hyphal elements produced in 10-day old potato dextrose agar culture. *Ea*, *b* represent stolon-like elements, by the elongation of which the mycelium becomes more extensive. ×500.

Fa–b.—Conidiophores of *H. leersii* produced on leaf of *Leersia virginica* under natural conditions. ×500.

PLATE 27

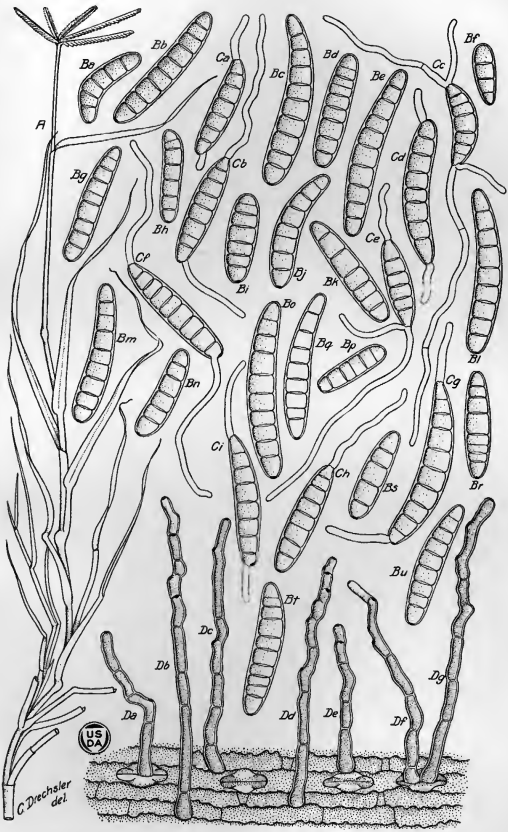
Helminthosporium cynodontis

A.—Portion of plant of *Cynodon dactylon* attacked by *Helminthosporium cynodontis*, showing withered condition of leaves bearing fructifications of fungus. ×¾. Drawn from material collected at Wauchula, Fla., April 19, 1921.

Ba–u.—Conidia from affected leaf of *Cynodon dactylon*, showing variation in size, shape, and septation. ×500.

Ca–i.—Conidia from leaf of *Cynodon dactylon* germinating in water, by production of two polar germ tubes. × 500.

Da–g.—Conidiophores of *H. cynodontis* emerging singly or in pairs from stomata or between epidermal cells of *Cynodon dactylon*. ×500.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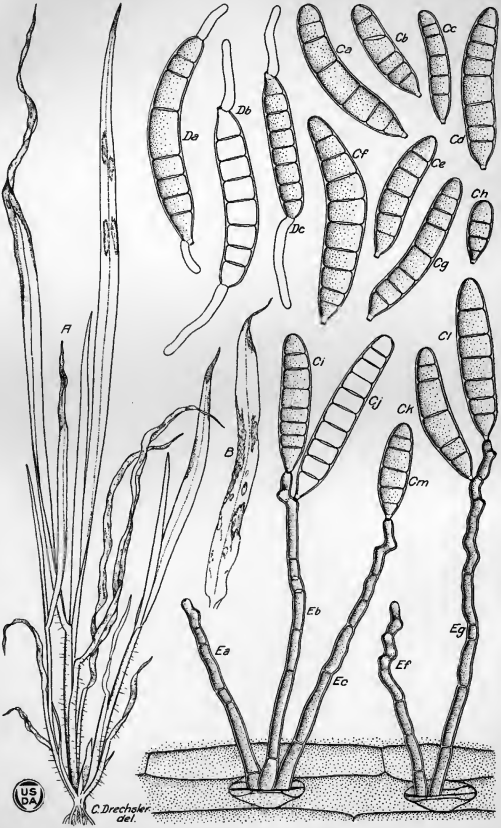
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PLATE 28

Helminthosporium micropus

A.—Young plant of *Paspalum boscianum* attacked by *H. micropus*, showing distribution of affected regions and the withering of foliage caused by fungus. ×¾.

B.—Leaf of young plant of *Paspalum boscianum* attacked by *H. micropus*, showing several affected regions and distortion and withering of diseased foliar organ. ×¾.

Ca–m.—Conidia from leaf of *Paspalum boscianum*, showing variation in size, shape, and septation. ×500.

Da–c.—Conidia from leaf of *Paspalum boscianum* germinating in water by production of two polar germ tubes. ×500.

Ea–g.—Conidiophores of *H. micropus* emerging in groups from stomata of *Paspalum boscianum*. ×500.

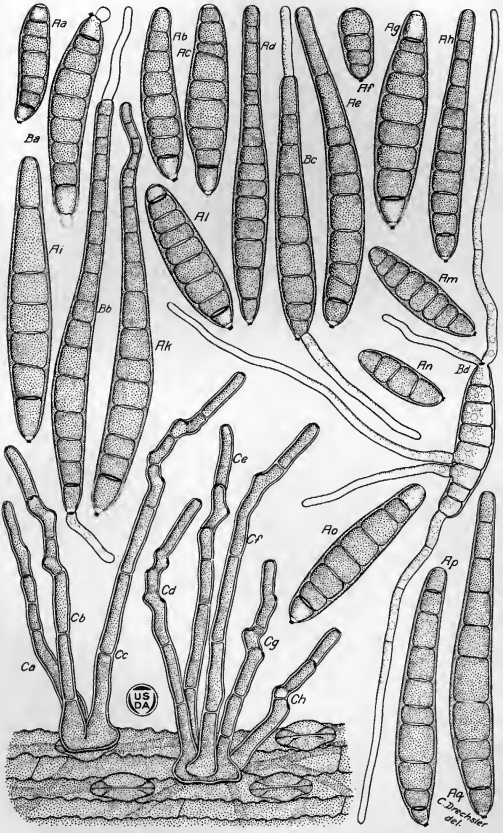
PLATE 29

Helminthosporium rostratum

Aa–q.—Conidia of *H. rostratum* from dry leaves of *Eragrostis major* collected near Washington, D. C., October 13, 1921, showing variation in size, shape, and septation. ×500.

Ba–d.—Conidia from dry leaf of *Eragrostis major* germinating in tap water, the mature spores (*Ba–c*) by the production of two polar germ tubes; the newly proliferated spore (*Bd*) by the production of two lateral germ tubes in addition to polar tubes. ×500.

Ca–h.—Conidiophores showing origin in groups from an expanded base and relation of latter to stomata or epidermal cells. × 500.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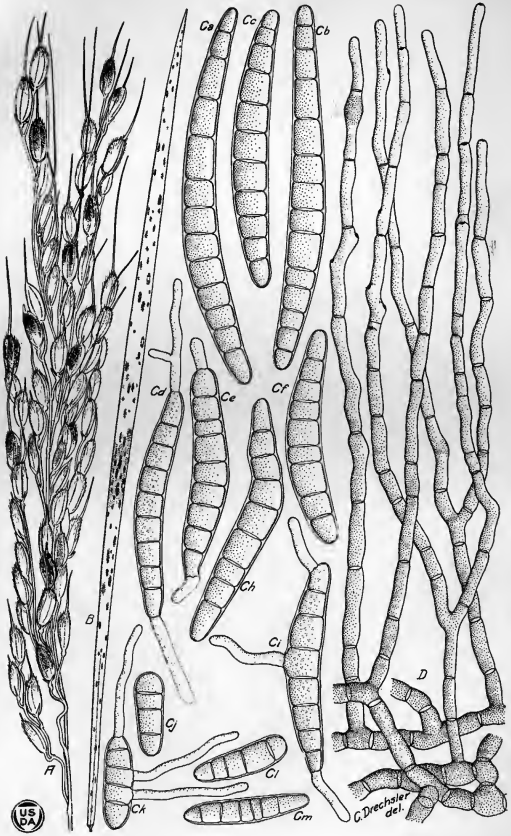
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PLATE 30

Helminthosporium oryzae

A.—Panicle of rice attacked by *H. oryzae*, showing some spikelets partly covered with dark growth composed of fructifications of the fungus. × 1.

B.—Leaf of rice showing numerous discolored spots resulting from inoculation with *H. oryzae*. ×¾.

Ca–m.—Conidia of *H. oryzae* produced on diseased floral parts. *Ca–b*, very large spores from well developed mats of fructification; *Cc, f, h, j, l, m*, conidia more typical in size from scattered fructifications; *Cd, e*, conidia germinating typically by the production of two polar germ tubes; *Ci, k*, relatively immature conidia germinating atypically by production of lateral germ tubes from intermediate segments in addition to polar germ tubes. × 500.

D.—Conidiophores of *H. oryzae* from infected floral scale showing origin from stout prostrate hyphae. × 500.

PLATE 31

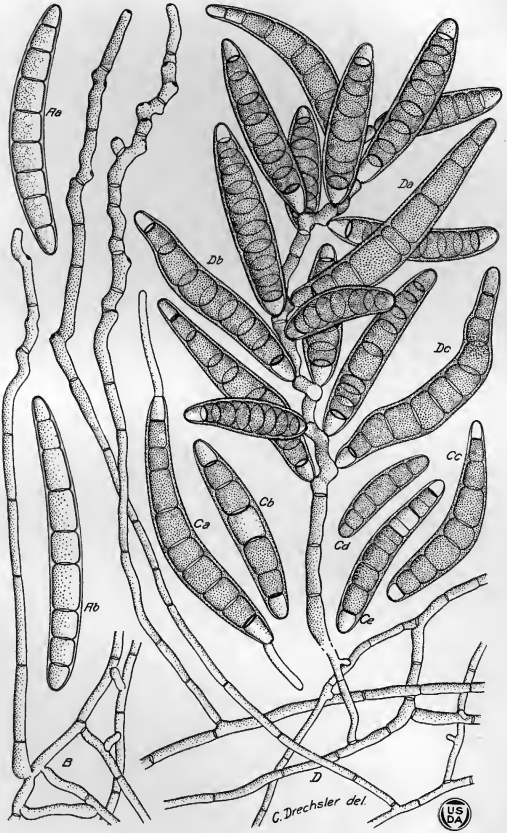
Helminthosporium oryzae

Aa–b.—Subhyaline conidia of *H. oryzae* from tap-water agar culture, 30 days old. × 500.

B.—Conidiophore from tap-water agar culture, 30 days old, showing relation to vegetative mycelium. × 500.

Ca–e.—Conidia from potato-dextrose agar culture, 30 days old; *Ca*, conidium germinating typically by production of two polar germ tubes; *Cb*, *e*, Conidia showing subhyaline end and intermediate segments; *Cc*, Conidium with two subhyaline end cells; *Cd*, Conidium of uniform color without subhyaline segments. × 500.

D.—Fructification and denuded conidiophores developed on potato dextrose agar, 30 days after inoculation, showing relation of sporophores to vegetative hyphae, angle at which conidia are attached, and variation of the latter in shape, size, and septation. × 500.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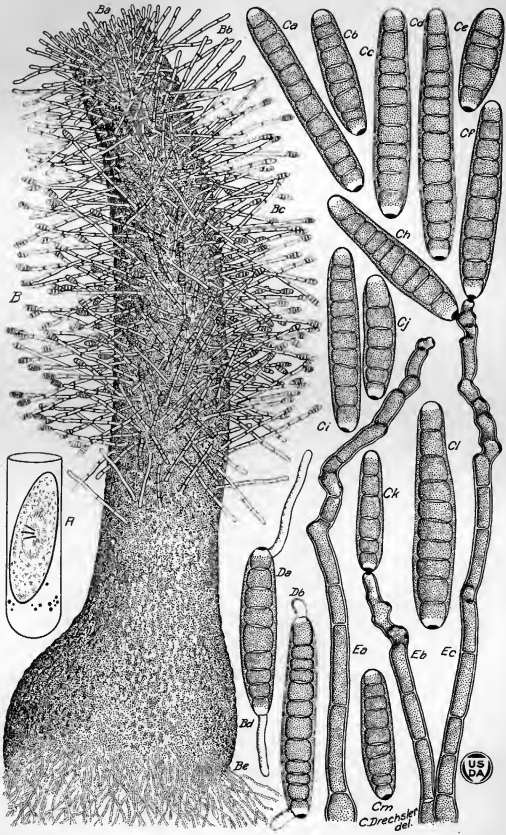
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PLATE 32

Helminthosporium cyclops

A.—Culture of *H. cyclops* on Beijerinck's agar, 20 days after inoculation, showing subspherical sclerotia imbedded in substratum, discrete fructifications scattered sparingly over surface of substratum, fluffy mycelial growth near point where inoculum was planted, and three compound fructifications in center. × 3/4.

B.—Compound conidial fructification on Beijerinck's agar, showing origin from imbedded hyphae at base (*Be*), sterile basal portion (*Bd*), conidiophore more or less radially arranged (*Bc*), and growing apex (*Ba*) where new conidiophores are proliferated (*Bb*). × 500.

Ca–m.—Conidia from leaves of *Danthonia spicata*, showing thick peripheral wall, conspicuous hilum, and variation in size, shape, and septation. × 500.

Da–b.—Conidia from leaves of *Danthonia spicata* germinating in water by the production of two polar germ tubes. × 500.

Ea–c.—Conidiophores from leaf of *Danthonia spicata*. × 500.

PLATE 33

Helminthosporium cyclops

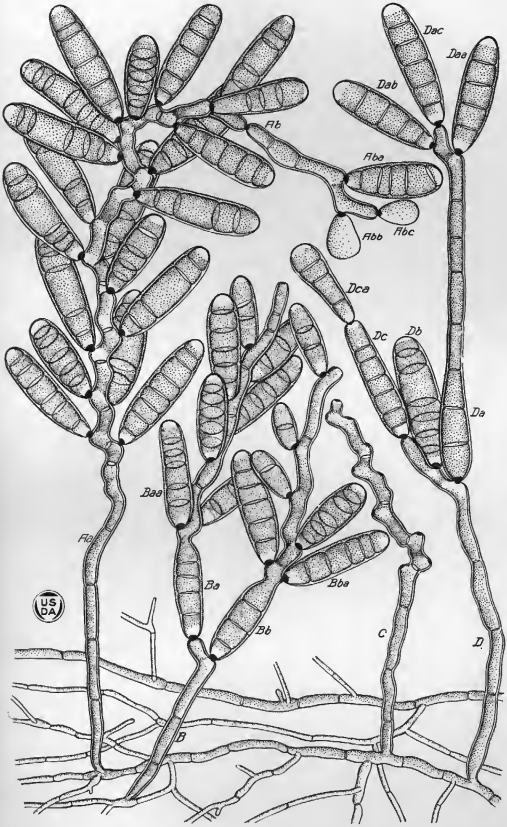
Mycelium, fructifications, and denuded sporophore of *H. cyclops* developed on Beijerinck's agar and drawn 30 days after inoculation of substratum. × 500.

Aa.—Conidiophore which, after producing many spores, has proliferated the distal sporophoric element *Ab* by a budding process similar to the proliferation of a conidium. The distal sporophoric element has given rise to three spores, *Aba–bc.*

B.—Conidiophore, the typical development of which has been replaced after the proliferation of two primary conidia *Ba–b*, by the growth of the latter into sporophoric elements, on which were developed numbers of secondary conidia, *Baa*, *Bba*, etc., respectively.

C.—Denuded conidiophore.

D.—Conidiophore bearing three primary conidia, one of which (*Da*) has grown out into a sporophoric process bearing three secondary spores *Daa–ac;* while another (*Dc*) has given rise directly to a secondary conidium *Dca* by apical proliferation.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JUNE 2, 1923 No. 9

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CONTENTS

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CONTROL OF SNOW MOLDING IN CONIFEROUS NURSERY STOCK [1]

By C. F. KORSTIAN [2]

Associate Silviculturist, Appalachian Forest Experiment Station, Forest Service, United States Department of Agriculture

During the time that the Cottonwood Nursery of the United States Forest Service was in operation (1906–1921) very serious winter losses of Douglas fir (*Pseudotsuga taxifolia*) seedlings and transplants occurred under the snow. Several other species were also injured, the extent of loss varying with the species, age class, and time of snow disappearance in the spring. All age classes of Norway spruce (*Picea excelsa*) were especially susceptible, while Engelmann spruce (*Picea engelmanni*) and the Pacific coast form of western yellow pine (*Pinus ponderosa*) suffered only moderate injury, and lodgepole pine (*Pinus contorta*) was practically immune.

The Cottonwood Nursery is situated 25 miles southeast of Salt Lake City, Utah, on the Wasatch National Forest, at an elevation of 7,450 feet. Here the snow usually covers the ground continuously from November 1 to May 10, lasting occasionally even until May 20 or 25. The maximum depth varies from 6 to 8 feet. Under this heavy blanket of snow the ground seldom freezes, and even where freezing has occurred in the autumn before the advent of permanent snow, the soil soon thaws out after the snow begins to accumulate. Invariably the injury was greatest during years of heaviest snowfall and consequent late melting of snow in the spring.

Hartley, Pierce, and Hahn [3] studied this type of injury and found that it was caused by weakly parasitic fungi, attacking the leaves. They succeeded in isolating, by cultural methods, a number of organisms from recently snow-molded seedlings of Douglas fir, of which they most strongly suspected *Botrytis cinerea* and a dark sterile mold, as yet unidentified, as causing the disease. These investigators conducted spraying experiments with fungicides, including sulphuric acid, formaldehyde, zinc chloride, copper sulphate, copper acetate, and ammoniacal copper carbonate, none of which gave promise of effectively controlling the disease. A lime-sulphur mixture gave only very slightly beneficial results. Mulches consisting of thin layers of sawdust, sterilized sand, and gravel gave no promise. At the beginning of the present investigation

[1] Accepted for publication Sept. 2, 1922.

[2] The writer is indebted to former Forest Planting Assistant N. J. Fetherolf for painstaking assistance in carrying out the experiments here reported.

[3] HARTLEY, Carl, PIERCE, Roy G., and HAHN, Glenn G. MOULDING OF SNOW-SMOTHERED NURSERY STOCK. *In* Phytopathology, v. 9, p. 521–531. 1919.

the writer submitted diseased specimens to Dr. Carl Hartley, who reported that the organisms were evidently the same as those with which he had worked.

For several years the practice of sowing black soil on the snow early in the spring to hasten its melting was followed by the nurserymen, but even with this practice the losses were still so heavy that the growing of Douglas fir on a large scale at the Cottonwood Nursery was discontinued in 1916.

TABLE I.—*Percentage of injury by snow-molding fungi in the several species and age classes, spring of 1920*

Species.	Age class.	Dead.	Alive but injured.
		Per cent.	*Per cent.*
Picea engelmanni	1–0	31.3	0
	2–0	27.7	0
	2–1	9.9	0
	3–2	8.1	0
Picea excelsa	2–0	77.0	0
	2–1	63.0	22.3
	3–2	59.3	0
Pseudotsuga taxifolia	1–0	62.9	0
	2–0	62.7	0
Pinus ponderosa	1–0	93.2	6.8
	2–0	52.2	44.7
	2–1	15.2	12.3
Pinus ponderosa scopulorum	1–0	16.8	2.5
	2–0	7.8	3.4
	2–1	3.5	15.8

The extent of the injury by these fungi among the different species and age classes is shown in Tables I and II, which are based upon counts of from several hundred to as many as 2,400 seedlings in each age class. It is recognized that a small loss occurred during the summer seasons but it was found to be negligible in comparison with the loss due to snow molding. Table I and Plate 1 show the much greater susceptibility of the exotic Norway spruce to the disease as compared with that of the native Engelmann spruce. Table I shows quite clearly that the younger age classes, 1–0 and 2–0, suffer by far the greatest loss and injury. Table II fails to bring out this relationship so clearly mainly because of the fact that a large part of the older age classes of stock had been abandoned, receiving no water or care of any kind during the summer of 1920. This left the stock in a weakened condition and an easy prey for the fungi. For example, 3–3 Engelmann spruce shows (not in Table II) 41.5 per cent of dead and injured trees as compared with 3.4 per cent for the 3–2 stock and 7.1 per cent for the 3–1 stock. The first age class had been abandoned in 1920, while the latter two classes of stock were watered and otherwise properly cared for during 1920. It should be noted, therefore, that among the older age classes, especially transplants, it is usually the weak or previously injured trees that are killed or severely injured. It is not uncommon to find some of the leaves of vigorous transplants infected when the snow melts, but the development of the fungi is checked as soon as the trees are exposed to the sunlight, and complete recovery may follow. Jack pine (*Pinus banksiana*), however,

was found to be a notable exception. During the winter of 1919–1920 a bed of some 300 vigorous 4-year-old jack pine transplants, 18 inches tall, was completely killed by the disease. Tables I and II clearly show that the Pacific Coast form of western yellow pine (*Pinus ponderosa*), grown from seed collected in central Idaho, is much more susceptible to the snow-molding fungi than the Rocky Mountain variety of the same species (*Pinus ponderosa scopulorum*), grown from seed collected in the region near the nursery.

TABLE II.—*Percentage of injury by snow-molding fungi in the several species and age classes, spring of 1921*

Species.	Age class.	Dead.	Alive but injured.
		Per cent.	*Per cent.*
Picea engelmanni	3–0	8.4	13.3
	3–1	0.8	6.3
	3–2	0	3.4
Picea excelsa	3–0	80.4	11.7
	3–3	80.3	19.6
Picea parryana	3–0	60.2	30.8
	3–3	3.0	31.5
Pseudotsuga taxifolia	1–0	5.1	6.9
	2–0	16.2	19.3
	[a] 3–0	2.0	5.4
	[b] 3–0	31.1	30.4
Pinus ponderosa	1–0	11.5	3.5
	3–0	0	83.0
	2–1	37.2	38.0
	2–2	36.8	35.6
Pinus ponderosa scopulorum	1–0	0.8	1.6
	3–0	4.5	18.5
	2–1	23.7	26.8
	2–2	6.3	24.7
Pinus contorta	2–1	0	3.0
	2–2	0	1.5
Abies concolor	3–0	22.1	30.8
Pinus resinosa	2–1	0	15.8
Pinus strobus	2–1	60.2	39.8
Libocedrus decurrens	.....	4.1	8.4
Thuja occidentalis	.....	100.0	0
Juniperus monosperma	.....	100.0	0

[a] Bed protected by framework of 2 by 6 lumber. [b] Unprotected bed.

Since most fungi will gain a foothold more readily at some previously injured part of a plant, it seems quite probable that the fungous injury during the winter of 1919–1920 was greatly increased as a result of the severe injury to the ends of the twigs by frost in the spring of 1919.[4]

In the spring of 1918 the problem of working out an efficacious method of controlling the snow molding of coniferous nursery stock was taken up by the writer. Six 4 by 12 foot beds were sown to the same amounts of Douglas fir, the time and method of sowing and subsequent care throughout the summer season being the same. Uniformly good stands were secured on all of the beds. The seed beds were covered with shade frames giving half shade during the hot, dry portion of the summer. In the autumn the shade frames were removed and just before the

[4] KORSTIAN, Clarence F. EFFECT OF A LATE SPRING FROST UPON FOREST VEGETATION IN THE WASATCH MOUNTAINS OF UTAH. *In* Ecology, v. 2, p. 47–52, 1 fig. 1921. Literature cited, p. 52.

permanent winter snow came, different treatments were applied to five of the beds, while the sixth was left untreated as a control. The object of these special treatments was mainly to afford mechanical protection from the disease. The treatment which gave much the best results consisted of a framework of 2 by 6 inch planks loosely placed with a spacing of 1 to 2 inches upon logs or squared timbers lying lengthwise along the sides of the beds. This type of protective covering, which was used on Bed 14, is shown in Plate 2, B. Two-inch lumber was used, mainly because it was available. It is possible that one-inch lumber could be used, if it were adequately supported to hold the heavy mass of snow above the tops of the seedlings. As soon as the snow had melted sufficiently in the spring to permit, the framework was removed. It was placed on the bed each autumn before the advent of the heavy winter snowfall.

TABLE III.—*Survival of Douglas fir seedlings in beds receiving different treatments as indicated; sowing spring of 1918.*

Bed No.	Date of count.	Total germination.	Survival.		Special treatment.
			Total.	In per cent of total germination.	
		Per square foot.	*Per square foot.*	*Per cent.*	
12	Oct. 9, 1918	126	103	81.8	None; control.
	Sept. 29, 1919		81	64.2	
	July 1, 1920		45	35.7	
	Oct. 6, 1920		42	33.3	
	May 22, 1921		14	11.1	
13	Oct. 9, 1918	163	131	80.3	Mulched with timothy hay winters of 1918–19 and 1919–20, 1920–21.
	Sept. 29, 1919		24	14.7	
	July 1, 1920		0	0	
	Oct. 6, 1920		0	0	
	May 22, 1921		0	0	
14	Oct. 9, 1918	128	98	76.6	Framework of 2 by 6's, 2 inches apart supported by timbers several inches above tops of trees. Winters 1918–19, 1919–20, 1920–21.
	Sept. 29, 1919		95	74.2	
	July 1, 1920		84	60.0	
	Oct. 6, 1920		84	60.0	
	May 22, 1921		84	60.0	
15	Oct. 9, 1918	69	58	84.0	Mulched with aspen leaves. Winters 1918–19, 1919–20, 1920–21.
	Sept. 29, 1919		24	34.8	
	July 1, 1920		13	18.8	
	Oct. 6, 1920		13	18.8	
	May 22, 1921		13	18.8	
16	Oct. 9, 1918	79	58	73.4	Covered with canvas throughout the winters of 1918–19, 1919–20, 1920–21.
	Sept. 29, 1919		28	35.4	
	July 1, 1920		3	3.8	
	Oct. 6, 1920		3	3.8	
	May 22, 1921		1	1.3	
17	Oct. 9, 1918	134	96	71.6	Mulched with sterilized sand. Winters of 1918–19, 1919–20, 1920–21.
	Sept. 29, 1919		78	58.2	
	July 1, 1920		8	6.0	
	Oct. 6, 1920		8	6.0	
	May 22, 1921		5	3.7	
7	Oct. 6, 1920	165	[a] 110	66.6	Framework, 2 by 6's.
	May 22, 1921		[a] 100	60.6	Same as bed No. 14.
9	Oct. 6, 1920	142	[a] 93	65.4	None; control.
	May 22, 1921		[a] 23	16.2	

[a] Sowing spring of 1920.

Since this investigation was completed Cornefert [5] has reported a somewhat different method which has given excellent results for several years in the protection of spruce nursery stock from *Herpotrichia nigra*. In the autumn peeled poles 10 to 15 cm. in diameter are placed between the rows of plants, so that the plants lean upon them when pressed down by the snow. In field planting each tree is placed so that it can be supported by a rock or a stump.

Table III shows the original germination per square foot, survival on various dates, and the special treatment applied to each of the beds sown in 1918. The use of the protective framework of 2 by 6 inch planks was again checked during the winter of 1920–21 on Douglas fir seedlings originating from seed sown in the spring of 1920, the survival data for which is also included in Table III. The general appearance of the 3–0 beds of the 1918 sowing are shown in Plate 2, A. From Table III it will be seen that the results are decidedly in favor of the plank framework (Bed 14 of the 1918 sowing and Bed 7 of the 1920 sowing), which keeps the heavy mass of snow from mashing the seedlings down flat against the soil. Sufficient snow sifts through the intervening spaces between the 2 by 6's to keep the soil moist during the winter. At the end of the third winter Bed 14 of the 1918 sowing showed an average survival of 84 seedlings per square foot, or 60 per cent of the original germination compared with the next highest survival of 14 seedlings per square foot, or 11.1 per cent of the original germination in Bed 12—the control bed to which no special treatment had been applied. During the winters of 1918–19 and 1919–20 the protected bed (No. 14) suffered losses of 2.4 per cent and 14.2 per cent and none at all during the succeeding winter, as compared with losses in the control bed (No. 12) during the three winters of 17.6, 28.5, and 22.2 per cent, respectively. Bed 7 of the 1920 sowing, which was given protection by the same type of framework covering as that described above, suffered a loss of 6 per cent during the winter of 1920–21, as compared with a loss of 49.2 per cent in the control bed during the same winter.

It is evident from Table III and from general observations in the nursery that a mulch of organic matter stimulates the activities of the fungi by providing a suitable substratum on which they may develop. Contact with various kinds of organic matter, dead snow-molded plants, and a surface even with the soil predisposes to the disease.

On May 25, 1921, the stock in Bed 14 (protected by framework) and in Bed 12 (control) was dug and graded with the result that 67 per cent of the stock from Bed 14 was suitable for transplanting, while only 34.4 per cent of the seedlings from Bed 12 was suitable. Even many of the firsts in the unprotected bed were infected with the snow-molding fungi, which probably would result in a heavier culling the following spring when the transplants were dug preparatory to field planting. The foliage was of a dull green color and numerous leaves were dead or partially killed. This lot of stock had very few healthy buds, only 25.5 per cent, while the seedlings from the protected bed had 90.7 per cent of good terminal buds. In each case the bud scales of the unhealthy seedlings were loose and flaccid, giving the buds a withered appearance. Small bunches of each lot of stock were allowed to stand in the laboratory with the roots submerged in water for 10 days. Practically all of the buds on the seedlings

[5] CORNEFERT, R. RÉGÉNÉRATION SUR LES HAUTS PLATEAUX DU JURA. *In* Soc. Forest. Franche-Comté et Belfort Bul. Trimest., t. 14, p. 206. 1921.

from the protected bed developed into normal growing shoots, while those from the unprotected bed remained in a dormant condition, apparently due to injury by these fungi.

Table IV and Plate 3 show the root, top, and terminal bud development of Douglas fir seedlings grown three years in protected and unprotected seed beds. The trees from the unprotected bed had heavier roots and tops and a greater stem diameter. This is probably due to the fact that the stand of seedlings in the unprotected bed is much more open than in the protected bed, and consequently each plant has more growing space in which to develop. At the same time none but the largest and most vigorous seedlings survived in the unprotected bed. On the other hand, the roots from the unprotected bed were heavier in proportion to the weight of the plant, the tops being correspondingly less. This is probably attributable to the fungous injury to the tops, which was unfavorable to their development and which was much less pronounced in the protected beds.

TABLE IV.—*Root, top, and terminal bud development*

Bed.	Average length of tops.	Average length of roots.	Average weight of tops.	Average weight of roots.	Ratio of weight of root to weight of entire plant.	Average diameter of stem at root collar.	Average length of terminal bud.	Average width of terminal bud.
	Inches.	*Inches.*	*Gm.*	*Gm.*	*Per cent.*	*Inches.*	*Inches.*	*Inches.*
Protected seedlings (Bed 14).....	6.03	11.43	1.62	0.46	22.1	0.08	0.24	0.11
Unprotected seedlings (Bed 12).....	5.77	10.68	1.78	.75	29.6	.10	.18	.09

The examination of field plantations revealed considerable fungous injury during the winter of 1919–1920, especially among jack pine, Engelmann spruce and Norway spruce. The jack pine were practically all dead and the few living trees so severely injured that ultimate recovery was impossible. With Norway spruce and Engelmann spruce, the greatest injury from these fungi occurred on soils rich in organic matter, the surface of which was covered with considerable plant litter. The underplanting of aspen (*Populus tremuloides*) stands with Engelmann spruce, Norway spruce, and Douglas fir has been accompanied by notable losses on areas on which the ground was covered with a deep layer of aspen leaves. The heaviest losses occur where the plants are bent over into intimate contact with the soil and litter which is the breeding ground of these weakly parasitic snow-molding fungi. Serious loss by molding under the snow can be prevented in spring plantings by removing the accumulation of leaf litter from an area of one or two square feet, in the center of which the hole for the tree is dug and also by the use of large vigorous planting stock, which will stand erect during leaf fall and through the wet snows of the early winter and in this way hold its leader out of the zone of greatest danger.

CONCLUSIONS

The snow-molding fungi have caused a greater loss of coniferous nursery stock at the Cottonwood Nursery than any other form of injury or disease.

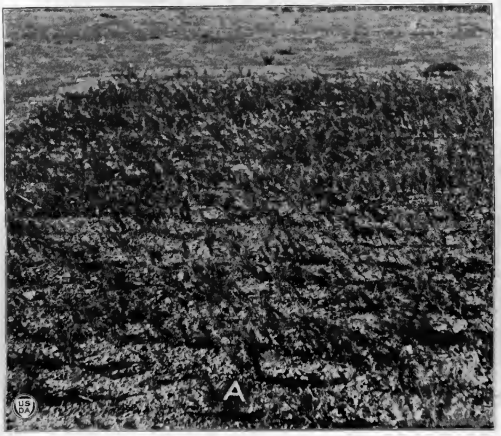
Examinations showed that practically all age classes and species grown in this nursery, excepting lodgepole pine, are subject to this disease and that it is more prevalent among young seedlings (1–0 and 2–0) than in the older age classes. Among older seedlings and transplants, the weak and previously injured trees are the ones generally attacked.

The disease can be controlled effectively in the nursery by placing a protective framework over the bed so that the full weight of the snow does not lie heavily on the trees, and press them down flat on the ground. It is by far the best and most efficient method of control which has been developed.

PLATE 1

A.—Bed of 3–1 Norway spruce as it appeared in June, 1920, about two weeks after the snow had disappeared, showing the very high mortality and large amount of injury due to snow-molding fungi.

B.—Bed of Engelmann spruce as it appeared in June, 1920, about two weeks after the snow had disappeared, showing its relative freedom from snow-molding injury, as compared with Norway spruce.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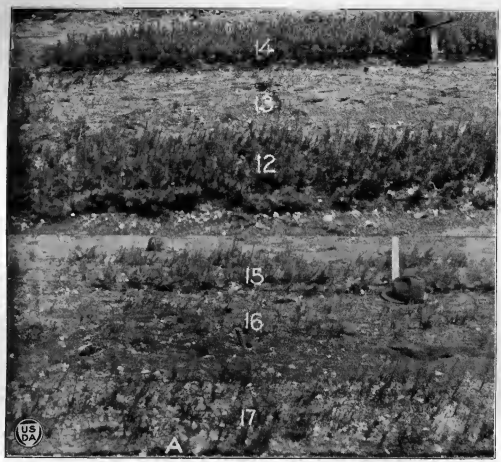
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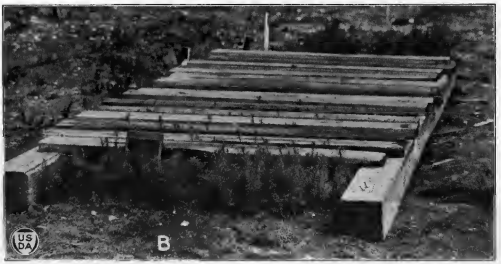
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PLATE 2

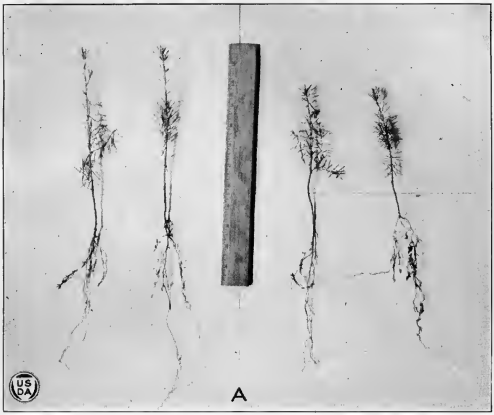
A.—Beds 12, 13, 14, 15, 16, and 17, 3–0 Douglas fir seedlings, as they appeared on May 27, 1921, about two weeks after the snow had disappeared. Bed 12 (control) received no special treatment, Bed 13 was mulched with timothy hay, Bed 14 was protected with a plank framework, Bed 15 was mulched with aspen leaves, Bed 16 was covered with canvas throughout the winter, Bed 17 was mulched with sterilized sand each autumn. Note the very poor stand on the mulched beds and the good stand and fine appearance of the protected bed (No. 14), as compared with the control bed (No. 12).

B.—The protective framework of 2 by 6's used on Bed 14, with a few of them removed from the front of the bed to show the 3–0 Douglas fir seedlings. Round logs may be used successfully instead of square timbers.

PLATE 3

A.—Healthy 3–0 Douglas fir seedlings from Bed 14, protected by framework of 2 by 6 planks, in which there was practically no injury from snow-molding fungi.

B.—Three-year-old Douglas fir seedlings from Bed 12, unprotected, showing unhealthy appearance due to injury from snow-molding fungi.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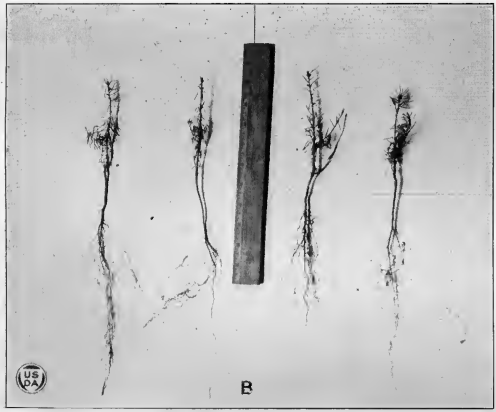
B

AN INFLUENCE OF MOISTURE ON BEAN WILT[1]

By LEWIS T. LEONARD[2]

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In 20 years' experience with the inoculation of legumes there have arisen only two noticeable failures in field work which could not be attributed to soil or climatic conditions or to the lack of efficiency on the part of the culture involved. The first case occurred with cowpea, evidenced by normal growth in the plants from untreated seed and sickly plants from the inoculated cowpeas. The other failure occurred in conjunction with the inoculation of navy bean, *Phaseolus vulgaris*, on the farm of the Office of Forage Crop Investigations at Redfield, S. Dak., in 1920 and was brought to my attention by Messrs. R. A. Oakley and H. L. Westover of that office. In the latter case many of the plants from beans which had been treated with a pure liquid culture, such as is distributed by the Department of Agriculture, died during the growing season, and the damage was sufficiently extensive to be rather noticeable in comparison with the rows of plants from the seed planted in the dry condition. It was estimated that 90 per cent of the crop was killed among the treated beans, whereas the loss was very slight among the plants which were uninoculated. A duplicate experiment with two different cultures, made on different pieces of ground at Redfield in 1921, gave an average loss of 25 per cent, due to treatment with liquid culture.

The possibility that the bean legume organism assumes a definite pathological rôle was considered, but this theory has been completely discarded since the publication of the work of Miss Florence Hedges,[3] who isolated and described an entirely different organism as the cause of this disease. The material used by Miss Hedges was obtained at Redfield, S. Dak.

The elimination of the possibility first mentioned led to the consideration of a second theory regarding the prevalence of the disease—that is, that some constituent of the medium employed to grow the culture of legume bacteria exercises a stimulating influence on the pathogenic organisms already in or on the seed. Miss Hedges found that the causative organism of the disease in question was generally present in the seeds which were used in the experiment.

EXPERIMENTAL PLAN

Attempts made to reproduce this diseased condition by the application of water and solutions containing the constituents of the media to beans which were planted on the Arlington Experimental Farm, Rossyln, Va., gave practically no definite results in one year, despite

[1] Accepted for publication Sept. 2, 1922.

[2] A large amount of credit for the successful carrying out of this experiment is due to Mr. H. L. Westover and Mr. Samuel Garver of the Office of Forage Crop Investigations.

[3] HEDGES, Florence. A BACTERIAL WILT OF THE BEAN CAUSED BY BACTERIUM FLACCUMFACIENS NOV. SP. *In* Science, n. s., v. 55, p. 433–434. 1922.

the fact that the seed and treatment were similar to those employed at Redfield, S. Dak. Results similarly inconclusive were obtained in 1922 at Rossyln, Va., from an experiment planned to parallel the one at Redfield the same year. It became necessary, therefore, to collect in South Dakota, entirely, the data concerning this work.

The plan of experiment was as follows: Allowance was made for duplicate rows of each treatment with each lot of beans and quadruplicate rows of untreated beans; rows were 3 feet apart and approximately 66 feet long; observations were made on nodule formation and the presence of disease.

Plan of Navy Bean Experiment at Redfield, S. Dak., in 1922

REDFIELD BEANS									
None	Water	Ashby Medium	Soil Medium	None	Redfield Soil	Farmogerm	407	162	342

REDFIELD BEANS									
None	Water	Ashby Medium	Soil Medium	None	Redfield Soil	Farmogerm	407	162	342

SELECTED BEANS									
342	162	407	Farmogerm	Redfield Soil	None	Soil Medium	Ashby Medium	Water	None

SELECTED BEANS									
342	162	407	Farmogerm	Redfield Soil	Ashby Medium	Water	None	Soil Medium	None

Two entirely different lots of beans were employed, consisting of the unsorted Redfield seed, the progeny of seed which gave such disastrous results in previous experiments, and a lot purchased from a seedsman of Washington, D. C., which was sorted with special care to remove such beans as gave evidence of harboring the bean wilt organism. Yellow spots on the beans, indicative of the disease, were more prevalent in the Redfield seed.

The two cultures of *B. radicicola*, bean strain, one of which was used in 1920, and both of which were tried in 1921, were supplemented by a commercial culture and one from Wisconsin.[4] All cultures were liquid except the commercial culture, which was agar, but for application to seed it was necessary to add water.

Since it was not known which of two media was used for growing the cultures employed in the 1920 and 1921 experiments, it was necessary to include both Ashby[5] broth and soil broth made from a modified formula of Löhnis[6] in the experiment as sterile control media. The latter medium, however, was used for the propagation of the cultures of the bean nodule organism. Soil from Redfield which had produced bean plants with nodules on their roots under greenhouse conditions comprised the source of soil inoculation.

[4] Kindly supplied by Dr. E. B. Fred, University of Wisconsin, Madison, Wis.

[5] ASHBY, S. F. SOME OBSERVATIONS ON THE ASSIMILATION OF ATMOSPHERIC NITROGEN BY A FREE LIVING SOIL ORGANISM.—AZOTOBACTER CHROOCOCCUM OF BEIJERINCK. *In* Jour. Agr. Sci., v. 2, p. 38. 1907.

[6] LÖHNIS, F. LABORATORY METHODS IN AGRICULTURAL BACTERIOLOGY. Tr. by Wm. Stevenson and J. H. Smith. xi, 136 p., 40 fig., 3 pl. 1913.

APPLICATION OF MATERIALS

The seed was divided into portions suitable for sowing an individual row. To each lot of seed was added its respective preparation in quantity sufficient to allow for the complete moistening of the beans. After they were treated they were spread out to dry; following this operation, they were planted. All seed used in the experiment was planted by hand on May 26, 1922, care being taken to cover the seed promptly to prevent the undue exposure of the inoculated seed to the rays of the sun. The soil which was practically bone-dry, was sowed at the approximate rate of 1,000 pounds per acre in the rows with the seed and covered promptly.

BASES OF OBSERVATIONS

Wilting symptoms (Pl. 1, B) were taken as the criteria for collecting disease data. Hand sections of the stems of the bean plants in the wilting stage invariably showed the presence of numerous bacteria in the tissues, and even in the healthy appearing tissues of these plants bacteria were noticeable. The wilting condition occurred either in the main stem or side shoot or in both (Pls. 1, A, B, and 2, A, B) and at practically all periods of growth. In connection with the figures reported in Table I it should be mentioned that dead plants were assumed to have been killed by the wilt disease organism. In a few cases partial recovery was noted, but only for a very short time. These data were collected on July 24 and July 25.

TABLE I.—*Influence of moisture on the bacterial wilt of navy beans in Redfield, S. Dak., soils*

Treatment.	Redfield seed.			Selected market seed.		
	Number of plants.	Diseased.	Totally diseased.	Number of plants.	Diseased.	Totally diseased.
		Per cent.	*Per cent.*		*Per cent.*	*Per cent.*
None [1]	933	2.0	0.7	1,042	2.9	0.1
Water	378	19.5	7.8	383	6.8	1.3
Ashby broth	365	22.0	10.3	475	5.6	.0
Lohnis broth	323	15.3	7.0	440	5.0	.6
Inoculated soil	481	7.0	3.4	507	2.8	.2
Commercial culture	398	17.0	9.0	465	9.5	1.6
407 Wisconsin	404	17.5	4.7	394	9.6	1.7
342 United States Department of Agriculture	324	34.5	10.0	384	7.5	1.0
162 United States Department of Agriculture	395	11.5	4.5	417	8.8	1.5

[1] The figures for this treatment represent the combined results from four rows of bean plants as indicated in the plan of experiment, whereas other data given represent only the average of two similar sized rows.

It is quite uniformly indicated that the plants from the Redfield beans were affected by the wilt to a greater extent than the selected market beans and that the application of moisture to the seed previous to planting is evidently the primary cause for the stimulation of the activities of the bean wilt organism.

Observations on the roots revealed that nodules were quite generally absent and that their occasional occurrence seemed to be more closely connected to the presence of moisture in the soil than to the application of inoculating material.

CONCLUSION

It is indicated that a slight application of moisture either in the form of water, certain culture media in the broth form or legume bacteria culture will cause a stimulation of the bean wilt disease under conditions such as obtain at Redfield, S. Dak.

If the application of legume bacteria culture to beans is necessary, it is indicated that satisfactory results may be obtained by adding this material in the form of naturally or artificially inoculated soil in a dry condition at the rate of not less than 300 pounds per acre. If only a small amount of soil is available, it may be mixed with the seed and sown with them.

This stimulation of disease organisms is apparently not produced with similar treatment under the humid conditions and soils of the Arlington Farm, Rosslyn, Va.

PLATE 1

A.—Plants in field showing symptoms of wilt beside a normal plant.
B.—A plant showing the early stages of complete destruction of life by the inroads of the wilt disease organism.


B
USDA


A
USDA


B


A

PLATE 2

A.—Plant in which disease has caused main shoot to wilt, but a side shoot is apparently unaffected.
B.—A completely destroyed bean plant which had formed pods.

43323—23——2

PLATE 3

A.—A part of a row of beans which were from seed planted dry.
B.—A part of a row of beans from seed which had been treated with Ashby broth. White cloths indicate the location of wilted specimens.


B


A

THE PSEUDO-ANTAGONISM OF SODIUM AND CALCIUM IN DILUTE SOLUTIONS[1]

By H. S. REED, *Professor of Plant Physiology*, and A. R. C. HAAS, *Assistant Professor of Plant Physiology, College of Agriculture, University of California*

The effects of calcium and of sodium ions upon living organisms are so diverse that many physiologists have advanced the idea that these ions antagonize each other at the surface of the cell. Osterhout (*6*) [2] has suggested that antagonism depends upon the production of a union of NaCl and $CaCl_2$ with some constituent of the protoplasm, and that the surface may become saturated with the antagonizing salts. Below the saturation point it is claimed that the relative proportions of the salts will be of less importance than their total concentration. In other words, it is considered that no distinctly favorable ratios exist in solutions of low concentration.

An attempt to demonstrate antagonism between Na and Ca in dilute solutions was made by Breazeale and Reed (*7*). The results of their inquiry showed an apparent antagonism existing in solutions containing 230 parts per million of total solutes. At any rate, wheat plants made somewhat better growth in ratios of $CaCl_2$ and NaCl in which Osterhout had found the greatest amount of antagonism than in other ratios.

The large amounts of calcium found by Kelley and Cummins (*5*) in citrus plants, suggested the suitability of these plants as material for the further study of this problem. In the first experiment rough-lemon (*Citrus limonia*) seedlings were grown in solutions of NaCl and $CaCl_2$ containing the following mixtures (the pure solutions each being 0.004M) 100 Na:0 Ca; 0 Na:100 Ca.

The distilled water used in experiments 1 and 2 was found to be calcium free as determined by evaporating 500 cc. of the water to a small volume and testing for calcium.

Liter glass jars, each containing six seedlings, were filled with culture solution on April 23. The seedlings were supported in paraffined-cork stoppers in such a way as to exclude foreign matter from the culture jars. The solutions were renewed on May 5, 13, 24, and June 1. The cultures were grown out of doors under half shade (in a lath room about 3 feet high with sides of cheesecloth) in a south exposure. On June 7 the experiment was concluded. The seedlings were momentarily dipped into and well rinsed in distilled water in order to remove adhering solution and also to remove dust from the leaves. In preliminary experiments it had been found that 0.033 gm. of dust had accumulated on 39 rough-lemon seedlings weighing 14.928 gm. green weight, 2.950 gm. dry weight, and 0.233 gm. ash.

The seedlings were freed from adhering moisture by means of filter paper, and the green weight was obtained. The seedlings were dried to constant weight at 70° C. and were then ignited in a porcelain dish at low heat. The salts in the ash were extracted with hot water and the residue

[1] Accepted for publication Sept. 2, 1922. Paper No. 97, University of California, Graduate School of Tropical Agriculture, and Citrus Experiment Station, Riverside, Calif.

[2] Reference is made by number (italic) to "Literature cited," p. 757.

and the filters were then returned to the dish, dried, and ignited. The filtrate was added to the dish, evaporated to dryness and heated at low heat to constant weight in order to obtain the weight of ash. By means of dilute HCl, practically all of the ash was brought into solution, the residue being inappreciable.

TABLE I.—*Rough-lemon seedlings grown 45 days in solution cultures*

Ratio of Na and Ca in solution.	Number of seedlings analyzed.	Composition calculated to a basis of 100 plants.					
		Green weight.	Dry weight.	Ash.	Ca.	K.	Na.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
100 Na:0 Ca.....	48	21.83	5.90	0.2781	0.0105	0.0515	0.0376
98 Na:2 Ca.....	48	26.16	6.83	.3739	.0335	.0587	.0420
0 Na:100 Ca.....	49	38.95	8.79	.5873	.1259	.0609	.0194

The results of the first experiment (Table I) show that better plants were obtained in cultures containing calcium; in fact, both green weight and dry weight increased where the amount of calcium was increased. In the pure NaCl solution the root tips appeared stunted and brown in color. The sodium content of the plants was slightly greater in cultures containing 98 Na:2 Ca than in pure NaCl solution. In this respect the results agree with those of Reed (7), though not in regard to the relative dry weight of plants produced.

Experiment 2 was begun April 27 and concluded June 7. The solutions were renewed at the same time as were those in experiment 1. Grapefruit (*Citrus grandis*) seedlings were used.

TABLE II.—*Grapefruit seedlings grown 42 days in solution cultures*

Ratio of Na and Ca in solution.	Number of seedlings analyzed.	Composition calculated to a basis of 100 plants.					
		Green weight.	Dry weight.	Ash.	Ca.	K.	Na.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
100 Na:0 Ca.....	29	36.29	8.34	0.4807	0.0124	0.0936	0.0547
98 Na:2 Ca.....	38	29.62	6.34	.4084	.0161	.0850	.0470
0 Na:100 Ca.....	42	36.20	7.11	.5195	.0646	.0948	.0235

From the above table it appears that the 98 Na:2 Ca ratio had no superiority over the 100 Na:0 Ca as measured by weight of plants produced. This apparent contradiction to the foregoing experiment may be due to the fact that many of the seedlings in the pure NaCl solution died and were removed. Such seedlings were discarded, for it has been shown by Johnson (4) that absorption of ions may differ appreciably in living and in dead cells. This left only the best plants in this lot; consequently we are dealing with what may be regarded as an inherently superior lot of individuals, while in the other cases we are dealing with the entire population, weak and strong.

In experiment 3, which was begun on May 4 and concluded on May 27, rough-lemon seedlings were grown in solutions of NaCl and $CaCl_2$. This

experiment was concluded early in order to avoid the possible killing of root tips. Even by so doing many of the seedlings in the solution having a ratio of 100 Na:0 Ca had dead root tips and were, therefore, discarded.

TABLE III.—*Rough-lemon seedlings grown 23 days in solution cultures*

Ratio of Na and Ca in solution.	Number of seedlings analyzed.	Composition calculated to a basis of 100 plants.				
		Green weight.	Dry weight.	Ash.	K.	Na.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
100 Na:0 Ca	28	23. 47	4. 27	0. 236	0. 0414	0. 0272
98 Na:2 Ca	40	26. 94	4. 64	. 273	. 0532	. 0225
85 Na:15 Ca	62	24. 56	4. 13	. 263	. 0446	. 0157

The results of this experiment are quite at variance with those of the preceding experiments. Here the differences in green weight and dry weight are so small as to be attributable to the inherent variability of the plants rather than to differences in the composition of the solutions.

Three experiments were conducted with the same mixtures as in experiment 2, using 60 seedlings for each ratio. African sour-orange (*Citrus aurantium*), grapefruit (*C. grandis*), and St. Michael orange (*C. sinensis*) seedlings were used, the solutions being renewed at the end of two weeks. After four weeks had elapsed the experiments were discarded because the root tips of the seedlings in the solution with the ratio 100 Na:0 Ca were dead.

On June 25 rough-lemon seedlings were placed in mixtures similar to those in experiment 3. On July 8 the solutions were renewed and on July 9 the experiment was discarded. In the solution having the ratio 100 Na:0 Ca the root of nearly every seedling had become slimy and gelatinous and was dead for a considerable distance back from the root tip. The roots of the seedlings in the solutions having the ratios 98 Na:2 Ca and 85 Na:15 Ca, respectively, were bright in appearance and although small had begun to develop lateral rootlets.

The above experiments indicate that citrus seedlings can not survive many days in a solution of 230 parts per million NaCl (0.004 M; 100 Na:0 Ca). One might be led to believe that antagonism has occurred with the ratio 98 Na:2 Ca, since the seedlings are found to grow when a trace of calcium is present, whereas, in the solution with a 100 Na:0 Ca ratio they are soon stunted and shortly afterward die. Experiment 4 indicates, however, that this is an instance of calcium starvation rather than of NaCl toxicity. It was found by analyses of culture solutions that citrus seedlings do not require a large concentration of calcium provided the supply is maintained. The usual methods of chemical analysis are not sufficiently delicate to detect significant absorption differences in the brief periods required by the conductivity method of studying permeability.

Experiment 4 was carried on with St. Michael orange seedlings, which were placed in the culture jars on September 4. Each set of cultures consisted of 12 jars with 3 seedlings per jar in the first set and 2 seedlings per jar in the remainder.

The first culture solution was that employed by Hoagland (*3*). The cultures were still growing on November 1. The second set of cultures

was grown in Hoagland's nutrient solution in which the calcium had been replaced by potassium; 500 cc. of this solution was evaporated to a small volume and was then tested and found to be calcium-free. Within 3 days after the experiment was begun, the root tips of the seedlings in the calcium-free cultures were becoming brown and failed to show any evidence of growth. After 7 to 10 days the root tips of most of the seedlings were becoming slimy and gelatinous. At the end of 2 weeks most of the roots appeared to be dead. The full amount of calcium of Hoagland's nutrient solution was then added in the form of calcium nitrate to six of the jars. Within a few days many of the seedlings began to recover, although several of the seedlings in advanced stages of decomposition failed to respond to the addition of the calcium nitrate.

In Plate 1, A, B, are shown various stages in the progressive killing of the primary root, beginning with the root tip and proceeding toward the cotyledons. Plate 1, B, shows some of the roots of Plate 1, A, somewhat enlarged. (The material closely held by the roots is composed of small pieces of sphagnum from the germination box.) It will be seen that when calcium was added to the solution, laterals were developed, the lowermost lateral always sharply separating the living from the dead portions of the primary root. Frequently in the absence of calcium the primary root was unable to grow. In such cases, when calcium was added, a lateral rootlet was produced immediately back of the root tip and this lateral then assumed the position of the primary root (Plate 1, A, B). One of the most characteristic effects of a lack of calcium is the gelatinization of the surface layers of the root. This process is most active in the apical region of the root and progressively decreases in intensity toward the upper portion. As time goes on the gelatinization proceeds inward until finally the root becomes translucent.

In the initial stages of this process of gelatinization, recovery is possible if a suitable amount of a calcium salt is added. The root then regains its firm white appearance and functions properly. In advanced stages of gelatinization recovery does not follow the addition of a calcium salt, although new laterals may be produced from the upper portion of the root. Herbst (*2*), Hansteen Cranner (*1*), and others have observed a similar gelatinization in other organisms in the absence of calcium and likewise have observed recovery when calcium salts were added.

There is thus evidence that the stunted appearance of roots in pure NaCl solutions above discussed is not due to the toxicity of NaCl but to the lack of calcium. Further study of this factor was made in which more concentrated solutions of NaCl, having toxic properties, were used. Seedlings placed in Hoagland's solution minus calcium failed to develop although it contained but 30 parts per million NaCl. They speedily recovered and grew when $CaCl_2$ was added. Another series in calcium-free Hoagland's solution plus 1,000 parts per million NaCl likewise failed to develop. When $CaCl_2$ was added to this latter solution the plants made good growth in spite of the amount of NaCl present.

It appears, therefore, that the harmful effect of 100 Na:0 Ca in dilute solutions is not due to the lack of ions which antagonize the sodium, but rather to calcium starvation, and that the result is conspicuous in the case of a plant like citrus which is extremely sensitive to calcium. In solutions containing only 0.004 M. NaCl, it seems improper to speak of a toxic action upon the roots. (Cf. True, *8*.)

In the study of the antagonism of Na and Ca with citrus seedlings it seems impossible to utilize solutions as dilute as those commonly employed as nutrients on account of the starvation which results when the calcium is reduced to extremely low concentrations. In other words, the assumption that no distinctly favorable ratios exist in solutions of low concentration is supported.

SUMMARY

Citrus seedlings grown in absence of calcium soon show injury to the root system, as evidenced by the gelatinization of the superficial layers and the ultimate death of the root. The tops, however, may not show the effect for some time after the injury to the roots has become severe.

If the injury has not progressed too far, the addition of calcium to cultures in which the roots are gelatinized induces the production of lateral rootlets the lowermost of which definitely delimits the dead from the living portion of the root.

The varieties of citrus here studied are known to possess a marked capacity for the absorption of calcium ions. Hence in very dilute solutions the amounts present may be too far below the equilibrium point within the plant to avoid a condition of starvation. In such cases we are dealing with the phenomenon of starvation rather than that of antagonism. These experiments with citrus seedlings have not demonstrated the existence of antagonism between sodium and calcium when the plants are grown in very dilute solutions.

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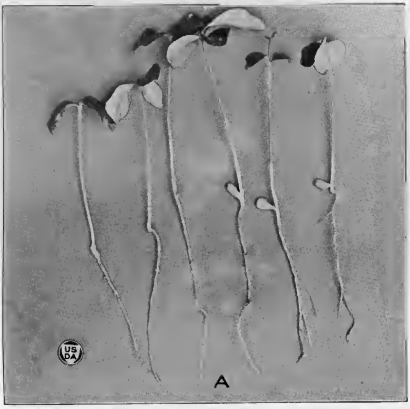
PLATE 1

A.—Orange seedlings grown in calcium-free Hoagland's solution. At the end of two weeks the full amount of calcium of Hoagland's solution was added in the form of calcium nitrate. The point at which recovery occurred is seen on each root.

B.—Showing the point of recovery of roots of orange seedlings (Pl. 1, A) when calcium is added to the solution after two weeks' exposure to calcium-free Hoagland's solution.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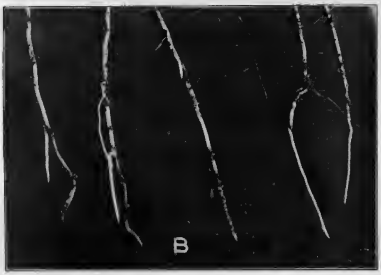
B

INFLUENCE OF THE HYDROGEN-ION CONCENTRATION ON THE GROWTH AND FIXATION OF NITROGEN BY CULTURES OF AZOTOBACTER [1]

By P. L. GAINEY, *Professor of Bacteriology*, and H. W. BATCHELOR, *Laboratory Assistant in Bacteriology, Kansas Agricultural Experiment Station*

Investigations in this laboratory have shown that the presence or absence of Azotobacter in natural soils is very closely associated with, if not dependent upon, the absolute reaction of the soil solution (*4*).[2] It has also been shown that in the laboratory the presence of this group of organisms in a soil can be controlled by varying the hydrogen-ion concentration of the soil (*5*).

In connection with these investigations it seemed desirable to study the influence of the hydrogen-ion concentration of laboratory culture media upon the growth and nitrogen fixing ability of pure cultures of Azotobacter.[3]

In their original investigations on Azotobacter, Beijerinck and Van Delden (*1*) failed to secure appreciable fixation of nitrogen by pure cultures. On the other hand, Lipman (*6*) experienced no difficulty in securing marked fixation by pure cultures, and he presents data to show that the ability of pure cultures to fix nitrogen in the media usually employed for their culture depended upon the neutralization of the acidity arising from the potassium phosphate present. Lipman further demonstrated that, quantitatively, the nitrogen fixed by pure cultures was inversely proportional to the titratable acidity of the media, and called attention to the probability that Beijerinck's failure to secure nitrogen fixation was due to the unfavorable reaction of the medium employed. Since Lipman's work appeared, it has been universally accepted that these organizations will not function in a high concentration of acid.

With regard to the effect of different degrees of acidity, or hydrogen-ion concentrations, upon the growth of pure cultures of this group of organisms little information has been published. Fred (*3*) observed the growth of two different cultures in a medium of varying hydrogen-ion concentrations and noted no growth at P_H 6.4 to 6.6, while growth occurred at P_H 6.6 to 6.8. On the other hand, when grown in a medium of P_H 7.2 the final hydrogen-ion concentration was found to be P_H 5.1. As already mentioned, previous investigations in this laboratory indicated that the hydrogen-ion concentration of the soil solution is the major factor in controlling the presence of this group of organisms in soils. Also, that the maximum concentration of hydrogen ions tolerated by this group of

[1] Accepted for publication Oct. 16, 1922. Contribution No. 50, Department of Bacteriology, Kansas Agriculture Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 767.

[3] The term "pure culture" is here used in a restricted sense. Owing to the extreme morphological variations exhibited by this group of organisms it is frequently very difficult to ascertain with certainty the purity of a given culture. All cultures used in these experiments conformed to the description given in the text of this paper.

organisms appear to be very close to P_H 5.9 to 6.0. It seemed of interest, therefore, to ascertain the effect of varying the hydrogen-ion concentration of laboratory media upon the activity of a number of strains of Azotobacter.

METHODS EMPLOYED

Crude cultures of Azotobacter were prepared from a number of soils by inoculating a mannite or dextrose cultural solution and incubating until a characteristic Azotobacter film developed. Portions of these films were then streaked upon mannite or dextrose agar and the streaking process repeated from isolated colonies until all colonies developing were similar and yielded, when stained, only typical Azotobacter cells. No effort was made to identify the various cultures.

The medium employed had the following composition: Monobasic potassium phosphate, 0.2 or 5.0 gm.; magnesium sulphate, 0.2 gm.; sodium chlorid, 0.5 gm.; ferric chlorid, 2 drops of a 10 per cent solution; dextrose or mannite, 20.0 gm.; distilled water 1,000 cc. Flasks of this medium, slightly alkaline to brom-thymol-blue, were inoculated from an agar streak culture and aerated vigorously at 28° C. until heavily clouded (two to four days). The cultures were again stained and examined, and all flasks showing morphological forms other than typical Azotobacter were discarded. One to five per cent of such a culture was used as an inoculum.

The quantity of sterile media necessary to set up an experiment was prepared and inoculated. Fifty cubic centimeter quantities were measured aseptically into carefully washed sterile 300 cc. Erlenmeyer flasks. The quantity of NaOH or HCl necessary to adjust the 50 cc. of media to approximately the desired reaction was determined and this quantity, previously sterilized, was carefully added to four flasks. The hydrogen-ion concentration of the contents of one of these flasks was immediately determined and recorded as the initial reaction. Hydrogen-ion determinations were made colorimetrically, a control being occasionally run electrometrically. A small quantity of formaldehyde was added to a second flask to act as a control with which to compare growth and nitrogen fixation. The remaining two cultures were incubated at 28° C. for two weeks. The quantity of acid or alkali required to produce a given change in reaction varied only slightly from time to time.

In the earlier experiments, where only 0.02 per cent KH_2PO_4 was used, the buffer index was very low and necessitated the use of very dilute (N/80) NaOH in adjusting the reaction. Where 0.5 per cent KH_2PO_4 was used (experiments 16 to 20 inclusive) the buffer index was much higher, requiring approximately ten times as much acid or alkali to produce a given change in reaction, and N/10 NaOH was used to correct the reaction.

During incubation the cultures were examined at frequent intervals and the presence or absence of growth recorded. The formaldehyde produced a slight change in the physical appearance of the medium in the control flasks and rendered it somewhat difficult to detect very slight changes in the appearance of cultures. Where it was impossible to determine definitely whether growth had taken place it has been indicated in Tables I and IV with a question mark.

After two weeks' incubation a final examination for growth was made, the hydrogen-ion concentration of the cultures recorded, and the total nitrogen present determined by the Kjeldahl method. The quantity of

nitrogen in the control flasks and in the cultures where no growth was visible was very small and, as will be observed in Table II, the experimental error for the controls was large. In examining the data relative to the quantity of nitrogen fixed this experimental error must be taken into consideration, otherwise erroneous conclusions are likely to be drawn. In the data recorded in Tables II and IV the quantity of nitrogen present in controls has been deducted from that present in the inoculated flasks and only the net gain recorded. Nitrogen determinations were made on at least four control flasks to determine the probable error where only small quantities of nitrogen were present.

INFLUENCE OF REACTION UPON GROWTH

In Table I data are recorded relative to the influence of the hydrogen-ion concentration of the culture medium upon the growth of several strains of Azotobacter. A study of these data will show that the maximum acidity permitting appreciable growth to take place is very close to P_H 5.9. There is only one instance where definite growth is recorded in a higher degree of acidity (experiment 15, P_H 5.8) and the duplicate of this culture failed to show growth. Another strain, 12–323–6, not cultured at P_H 5.9 in the experiment here recorded grew at P_H 5.9 in an unrecorded experiment. All cultures showed some growth at P_H 5.9–6.0. The amount of growth that took place at P_H 5.9 was in most instances very slight, and growth increased as the acidity decreased until P_H 6.1 to 6.3 was reached. At this reaction growth appeared in most instances to be as vigorous as at lower hydrogen-ion concentrations. Cultures 407–5 and 449 did not grow as abundantly as did the other cultures, even when the reaction was favorable. These two cultures failed to produce a surface film, and when growth is compared with nitrogen fixation it will be noted that they also fixed small quantities of nitrogen.

TABLE I.—*The influence of the hydrogen-ion concentration of culture media upon the growth of Azotobacter*

Experiment No.	Culture.	Initial reaction expressed as P_H (growth records for 2 weeks).																		
		Below 5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.2	Above 7.6	Ca CO_3
6	426–21	−	−	.....	−	−	+	++	++++	++++	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
		−	−	.....	−	−	+	++	++++	++++	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	C	−	.....	−	.....	−	.....	+	++	.....	.....	++++	++++	.....	.....	.....	.....	.....	.....	.....
		−	.....	−	.....	−	.....	+	++	.....	.....	++++	++++	.....	.....	.....	.....	.....	.....	.....
9	19–399	.....	−	−	.....	.....	−	.....	.....	+	++++	.....	.....	++++	.....	.....	.....	.....	.....	.....
		.....	−	.....	.....	.....	+	.....	.....	+	++++	.....	.....	++++	.....	.....	.....	.....	.....	.....
11	426–21	.....	.....	−	.....	.....	.....	+	+	.....	.....	++++	++++	++++	.....	++++	++++	.....	.....	.....
		.....	.....	?	.....	.....	+	+	++++	.....	.....	++++	++++	++++	.....	++++	++++	++++	.....	.....
12	40–399	−	.....	?	.....	.....	+	++	.....	.....	++++	.....	++++	.....	.....	.....	.....	++++	++++	.....
		−	.....	?	.....	.....	+	++	.....	.....	++++	.....	++++	.....	.....	.....	.....	++++	++++	.....
13	12–323–6	−	.....	−	.....	.....	.....	+	.....	++	++++	.....	.....	++++	.....	.....	.....	++++	++++	.....
		−	.....	?	.....	.....	.....	+	.....	++	++++	.....	.....	++++	.....	.....	.....	++++	++++	.....
14	C	.....	.....	?	?	.....	+	+	+	.....	.....	.....	++++	.....	.....	.....	.....	++++	++++	.....
		.....	.....	?	?	.....	+	+	+	.....	.....	.....	++++	.....	.....	.....	.....	++++	++++	.....
15	40–399	.....	−	−	−	−	+	++	++	.....	.....	.....	++	.....	++	++++	.....	.....	.....	++++
		.....	−	−	−	+	++	++	++	.....	.....	.....	++++	.....	++	.....	.....	.....	.....	.....
16	407–5	−	.....	−	−	−	?	+	.....	.....	.....	+	+	.....	.....	.....	.....	.....	.....	.....
		−	.....	?	−	−	?	+	.....	.....	.....	+	+	.....	.....	.....	.....	.....	.....	.....
17	14–399–6	−	.....	.....	?	.....	+	.....	+	.....	++++	.....	++++	.....	.....	++++	.....	.....	.....	++++
		−	.....	.....	?	.....	+	.....	+	.....	++++	.....	++++	.....	.....	++++	.....	.....	.....	++++
18	E	−	.....	.....	−	.....	+	++	++	++++	++++	.....	.....	.....	.....	++++	.....	.....	.....	.....
		−	.....	.....	−	.....	+	++	++	++++	++++	.....	.....	.....	.....	++++	.....	.....	.....	.....
19	407–5	−	.....	.....	.....	−	−	.....	?	.....	+	++	.....	.....	.....	++	.....	.....	.....	.....
		−	.....	.....	.....	−	+	.....	?	.....	+	++	.....	.....	.....	++	.....	.....	.....	.....
20	499	−	−	.....	−	.....	−	+	+	+	.....	.....	.....	.....	.....	++	.....	.....	.....	++++
		−	−	.....	−	.....	?	?	+	+	.....	.....	.....	.....	.....	++	.....	.....	.....	++++

− No visible growth.
? Questionable growth.
+ Slight but distinct growth.
++ Good growth, but no film.
++++ Heavy growth and film formation.

INFLUENCE OF REACTION UPON NITROGEN FIXATION

Data relative to the influence of the hydrogen-ion concentration of the culture medium upon the fixation of nitrogen by cultures of Azotobacter are recorded in Table II. In examining these data the rather large experimental error for the controls of the individual experiments should be noted. In no instance is there recorded a definite fixation of nitrogen in a hydrogen-ion concentration greater than P_H 5.9. The data for P_H 5.9, 6.0, and 6.1 are inconclusive, the quantities of nitrogen fixed, if any, being small. At P_H 6.2 and above definite fixation occurred, the maximum in most experiments being recorded at P_H 6.3 to 6.5. Vigorous fixation of nitrogen appeared to be associated with the growth of a definite film on the surface of the medium.

TABLE II.—*The influence of the hydrogen-ion concentration of culture media upon the fixation of nitrogen by Azotobacter*

Experiment No.	Culture.	Initial reaction expressed as P_H. nitrogen fixed per culture of 50 cc. media (mgm.).																	
		Below 5.4.	5.5.	5.6.	5.7.	5.8.	5.9.	6.0.	6.1.	6.2.	6.3.	6.4.	6.5.	6.6.	6.8.	6.9.	7.2.	Above 7.6.	$CaCO_3$.	Probable error controls.
11	426–21	.....	.....	0.08	.....	.....	.....	0.34	0.17	.....	.....	4.12	2.94	4.81	.....	5.18	4.84	.....	.....	±0.51
		.....	.....	0.00	.....	.....	0.43	0.00	1.81	.....	.....	4.75	2.85	5.44	.....	5.27	.....	.....	.....	±0.51
12	40–399	0.00	.....	0.00	.....	.....	0.83	1.11	.....	.....	3.79	.....	3.70	.....	.....	.....	.....	3.97	.....	±0.95
		0.37	.....	0.74	.....	.....	0.00	0.00	.....	.....	4.06	.....	4.62	.....	.....	.....	.....	3.98	.....	±0.95
13	12–232–6	0.00	.....	0.18	.....	.....	.....	1.02	.....	1.20	2.14	.....	.....	4.43	.....	.....	.....	4.62	.....	±0.46
		0.00	.....	0.00	.....	.....	.....	0.18	.....	1.48	4.43	.....	.....	3.79	.....	.....	.....	4.53	.....	±0.46
14	C	.....	.....	0.51	0.35	.....	1.35	0.51	1.16	.....	.....	.....	5.31	.....	.....	.....	.....	5.59	.....	±1.01
		.....	.....	0.32	0.69	.....	0.60	0.60	0.10	.....	.....	.....	5.78	.....	.....	.....	.....	3.66	.....	±1.01
16	407–5	0.00	.....	0.28	0.19	0.47	.....	0.47	.....	.....	.....	0.85	2.48	.....	.....	.....	.....	.....	.....	±0.45
		0.28	.....	0.19	0.09	0.09	.....	0.57	.....	.....	.....	0.66	.....	.....	.....	.....	.....	.....	.....	±0.45
17	14–399–6	0.00	.....	.....	0.21	.....	0.31	.....	0.62	.....	0.93	.....	4.64	.....	5.37	.....	.....	.....	5.59	±0.43
		0.10	.....	.....	0.82	.....	0.21	.....	0.41	.....	0.72	.....	5.06	.....	4.75	.....	.....	.....	4.73	±0.43
18	E	0.00	.....	.....	0.17	.....	0.17	1.12	0.41	1.43	1.12	.....	.....	.....	1.84	.....	.....	.....	.....	±0.77
		0.00	.....	.....	loss	.....	0.20	1.63	0.92	1.22	1.43	.....	.....	.....	1.63	.....	.....	.....	.....	±0.77
19	407–5	0.00	.....	.....	.....	0.00	0.00	.....	0.32	.....	0.22	0.65	.....	.....	2.38	.....	.....	.....	.....	±0.05
		0.14	.....	.....	.....	0.00	0.47	.....	0.00	.....	0.54	0.76	.....	.....	1.73	.....	.....	.....	.....	±0.05
20	499	0.56	0.00	.....	0.00	.....	0.00	0.10	1.07	0.73	.....	.....	.....	.....	1.50	.....	.....	.....	4.28	±0.64
		0.31	0.21	.....	0.21	.....	0.63	1.58	1.26	1.89	.....	.....	.....	.....	3.78	.....	.....	.....	4.55	±0.64
Average....		0.12	0.11	0.23	0.27	0.14	0.40	0.66	0.77	1.33	1.94	1.97	4.15	4.62	2.87	5.22	4.84	4.39	4.79	±0.27

CHANGES IN REACTION PRODUCED BY GROWTH

The initial and final reactions of the cultures are recorded in Table III. The medium used in all experiments below 16 contained only 0.02 per cent KH_2PO_4 and possessed a very low buffer index. The quantity of N/1 base or acid necessary to produce a change of 0.1 P_H in 100 cc. of this medium was only approximately 0.006 cc. In these experiments some rather marked changes in reaction were recorded. The changes sometimes indicated the production of acid, at other times the production of basic compounds. However, when calculated in terms of total acid or base the quantities are insignificant.

TABLE III.—*The influence of the growth of Azotobacter in culture media upon the reaction of the media*

Experiment No.	Culture.	Initial reaction expressed as P_H, after 2 weeks' growth.																	
		Below 5.4.	5.5.	5.6.	5.7.	5.8.	5.9.	6.0.	6.1.	6.2.	6.3.	6.4.	6.5.	6.6.	6.7.	6.8.	6.9.	7.2.	Above 7.6.	Per cent KH_2PO_4.
6	426–21	*Less than*- 5.4—	5.4—	.....	5.6	5.8	5.8	5.9	6.1	6.0	.....	5.8	.....	.....	.....	.....	.....	.....	.....	[a] 0.02
		5.4—	5.5	.....	5.7	5.8	5.9	6.0	6.1	6.2	.....	6.4	.....	.....	.....	.....	.....	.....	.....	.02
7	12–232–6	.....	5.4	.....	5.8	.....	5.9	.....	.....	.....	6.1	.....	6.1	.....	6.8	.....	.....	.....	.....	.02
		.....	5.4	.....	5.8	.....	5.9	.....	.....	.....	6.1	.....	6.3	.....	6.8	.....	.....	.....	.....	.02
8	C	.....	.....	.....	.....	.....	.....	5.8	6.3	.....	.....	6.3	6.6	.....	.....	.....	.....	.....	.....	.02
		.....	.....	.....	.....	.....	.....	6.0	5.9	.....	.....	6.4	6.6	.....	.....	.....	.....	.....	.....	.02
1	426–21	.....	.....	5.4—	.....	.....	5.4—	5.4—	5.4—	.....	.....	6.7	5.4—	7.3	.....	.....	7.3	7.4	.....	.02
		.....	.....	5.4—	.....	.....	5.6	5.4—	5.5	.....	.....	6.7	6.4	7.3	.....	.....	7.5	.....	.....	.02
12	40–399	5.4—	.....	5.7	.....	.....	5.7	5.8	.....	.....	6.5	.....	5.6	.....	.....	.....	.....	.....	5.7	.02
		5.6	.....	5.7	.....	.....	5.7	5.7	.....	.....	6.5	.....	6.4	.....	.....	.....	.....	.....	5.9	.02
13	12–232–6	5.4—	.....	5.5	.....	.....	.....	5.4—	.....	5.4—	6.4—	.....	.....	7.3	.....	.....	.....	.....	7.5	.02
		5.4—	.....	5.4	.....	.....	.....	5.4	.....	5.4—	5.4—	.....	.....	6.5	.....	.....	.....	.....	7.2	.02
14	C	.....	.....	5.4	5.4	.....	5.4	5.7	5.8	.....	.....	.....	6.8	.....	.....	.....	.....	.....	7.0	.02
		.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6.7	.....	.....	.....	.....	.....	7.2	.02
16	407–5	5.4—	.....	5.8	5.8	.....	5.9	6.1	.....	.....	.....	6.4	6.4	.....	.....	.....	.....	.....	.....	[b] 0.50
		5.4—	.....	5.7	5.9	.....	5.9	6.1	.....	.....	.....	6.4	6.5	.....	.....	.....	.....	.....	.....	.50
17	14–399–6	5.4—	.....	.....	5.6	.....	5.9	.....	6.1	.....	6.3	.....	6.7	.....	.....	6.7	.....	.....	.....	.50
		5.4—	.....	.....	5.8	.....	5.8	.....	6.0	.....	6.3	.....	6.6	.....	.....	6.7	.....	.....	.....	.50
18	E	5.4—	.....	.....	5.8	.....	5.9	6.4	6.1	6.1	6.3	.....	.....	.....	.....	6.6	.....	.....	.....	.50
		5.4—	.....	.....	5.6	.....	5.9	6.4	6.1	6.2	6.2	.....	.....	.....	.....	6.6	.....	.....	.....	.50
19	407–5	5.4—	.....	.....	.....	5.6	5.8	.....	6.0	.....	6.4	6.4	.....	.....	.....	6.8	.....	.....	.....	.50
		5.4—	.....	.....	.....	5.8	5.8	.....	6.0	.....	6.1	6.4	.....	.....	.....	6.8	.....	.....	.....	.50
20	449	5.4—	5.4—	.....	5.4—	.....	5.6	5.9	6.0	6.1	.....	.....	.....	.....	.....	6.8	.....	.....	.....	.50
		5.4—	5.4—	.....	5.7	.....	5.8	5.9	6.1	5.4—	.....	.....	.....	.....	.....	6.8	.....	.....	.....	.50

[a] Media containing 0.02 per cent KH_2PO_4 required only 0.006 cc. N/1 NaOH per 100 cc. to change the P_H 0.1.
[b] Media containing 0.50 per cent KH_2PO_4 required 0.05 cc. N/1 NaOH per 100 cc. to change the P_H 0.1.

These results led to the conclusion that no satisfactory data as to changes in reaction could be secured in a medium so poorly buffered. The quantity of KH_2PO_4 was accordingly increased to 0.5 per cent. Higher concentrations of phosphate were sometimes found to be toxic. This increase in phosphate increased the buffer effect approximately ten times, the media containing 0.5 per cent requiring 0.05 cc. N/1 NaOH to effect a change of 0.1 P_H in 100 cc. The buffer effect was still low, but an examination of experiments 16 to 20 will show that changes in P_H produced by the growth of the various cultures were practically eliminated. If acid or basic metabolic by-products are produced under these experimental conditions the quantities are inappreciable.

Fred (*3*) recorded a change in P_H from 7.2 to 5.1. The media employed by him, however, contained only 0.02 per cent phosphate, and the actual quantity of acid necessary to produce the recorded change in P_H was probably very small. Stoklasa (7) recorded the production of as high as 3.3 cc. N/1 acid per 100 cc. media. This quantity would have produced very marked changes in the P_H of our media. However, the purity of Stoklasa's cultures has been questioned by Bonazzi (*2*).

In Table IV are recorded in parallel columns the initial reaction, growth, final reaction, and milligrams of nitrogen fixed for four experiments. This table is included in order that these various factors may be compared one with the other without the necessity of examining several tables. In figure 1 the influence of the reaction on nitrogen fixation is shown graphically.

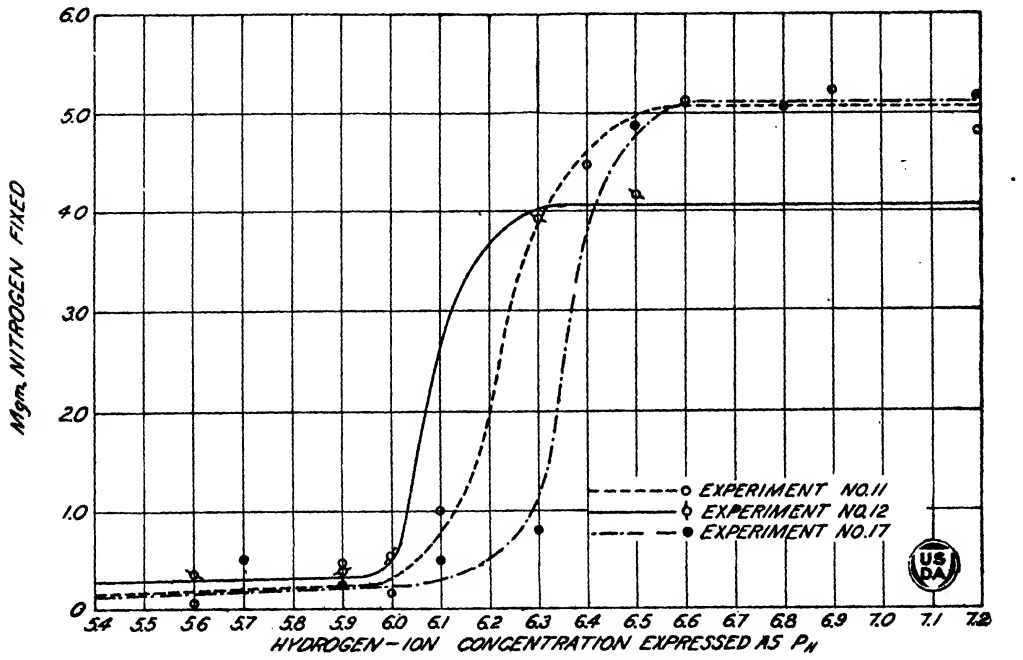

FIG. 1.—Effect of hydrogen-ion concentration on nitrogen fixation.

TABLE IV.—*Comparison of initial reaction, growth, final reaction, and nitrogen fixed by four cultures of Azotobacter*

Experiment 11, Culture 426–21.				Experiment 12, Culture 40–399.				Experiment 17, Culture 14–399–6.				Experiment 18, Culture E.			
Initial P_H.	Growth.	Final P_H.	Nitrogen fixed.	Initial P_H.	Growth.	Final P_H.	Nitrogen fixed.	Initial P_H.	Growth.	Final P_H.	Nitrogen fixed.	Initial P_H.	Growth.	Final P_H.	Nitrogen fixed.
			Mgm.				*Mgm.*				*Mgm.*				*Mgm.*
5.6	—	5.4—	0.08	5.4—	—	5.4—	0.00	5.4—	—	5.4—	0.00	5.4—	—	5.4—	0.00
5.6	?	5.4—	.00	5.4—	—	5.6	.37	5.4—	—	5.4—	.10	5.4—	—	5.4—	.00
5.9	.....	.....	.....	5.6	?	5.7	.00	5.7	?	5.6	.21	5.7	—	5.8	.17
5.9	?	5.6	.43	5.6	?	5.7	.74	5.7	?	5.8	.82	5.7	—	5.6	.00
6.0	?	5.4—	.43	5.9	+	5.7	.83	5.9	+	5.9	.31	5.9	+	5.9	.17
6.0	?	5.4—	.00	5.9	+	5.7	.00	5.9	+	5.8	.21	5.9	+	5.9	.20
6.1	?	5.4—	.17	6.0	++	5.8	1.11	6.1	+	6.1	.62	6.0	++	6.4	1.12
6.1	++++	5.5	1.81	6.0	++	5.7	.00	6.1	+	6.0	.41	6.0	++	6.4	1.63
6.4	++++	6.7	4.12	6.3	++++	6.5	3.79	6.3	++++	6.3	.93	6.1	++	6.1	.41
6.4	++++	6.7	4.75	6.3	++++	6.5	4.06	6.3	++++	6.3	.78	6.1	++	6.1	.92
6.5	++++	5.4—	2.94	6.5	++++	5.6	3.70	6.5	++++	6.7	4.64	6.2	++++	6.1	1.43
6.5	++++	6.4	2.85	6.5	++++	6.4	4.62	6.5	++++	6.6	5.06	6.2	++++	6.2	1.22
6.6	++++	7.3	4.81	7.6	++++	5.7	3.97	6.8	++++	6.7	5.37	6.3	++++	6.3	1.12
6.6	++++	7.3	5.44	7.6	++++	5.9	3.98	6.8	++++	6.7	4.75	6.3	++++	6.2	1.43
6.9	++++	7.3	5.18	.....	.....	.....	.....	$CaCO_3$	++++	.....	5.59	6.8	++++	6.6	1.84
6.9	++++	7.5	5.27	.....	.....	.....	.....	$CaCO_3$	++++	.....	4.73	6.8	++++	6.6	1.63
7.2	++++	7.4	4.84	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

— No visible growth.
? Growth indefinite.
+ Slight but distinct growth.
++ Good growth with no film formation.
++++ Heavy growth with film formation.

SUMMARY

The data presented in this paper point very definitely to a limiting hydrogen-ion concentration of P_H 5.9 to 6.0 for the various cultures of Azotobacter employed when grown under the conditions of these experiments. Vigorous growth and nitrogen fixation took place at P_H 6.1 to 6.5, the optimum P_H for nitrogen fixation apparently being somewhat higher than the optimum for growth. Very slight, if any, changes in the reaction of the media are produced by the growth of the various strains of Azotobacter studied, indicating the production of inappreciable quantities of acid or basic metabolic by-products.

The data for pure cultures here presented agree very closely with our findings in soil and tend to substantiate former conclusions that this group of organisms will not exist and function in soils, the hydrogen-ion concentration of which is greater than P_H 5.9 to 6.0.

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SUNFLOWER INVESTIGATIONS[1]

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INTRODUCTION

The value of sunflowers for a silage crop in the Pacific Northwest has become quite well established. Their resistance to drought and early frosts, and the fact that they yield a large tonnage of green material per acre, justify a more complete knowledge of the proper spacing of plants in the row and the best stage of cutting the plant for silage purposes. With these facts in mind, an investigation was planned which would yield data on the proper method of planting and the proper time of harvesting.

The composition of the sunflower plants was first studied when harvested at various stages during growth, and when grown under two different spacings, namely, 4 to 8 inches, and 36 inches, apart in the row. The sunflower plants grown under the two systems of planting were compared for the percentages of leaves, stalks, stems, and flowers, and analyses were made of composite samples of each of these plant parts. Not only was the composition of the sunflowers determined in these two systems of planting and at various stages of maturity, but also the composition of the silage made from the sunflowers cut at each of these stages of maturity and from each of the two different spacings of plants in the row.

PREVIOUS WORK

Shaw and Wright,[2] of the United States Department of Agriculture, published data on the composition of sunflowers grown at the Dairy Division Experiment Farm at Beltsville, Md. Their investigation included the composition of sunflowers cut at seven different stages of maturity.

Blish,[3] of the Montana Experiment Station, has recently published data on the effect on the composition and quality of silage of cutting sunflowers at different stages of growth. All results showed that silage of good quality resulted from sunflowers cut at the different stages of maturity selected by him. The problems, then, that present themselves in Idaho are: First, what is the most favorable stage of maturity for harvesting the sunflowers for silage purposes, and, second, what is the proper distance apart of planting in order that the maximum feeding value may be secured?

EXPERIMENTAL WORK

The sunflowers used in this investigation were grown at the Idaho State Experiment Station at Moscow, Idaho, in the summer of 1920, by officials of the agronomy department, who kindly allowed us to sample the

[1] Accepted for publication Oct. 16, 1922.

[2] SHAW, R. H., and WRIGHT, P. A. A COMPARATIVE STUDY OF THE COMPOSITION OF THE SUNFLOWER AND CORN PLANTS AT DIFFERENT STAGES OF GROWTH. *In* Jour. Agr. Research, v. 20, p. 787–793. 1921. Literature cited, p. 792–793.

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sunflowers at all stages of maturity. Additional work on the 1921 crop of sunflowers is included later in this paper.

The plants were grown from seed of the Giant Russian variety on Palouse silt loam soil. The plants grew luxuriantly, many of the largest of them reaching a height of from 10 to 12 feet.

STAGES OF MATURITY SELECTED

In selecting the proper stages at which to sample the sunflowers considerable difficulty was encountered in choosing definite stages of growth, since the sunflower plant does not offer any definite and sharply defined differences at any time during its growth. After due consideration, the stages were arbitrarily chosen in terms of budding and flowering in the early period of growth and, later on, of the degree of hardness of the seeds in the main or top flower. Five stages were selected, as follows: First, when the first bud was appearing on the top of the plant; second, when the first flower was about 3 inches in diameter but no seed had developed; third, just before the seeds of the first flower were in the dough stage; fourth, when the seeds of the first flower were well into the dough stage and the rays were just beginning to fall; fifth, when the seeds of the first flower were quite hard and its rays had fallen.

METHOD OF SAMPLING

Representative samples were secured by collecting a number of plants of uniform development and weighing and measuring each plant; the average was then recorded. Composite samples of sufficient size were made by cutting in a small silage cutter the constituent plants and then mixing thoroughly. A portion of each sample was dried in an electric oven to secure the percentage of moisture. A second portion was air dried and reserved for the approximate analysis. The remaining portion was used for making silage. For this purpose quart milk bottles were tightly filled and each stoppered with a rubber stopper containing a bent glass tube which had its outlet in a beaker of mercury. This arrangement allowed the fermentation gases to escape but prevented air from gaining access to the silage. This procedure was found to be very satisfactory and in every case resulted in a good quality of silage.

DIFFERENT SPACINGS OF PLANTS

In the early stages of growth, when the plants were about 6 inches high, the plants were thinned in order to secure data on the yield and composition of sunflowers when grown at various distances apart in the row.

In order to secure chemical data, two distances apart were chosen, which represented as nearly as possible the extremes of planting and also the two extreme types of sunflowers.[4] The two types selected were plants grown 36 inches apart and plants grown from 4 to 8 inches apart. The former system resulted in plants with an abundance of leaves and flowers on one large stalk, while the latter resulted in rather tall, spindly stalks with much smaller leaves and flowers. The data on the composition of sunflowers and sunflower silage are given in Table I.

[4] The spacings of plants intermediate between 4 inches to 8 inches and 36 inches were not included in this investigation, as the analytical work would have been enormously increased, and it was not certain that the results would show differences sufficient to warrant the additional analyses.

TABLE I.—*Composition of sunflowers and sunflower silage (1920)*

Stage of growth.	Average height.		Average weight.		Distance apart in rows.	Moisture.	Anhydrous material.	Crude protein.	Ether extracted.	Crude fiber.	Nitrogen-free extract.	Ash.	Type of material analyzed.
	Ft.	*in.*	*Lbs.*	*oz.*	*Inches.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Budding	8	10	13	2	36	84.45	15.55	15.82	1.95	24.07	45.80	12.36	Original.
Do	8	10	13	2	36	86.00	14.00	18.96	1.68	21.55	43.88	13.93	Silage.
Do	9	2	3	13	4 to 8	84.25	15.75	8.82	2.01	26.68	50.37	12.12	Original.
Do	9	2	3	13	4 to 8	86.35	13.65	11.56	1.32	23.99	48.73	14.40	Silage.
First flower in blossom	9	10	9	9	36	84.15	15.85	12.20	2.39	20.25	52.40	12.76	Original.
Do	9	10	9	9	36	85.00	15.00	15.93	3.13	18.32	48.47	14.15	Silage.
Do	8	0	1	8	4 to 8	79.75	20.25	7.13	2.13	28.89	51.16	10.69	Original.
Do	8	0	1	8	4 to 8	83.00	17.00	12.12	3.68	24.79	46.00	13.41	Silage.
Seeds not quite in dough stage	9	3	9	6	36	81.70	18.30	12.50	3.04	29.25	43.47	11.74	Original.
Do	9	3	9	6	36	83.50	16.50	14.62	1.65	27.57	42.46	13.70	Silage.
Do	7	7	2	3	4 to 8	79.00	21.00	10.25	2.61	24.95	50.50	11.69	Original.
Do	7	7	2	3	4 to 8	82.70	17.30	12.86	2.39	24.57	47.00	13.18	Silage.
First flower seeds well in dough stage.	9	0	10	1	36	79.85	20.15	10.25	2.18	20.66	57.05	9.86	Original.
Do	9	0	10	1	36	81.72	18.28	11.56	3.88	21.46	52.37	10.73	Silage.
Do	10	3	6	0	4 to 8	83.00	17.00	9.76	4.04	27.60	46.63	11.97	Original.
Do	10	3	6	0	4 to 8	84.00	15.90	11.06	5.14	22.10	48.75	12.95	Silage.
First flower seeds well beyond dough stage.	7	6	10	3	36	80.35	19.65	12 38	5.78	20.37	48.47	13.00	Original.
Do	7	6	10	3	36	80.60	19.40	13.63	5.35	23.45	42.49	15.08	Silage.
Do	8	10	6	0	4 to 8	81.80	18.20	11.75	1.68	24.84	49.83	11.90	Original.
Do	8	10	6	0	4 to 8	83.96	16.04	12.75	3.70	21.90	48.36	13.29	Silage.

DISCUSSION OF THE COMPOSITION OF SUNFLOWERS

In studying the results on the five stages of maturity, consideration must be given to the general trend of results rather than to slight differences which may occur at the different stages of growth, for the reason that it is extremely difficult to secure uniform samples. All percentages are based on the original weight of the plant, and were found by analysis of the anhydrous material.

DRY MATERIAL

In comparing the sunflower plants cut at different stages of maturity in the 4 to 8 inch spacing and the 36-inch spacing, there is seen very little difference in percentage of dry material, the 36-inch spacing showing only a slightly higher percentage of dry material over the 4 to 8 inch spacing. The plants in the 36-inch spacing show a more gradual increase in dry material throughout the five stages of maturity than the plants in the 4 to 8 inch spacing. The fifth stage of maturity of the sunflowers represents the stage of growth that was siloed in the large experiment station silos.

PROTEIN

Protein is consistently higher in all the stages of maturity when the plants were spaced 36 inches apart than when they were spaced from 4 to 8 inches. The first two stages of maturity of the 36-inch spacings were considerably higher in protein than the first two stages of the 4 to 8 inch spacings. In the last three stages, the advantage of high protein remains with the 36-inch spacings, but the differences in the percentage of protein in the two spacings were not so marked as they were in the first two stages.

CRUDE FIBER

Contrary to the general belief, it is seen that the crude fiber content of the sunflowers in both systems of plantings is not greater at the latter two stages than at the earlier stages of maturity. In fact, this table shows that the crude fiber is slightly less at the latter stage of maturity than at the earliest stage analyzed. The 36-inch spacings have less crude fiber than the 4 to 8 inch spacings. From the results it appears that the percentage of crude fiber does not increase until near the end of the growing period of the sunflower, when the seeds are matured and the stalk and leaves are dead. At this stage the percentage of moisture has decreased, the stalk has become hard and woody, and many leaves have fallen, thus increasing the crude fiber and materially lessening the feeding value of the sunflowers.

ETHER EXTRACT

No uniformity seems to exist in the relation of the ether extract in the two different systems of spacings at the different stages of maturity. In general, the 36-inch spaced plants show a larger percentage of ether extract than the 4 to 8 inch spaced plants. Sunflowers when planted closely together produce much smaller flowers, which do not always mature, hence the ether extract is less in amount. Plants spaced far apart not only produce larger flowers but in many cases more mature ones containing a considerable quantity of seed, which in turn contain a large amount of oil, thereby increasing the percentage of ether extract.

COMPOSITION OF LEAVES, STALKS, AND FLOWERS

The composition of leaves, stalks, and flowers was also determined. The results are given in Table II.

TABLE II.—*Composition of sunflower leaves, stalks, and stems (1920)*

Stage of growth.	Average height.	Average weight.	Distance apart in rows.	Moisture.	Anhydrous material.	Crude protein.	Ether extract.	Crude fiber.	Nitrogen-free extract.	Ash.	Part of the plant analyzed.
	Ft. in.	*Lbs. oz.*	*Inches.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
Budding		2 8	36	77.20	22.80	26.20	2.72	8.33	42.85	22.80	Leaves.
Do		0 14	4 to 8	77.20	22.80	22.38	3.69	9.88	44.57	22.80	Do.
Do	8 3	8 4	36	85.60	14.40	8.56	1.38	27.38	52.13	10.55	Stalk and stems.
Do	9 8	3 ½	4 to 8	82.20	17.80	6.88	0.83	28.66	54.65	8.98	Do.
First flower in blossom		3 0	36	77.70	22.30	23.57	2.94	9.06	44.16	20.27	Leaves.
Do		1 1½	4 to 8	75.90	24.10	21.69	3.78	11.04	48.40	19.09	Do.
Do		0 15	36	81.20	18.80	16.69	4.71	11.16	58.67	8.77	Flowers.
Do		0 10½	4 to 8	80.80	19.20	15.81	4.57	4.87	66.50	8.25	Do.
Do	11 2	8 1	36	80.30	19.70	5.31	0.63	21.85	63.80	8.41	Stalk and stems.
Do	10 2	4 0	4 to 8	80.40	19.60	5.74	0.56	31.84	52.10	9.76	Do.
Seeds not quite in the dough stage		1 7	36	76.40	23.60	21.94	2.05	9.87	45.06	21.08	Leaves.
Do		1 5	4 to 8	78.20	21.80	24.19	2.97	10.91	41.71	20.22	Do.
Do		1 0	36	83.90	16.10	15.62	5.66	10.28	60.12	8.32	Flower.
Do		0 15	4 to 8	84.70	15.30	14.81	5.03	12.88	58.83	8.45	Do.
Do	8 4	5 0	36	81.00	19.00	5.31	0.51	27.98	57.17	9.03	Stalk and stems.
Do	10 9	4 10	4 to 8	84.20	15.80	6.12	0.49	36.94	46.37	10.08	Do.
First flower seeds well into the dough stage		0 15	36	76.80	23.20	20.80	3.44	8.98	46.75	20.03	Leaves.
Do		0 12	4 to 8	75.60	24.40	22.50	4.05	9.71	43.70	20.00	Do.
Do		1 14	36	84.00	16.00	15.56	9.48	20.40	46.56	8.00	Flower.
Do		0 14	4 to 8	82.00	18.00	13.87	5.38	11.92	61.23	7.60	Do.
Do	8 4	4 5	36	85.70	14.30	5.19	0.86	31.74	50.72	11.49	Stalk and stems.
Do	10 0	3 12½	4 to 8	80.40	19.60	5.25	1.76	32.58	52.30	8.11	Do.
First flower seeds well beyond the dough stage		2 8	36	77.30	22.70	24.25	4.05	8.78	42.88	20.04	Leaves.
Do		0 10½	4 to 8	75.00	25.00	20.19	4.16	11.06	42.43	22.16	Do.
Do		2 14	36	82.30	17.70	16.12	6.27	17.44	53.27	6.90	Flower.
Do		0 14½	4 to 8	82.80	17.20	14.31	5.94	15.60	56.28	7.87	Do.
Do	8 6	9 2	36	83.50	16.50	5.56	0.72	32.24	48.74	11.84	Stalk and stems.
Do	8 2	2 4	4 to 8	78.50	21.50	4.25	0.88	29.21	57.11	8.55	Do.

Table II is presented with a view of showing the distribution of the food nutrients in the leaves, flowers, and stalks. The table is largely self-explanatory, but a few significant facts will be discussed. The largest protein percentage content of the plant is found in the leaves. The flowers rank second and the stalks contain the smallest proportion.

When the different stages of maturity are compared in the two systems of spacing, it is seen that the percentage of protein in the leaves is quite constant throughout the various stages of growth. The 36-inch spaced plants showed a slightly higher percentage of protein in the leaves at the final stage of maturity as compared with the plants of 4 to 8-inch spacing. The percentage of protein found in the stalks and stems is the reverse of that found in the leaves when the two spacings are compared, while the percentage of protein in the flowers of both systems of plants is quite uniform. In the percentage of ether extract the flowers rank first, the leaves second, and the stalks the last in amount. The percentage of crude fiber is greatest in the stalks and stems and least in the leaves. The flowers contain an amount intermediate between the leaves and stalks, and as the flowers mature they show an increase in amount of crude fiber.

TABLE III.—*Percentage of leaves, stalks, and flowers (1920)*

Stage of growth.	Height.	Average weight of plants.	Distance apart in rows.	Flower.	Leaves.	Stalk and stems.
	Ft. in.	*Lbs. oz.*	*Inches.*	*Per cent.*	*Per cent.*	*Per cent.*
Budding	8 3	10 12	36	.....	23.1	76.9
Do	9 8	3 15	4 to 8	.....	22.2	77.8
1st flower in blossom	11 2	12 0	36	7.8	25.0	67.2
Do	10 2	5 12	4 to 8	11.4	18.0	70.6
Seeds not quite in the dough stage	8 4	7 7	36	13.5	19.3	67.2
Do	10 9	6 14	4 to 8	13.6	19.1	67.3
1st flower seeds well into dough stage	8 4	7 2	36	26.3	13.0	60.7
Do	10 0	5 1½	4 to 8	17.7	14.7	67.6
1st flower seeds well beyond the dough stage	8 6	14 8	36	19.0	17.2	63.8
Do	8 2	3 13	4 to 8	23.8	14.0	62.2

SUNFLOWER PLANT PERCENTAGE OF LEAVES, STALKS, AND FLOWERS

Table III is inserted to show the percentage of leaves, stalks, and flowers. Since the analyses of these different parts showed such widely different amounts of food nutrients, this table was introduced to show whether the two systems of plantings resulted in a different percentage of leaves, stalks, and flowers. A study of Table III indicates that under these two systems of plantings no consistent variations were noted in the ratios of leaves, stalks, and flowers. The percentage of leaves and stalks decreased throughout the growing period, while the percentage of flowers increased; this was true in both systems of plantings. The actual percentage of flowers, leaves, and stalks remain quite uniform throughout the various stages of maturity in sunflower plants which are grown at the two extreme distances in the row—namely, 4 to 8 and 36 inches.

TABLE IV.—*Acidity of sunflower silage (1920)*

Stage of growth.	Distance apart in rows.	Moisture.	Acids in 100 gm. silage juice.		Total acids.
			Nonvolatile acids.	Volatile acids.	
	Inches.	*Per cent.*	*Gm.*	*Gm.*	*Gm.*
Budding	36	86.0	2.024	0.382	2.406
	4 to 8	86.35	2.616	.558	3.174
First flower in blossom	36	85.0	2.200	.566	2.766
Do	4 to 8	83.0	2.640	.653	3.293
Seeds not quite in dough stage	36	83.5	1.896	.509	2.405
Do	4 to 8	82.7	2.740	.600	3.340
First flower seeds well in dough stage	36	81.7	2.648	.728	3.376
Do	4 to 8	84.1	2.376	.613	2.989
First flower seeds well beyond dough stage	36	80.6	2.536	.700	3.236
Do	4 to 8	83.9	3.000	.623	3.623

Stage of growth.	Distance apart in rows.	Moisture.	Acids in 100 gm. wet silage.		Total acids.	Acids in 100 gm. anhydrous silage.		Total acids.
			Nonvolatile.	Volatile.		Nonvolatile.	Volatile.	
	Inches.	*Per cent.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Budding	36	86.0	1.741	0.329	2.070	10.69	2.020	12.71
	4 to 8	86.35	2.260	.482	2.742	14.30	3.049	17.35
First flower in blossom	36	85.0	1.870	.481	2.351	10.68	2.747	13.43
Do	4 to 8	83.0	2.191	.542	2.733	10.70	2.646	13.35
Seeds not quite in dough stage	36	83.5	1.585	.425	2.010	8.09	2.170	10.26
Do	4 to 8	82.7	2.267	.496	2.763	10.84	2.371	13.21
First flower seeds well in dough stage	36	81.7	2.162	.595	2.757	9.71	2.657	12.37
Do	4 to 8	84.1	2.000	.516	2.516	10.59	2.730	13.32
First flower seeds well beyond dough stage	36	80.6	2.045	.564	2.609	8.53	2.350	10.88
Do	4 to 8	83.9	2.518	.523	3.041	13.10	2.723	15.82

ACIDITY OF SUNFLOWER SILAGE

Table IV shows that there is more acidity formed when sunflowers are siloed in the later stages of growth than when siloed in the earlier stage. This fact is different in corn silage, since immature corn always produces more acidity than corn siloed at a mature stage. The total amounts of acidity, however, found in these samples of sunflower silage easily fall within the amounts usually found in good sunflower silage. It must be remembered that kind of acids present is more of a criterion of good silage than quantity of acids. All silage samples made at the different stages of growth were classed as good samples when acidity, odor, and color were used as the criterion. The earlier stages would not be as suitable for silage purposes as the later stages, due to the large percentage of moisture which they contained. Even the last stage cut contained slightly higher than 80 per cent of moisture, but it must be remembered that these samples were not allowed to be exposed to the

sun after cutting, hence they represent the amount of moisture present immediately at the time of cutting. In the general farm practice of siloing sunflowers there is usually a loss of a few per cent of moisture by the slight wilting of the plants which takes place during the time of cutting and hauling to the silage cutter. The plants cut at this last stage of maturity were siloed in the large experiment station silos and produced excellent silage.

COMPOSITION OF SUNFLOWERS IN 1921

Additional work was carried on in 1921 on sunflowers planted singly and in hills at different distances apart in the row. The different distances apart in the rows selected were 8, 24, and 42 inches. In the hill system of planting there were one, two, three, and four stalks to the hill. All hills were planted 42 inches apart in the row. In both the drilled and hill systems the rows were 42 inches apart, this being the width of the planter.

The results of the analyses of the five stages of plants are given in Table V. All results except acre yield are given on the anhydrous basis.

In comparing the different systems of planting, 8, 24, and 42 inches apart in the row, and the hill systems, one, two, three, and four plants in the hill, it is noticeable that the 8-inch spacings produced the highest yield of all. This system is closely followed by the hill system of planting where three stalks were allowed to grow in each hill.

ANHYDROUS MATERIAL

The same conclusions as those found in 1920 are drawn when the amount of anhydrous material is considered. The 8-inch spacing is highest in amount with the three stalks in a hill system closely following. Very little distance is noted in any of the other systems of planting.

CRUDE PROTEIN

Crude protein gradually decreases as the sunflowers become more mature. The amount of protein is quite similar for all systems of plantings, averaging between 9 and 10 per cent in the final stages, the only exception to this being the four stalks in a hill system, which shows only 7.98 per cent crude protein in the final stage analyzed.

CRUDE FIBER

It is a noticeable fact that crude fiber increases up to the fourth stage, then decreases somewhat in the final stage examined. This fact holds true in both 1920 and 1921, with the exception of the 8-inch spacings grown in 1921.

ETHER EXTRACT

Ether extract is considerably higher in the last two than in the first three stages analyzed.

TABLE V.—*Composition of sunflowers (1921)*

Stage of growth.	Height.	Weight.	Distance apart in rows.	Acre tons.[a]	Number in hill.	Moisture.	Anhydrous material.	Crude protein.	Ether extract.	Crude fiber.	Nitrogen free extract.	Ash.
	Ft. in.	*Lbs. oz.*	*Inches.*			*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Budding	6 6	1 5	8	.....	1	81.4	18.6	10.38	1.82	26.40	48.72	12.68
First flower in blossom	6 1	1 8½	8	.....	1	83.44	16.56	8.44	2.29	25.50	52.27	11.50
Seeds not quite in dough stage	6 1	1 14	8	.....	1	82.65	17.35	8.80	2.26	27.30	48.89	12.75
First flower seeds well into dough stage	6 1	2 1	8	.....	1	81.4	18.6	7.94	2.55	27.10	49.66	12.75
First flower seeds well beyond dough stage	6 0	1 15	8	11.36	1	77.4	22.6	9.32	10.02	28.15	41.76	10.75
Budding	6 0	2 6½	24	.....	1	82.4	17.6	11.60	1.72	26.10	46.53	14.05
First flower in blossom	6 0	2 13	24	.....	1	81.93	18.07	10.40	2.26	27.10	48.39	11.85
Seeds not quite in dough stage	6 4	3 8	24	.....	1	82.2	17.8	9.72	1.92	30.30	46.91	11.15
First flower seeds well into dough stage	6 6	4 5	24	.....	1	82.53	17.47	9.32	1.93	31.50	46.13	11.12
First flower seeds well beyond dough stage	6 0	4 14	24	7.72	1	79.4	20.6	10.90	7.38	29.10	41.83	10.79
Budding	5 10	5 3½	42	.....	1	84.14	15.86	13.90	1.63	26.20	43.30	14.97
First flower in blossom	5 5	5 2½	42	.....	1	85.53	14.47	12.80	1.70	27.50	44.63	13.37
Seeds not quite in dough stage	5 11	6 2	42	.....	1	83.57	16.43	11.35	1.76	25.90	49.39	11.60
First flower seeds well into dough stage	5 11	8 0	42	.....	1	83.8	16.2	11.98	2.25	31.70	42.40	11.67
First flower seeds well beyond dough stage	5 6	8 3	42	9.72	1	79.4	20.6	9.53	5.87	27.75	46.95	9.90
Budding	5 11	2 8½	42	.....	2	83.4	16.6	10.45	1.57	29.80	45.18	13.00
First flower in blossom	6 2	2 12	42	.....	2	83.67	16.33	10.65	1.97	27.80	46.11	13.47
Seeds not quite in dough stage	6 2	3 6½	42	.....	2	82.23	17.77	10.90	2.07	27.75	46.28	13.00
First flower seeds well into dough stage	5 10	4 5½	42	.....	2	82.07	17.93	10.71	3.75	30.15	43.24	12.15
First flower seeds well beyond dough stage	6 0	5 ½	42	10.28	2	79.2	20.8	9.75	5.56	27.46	47.98	9.25
Budding	6 4	1 15	42	.....	3	82.9	17.1	10.06	1.47	25.70	49.62	13.15
First flower in blossom	6 2	2 1	42	.....	3	81.87	18.13	9.35	2.11	25.70	51.39	11.45
Seeds not quite in dough stage	6 5	2 14	42	.....	3	82.43	17.57	10.00	1.75	31.30	44.30	12.65
First flower seeds well into dough stage	6 4	4 1	42	.....	3	83.0	17.0	10.50	2.43	30.40	45.02	11.65
First flower seeds well beyond dough stage	6 0	4 6	42	10.58	3	78.14	21.86	10.02	6.61	28.45	44.62	10.30
Budding	6 0	2 2	42	.....	4	84.54	15.46	10.25	1.32	23.80	52.38	12.35
First flower in blossom	6 1	2 ⅓	42	.....	4	82.4	17.6	10.30	1.90	27.04	49.01	11.75
Seeds not quite in dough stage	6 5	2 11⅓	42	.....	4	84.25	15.75	10.85	1.61	30.40	43.89	13.25
First flower seeds well into dough stage	5 10	2 12	42	.....	4	79.7	20.3	9.33	3.06	29.70	46.31	11.60
First flower seeds well beyond dough stage	6 3	2 11	42	9.58	4	78.6	21.4	7.98	6.32	26.10	48.70	10.90

[a] Acre yields were furnished by the agronomy department of the University of Idaho.

COMPARISON OF YIELDS, 1920 AND 1921

The yield per acre of sunflowers grown in 1921 was slightly less than half the yield secured in 1920, due to dry weather. The total precipitation for each month during the growing season is given in the following table:

TABLE VI.—*Precipitation during the growing season*

Month.	1920.	1921.	Month.	1920.	1921.
	Inches.	*Inches.*		*Inches.*	*Inches.*
April	2.72	2.86	July	0.54	0.19
May	1.35	1.80	August	1.22	0.30
June	1.16	1.47	September	2.52	1.43

CONCLUSIONS

A comparison of the results on sunflowers grown in Idaho and those grown by Shaw and Walters at Beltsville, Md., shows some striking facts. Probably, due to climatic conditions, Shaw and Wright were able to secure a more advanced stage of maturity of their sunflower crop as the last stage represented the production of good hard seed. As has been stated before, it is seldom possible to secure such mature sunflowers in certain portions of the Northwest. The crop grown for this experimental work is a good example, since the last stage analyzed represents the maximum growth. At this period a frost occurred and the sunflowers were siloed.

In content of dry material Idaho sunflowers when siloed represent Shaw and Wright sunflowers matured in stages between rays partly fallen and rays all fallen. It appears from these results, and results of previous years, that the crude protein is considerably higher in sunflowers grown in the Palouse country of Idaho than in Maryland, one crop grown in 1920 representing 12.38 per cent, and one in 1921 10 per cent, as against approximately 7 per cent in Maryland for the same stage of maturity. The sunflowers grown in Montana, analyzed by Blish, show lower percentages of protein than sunflowers grown in the Palouse section of Idaho. From these results it would appear unfair to compare sunflowers grown in different parts of the United States for relative food value, since the composition of sunflowers may vary widely, owing to the differences in climatic and soil conditions.

The 1920 results on the composition of sunflowers at different periods of growth show that the crude fiber does not continue to increase as the plant passes through the various stages of maturity. Similar results were obtained with the 1921 crop. The percentage of crude fiber found in the sunflowers cut at the last stage of growth was slightly less than in the more immature stages studied. From these data, it appears that there is practically no gain in crude fiber during the growth of the sunflower plant throughout the five stages of maturity included in this investigation. The crude fiber may increase materially if the sunflowers are allowed to mature completely. At this time the leaves are dead and partly fallen and the stalk becomes hard and woody. Silage made from sunflowers in this stage of maturity is high in crude fiber and is not palatable. It is possible that some of the unfavorable results which have been noted in isolated cases with sunflowers have been due to the fact that sunflowers were allowed to mature too far before they were cut for silage.

TIME FOR CUTTING

In considering the proper stage for cutting sunflowers in Idaho, either of the last two stages could be profitably used for silage, as there is little material difference in the analyses for the two stages in either the 1920 or 1921 crops. In some sections where sunflowers mature slightly more than in the Palouse country of Idaho, it might be profitable to cut them a little later than those of the last stage included in this investigation. In any event, cutting should not be delayed much beyond the last stage studied, for then the stalks become hard and woody and a silage is obtained which is unpalatable and not relished by the stock.

The moisture content of the sunflower plant grown in the last two stages is approximately 80 per cent. This percentage of moisture is slightly higher than is usually desired in a crop intended for the silo. When the green material contains too much moisture, there is danger of a loss of considerable juice, which means a loss of soluble food nutrients. In crops containing a high sugar content, a high moisture content means also a high acid content. Shaw and Wright state that "a high moisture in the plant is usually associated with high acid silage." This holds true for corn silage, but an inspection of Table I shows that it does not always hold true for sunflowers. In the case of sunflowers a high moisture content results chiefly in a loss of soluble food nutrients in the juice.

If the sunflowers seem to have too high a moisture content when cut, this can be materially decreased by allowing the plant to wilt for a short period. Blish, of Montana, has shown that excellent silage resulted when sunflowers were cut and allowed to wilt before siloing until the moisture content was 72.24 per cent. A moisture content for sunflower silage in Idaho can vary from 70 to 80 per cent, the writers recommending approximately 75 per cent as an average.

DISTANCE APART IN ROW

When the two different distances of planting in the row are considered for 1920, it appears that the single plants growing 36 inches apart in the row rank slightly higher in food nutrients than the sunflowers planted every 4 to 8 inches. The analyses of the sunflowers, and the sunflower silage, both show a slight advantage over the closer plantings. The yield must be considered in deciding which system of planting is most profitable. The yields for the two spacings determined by the agronomy department showed 22.32 tons for the 36-inch spacings and 20.23 tons for the 4-inch to 8-inch spacings. However, because of the difference in the contour of the land upon which the sunflowers were grown, these yields can not be considered as final.

The yields of sunflowers grown in 1921 show results which are contrary to the findings of 1920. The 8-inch spacings gave a higher yield than the 42-inch spacings. Under the conditions that existed in 1921, very little difference was noted in the yields of the sunflowers drilled 8 inches apart and the sunflowers planted in hills containing two or three stalks. Four stalks in the hill did not yield as well as the hills containing two and three stalks.

From the data presented on the two years of sunflowers it is apparent that the different climatic conditions, chiefly the proper distribution of moisture throughout the life cycle of the sunflower plant, affect the yield of sunflowers when planted at different spacings in the row. The results vary from year to year in the Palouse country, because of variable

climatic conditions, to such an extent that no specific recommendations can be made as to the best systems of planting. Where irrigation is practiced, and the moisture supply controlled, more regular yields would be expected under the different systems of planting. It is hoped that these studies on sunflowers grown under irrigation will be continued. The results in this paper are representative of what may be expected when sunflowers are grown in sections of the United States where climatic conditions and altitude are similar to those in the Palouse country. The choice of the system of planting sunflowers, grown without irrigation, must necessarily rest with the farmer, depending upon whether it is more feasible to harvest and silo the large type of sunflowers rather than the smaller type. In choosing the system of planting, the farmer should consider the difference in labor involved in harvesting the large or small type of sunflowers.

EFFECT OF DIFFERENT CONCENTRATIONS OF MANGANESE SULPHATE ON THE GROWTH OF PLANTS IN ACID AND NEUTRAL SOILS AND THE NECESSITY OF MANGANESE AS A PLANT NUTRIENT[1]

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INTRODUCTION

In a study of the literature on the nutrition of plants, one is impressed with the lack of definite knowledge concerning the necessity of a number of elements frequently found in appreciable amounts in the ash of different species.

For many years it has been taught and accepted by agronomists and plant physiologists that only 10 of the elements are essential for the normal growth and maturation of plants. These essential elements are carbon, hydrogen, oxygen, nitrogen, calcium, magnesium, potassium, phosphorus, sulphur and iron. In addition to these, it is also well known that as many as 20 or 30 others are frequently found in small amounts in plants which have grown under natural conditions.

Within recent years many data have been obtained which indicate that a few of these so-called nonessential elements may have important functions in the plant's economy. During the past 25 years, perhaps, more attention has been given to the acquisition of knowledge concerning the occurrence, distribution, and probable functions of manganese in soils and plants than to any other one of the elements referred to.

The number of contributions to the literature on the relation of manganese to various phases of agriculture number no less than 100. As a matter of convenience these contributions may be divided into two general classes, depending upon the object to be attained. Investigators of one class, apparently, have been interested in determining whether or not manganese has any commercial value from the standpoint of a necessary fertilizer, while those of the other class have sought to determine whether or not manganese is an essential element in the vegetable economy and, if so, to assertain its functions.

HISTORICAL

Manganese was discovered by Scheele (*9*)[2] in 1774. He found that the soil contained small amounts of this element and that it was assimilated by plants that grew in the soil; however, he made no effort to determine whether manganese was necessary for plant growth.

In 1864 Sachs (*8*) conducted experiments, the purpose of which was to determine whether or not manganese was necessary in plant economy. He proved that manganese could not replace iron in plant growth, but

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This investigation, begun at the Kentucky Agricultural Experiment Station in 1916, was continued, with the consent of the Director, at Cornell University where part of the results were offered in partial fulfillment of the requirements for the degree of doctor of philosophy. Since September, 1922, the work has been carried on at the Kentucky Station. The paper presents in part the results of the investigation at Cornell, the earlier results, and the recent findings at the Kentucky Station.

[2] Reference is made by number (italic) to "Literature cited," p. 793–794.

he did not prove that manganese was also necessary when iron was available.

In 1883 Yoshida (*12*) isolated from the sap of the lac tree a product possessing peculiar enzymic properties which he was unable to explain.

In 1894 Bertrand (*1*) undertook further work on the nature and composition of the product which had been isolated from the sap of the lac tree, and found that it contained considerable manganese, which gave to the product its peculiar enzymic properties. After several years of research on the relation of manganese to the growth of plants he concluded that this element is necessary for the normal growth of both autotrophic and heterotrophic plants and that its functions can not be performed by any other element.

In 1914 Brenchley (*2*) published the results of an investigation concerning the effect of manganese sulphate on the growth of barley seedlings. She found that with a concentration of 1/100,000 of $MnSO_4$ and less a decided stimulation in the growth was produced; with greater concentrations than this, however, toxic effect resulted. In her conclusion with respect to the function of manganese in plants she suggests the probability that manganese may prove to be an essential element in the economy of plant life, even though the quantity usually found in plants is very small.

Kelly (*5*) made a study of the effect produced on different species of plants when grown in a natural soil containing a large amount of manganese as compared with the effect produced on the same species when grown on a soil containing only a normal amount of manganese. From his results he concluded that the small amount of manganese occurring in soils probably performs a twofold function in plant growth: (1) It acts catalytically, increasing the oxidations in the soil and accelerating the auto-oxidations in plants; and (2) it tends to modify the absorption of calcium and magnesium, perhaps by partially replacing calcium from insoluble combinations, but especially through a direct effect by which the absorption of calcium is increased and that of magnesium is decreased.

McHargue (*6*) grew wheat plants to maturity in solution cultures free from and containing manganese and found that in the absence of manganese the plants became etiolated and did not develop in a normal way, thus showing that manganese is essential to the normal growth and maturation of this plant.

Previous to 1902 but little attention had been given to the occurrence and distribution of manganese in soils. During that year Ewell (*3*) made an interesting observation with respect to the occurrence of soluble salts of manganese in a certain small area of soil which failed to produce crops in a normal way. The results of his investigation showed that the small nonfertile area of soil contained relatively large amounts of soluble salts of manganese, while the adjacent fertile soil contained none. He assumed that the difference in the fertility of the two soils was due to the presence of the soluble compounds of manganese. The publication of his conclusions apparently awakened a wide interest regarding the rôle manganese plays in soils.

In 1907 the United States Bureau of Soils (*10*) inaugurated a series of experiments with manganese at the Arlington Experiment Farm. Manganese sulphate was applied at the rate of 50 pounds per acre to an acid silty clay loam soil, and crops were grown on the treated and untreated soil for six years. The application of manganese to the acid soil resulted

in decreased yields; however, upon neutralizing the acidity in the soil by the addition of calcium carbonate the plots receiving manganese produced very marked increases in yields, thus showing that the reaction of the soil is an important factor in determining whether or not an application of manganese sulphate will exert a beneficial effect on plant growth.

Funchess (*4*) has obtained data which tend to show that some acid soils contain soluble salts of manganese. He also offers the suggestion that the toxic effect associated with soil acidity may be due in part to soluble salts of this element. He describes experiments with acid soils that contained soluble salts of manganese and were toxic to the growth of plants. He found that the toxic effect of the soil was destroyed after neutralization with calcium hydroxid.

In 1920 Olaru (*7*) published results showing the effect of manganese on some of the more important microorganisms concerned with nitrogen transformations in soils and leguminous plants. With pure cultures of *Bacillus radiocicola*, *Azotobacter chroococcum*, *Clostridium pasteurianum*, and *Micrococcus ureae* and with concentrations of manganese sulphate varying from zero to 10 mgm. per liter he obtained a marked stimulation in the functions of each of these organisms. The maximum stimulation appears to have been obtained with concentrations of about 1 mgm. of manganese per liter. He therefore concludes that the small amount of manganese occurring in the soil serves a very useful function with respect to the organisms concerned in nitrogen transformation.

Robinson (*11*) has determined the manganese content of 26 different and representative soil types in this country. The maximum amount found in any soil was 0.51 per cent, the minimum 0.01 per cent, and the average 0.20 per cent of MnO, respectively. Various other reports concerning the amount of manganese contained in the soils of this and other countries will average approximately 0.10 per cent of this element, which is as much as the phosphorus and sulphur contained in soils that produce average yields of field crops.

In the foregoing review it has been the aim of the author to refer to those contributions on the subject which contain the more modern views concerning the probable function of manganese in its relation to agriculture.

The purpose of this investigation was, first, to show the effect of increased concentrations of manganese sulphate on the growth of plants in certain soils, and, secondly, to determine definitely if manganese is an essential element in the plant economy.

EXPERIMENTAL DATA

Citation (*4*) is a contribution whose data indicate that soluble salts of manganese are associated with and may in part be responsible for the toxicity of acid soils. To obtain further data on this subject, a series of experiments was planned in which plants were grown in acid and in neutralized portions of the same soil to which were added equal and graduated amounts of manganese sulphate. The soils selected for the experiments were not only acid but were lacking in capacity to produce good crops without the addition of certain plant nutrients.

EXPERIMENTS WITH VOLUSIA SILT LOAM SOIL

Volusia silt loam soil has a brownish-gray color, is low in organic matter, and contains considerable clay. It requires about 2,000 pounds of calcium carbonate per acre foot to neutralize its acidity.

About 500 pounds of this soil were obtained in a pasture field on Turkey Hill, Tompkins County, N. Y. The soil was well mixed and allowed to air-dry. Representative samples were taken for determination of the total and water-soluble manganese.

The total manganese content was determined by the potassium bisulphate fusion and the colorimetric periodate method. The amount of manganese found by this method was 0.08 per cent, or 800 parts per million of the air-dry soil. The amount of manganese dissolved by digestion with distilled water was 6.25 parts per million of the air-dry soil.

For pot experiments, 4,000 gm. portions of the air-dry soil were weighed into clean 1-gallon earthenware jars without drainage. Two series of 12 jars each were prepared, one with and the other without calcium carbonate, as shown in Table I, each treatment being made in duplicate. The mineral nutrients consisted of 10 gm. calcium nitrate, 10 gm. dipotassium phosphate, and 5 gm. magnesium sulphate per jar.

TABLE 1.—*Pot treatments in Series I and II*

Pot No.	Treatment, Series I.	Treatment, Series II.
1 and 1a...	No treatment.....	20 gm. $CaCO_3$.
2 and 2a...	Mineral nutrients only.....	Mineral nutrients and 20 gm. $CaCO_3$.
3 and 3a...	Mineral nutrients and 5 parts per million Mn.	Mineral nutrients, 20 gm. $CaCO_3$ and 5 parts per million Mn.
4 and 4a...	Mineral nutrients and 10 parts per million Mn.	Mineral nutrients, 20 gm. $CaCO_3$ and 10 parts per million Mn.
5 and 5a...	Mineral nutrients and 50 parts per million Mn.	Mineral nutrients, 20 gm. $CaCO_3$ and 50 parts per million Mn.
6 and 6a...	Mineral nutrients and 100 parts per million Mn.	Mineral nutrients, 20 gm. $CaCO_3$ and 100 parts per million Mn.

Purple-top radish seeds were sown in each pot and the moisture content of the soil in each pot was brought to one-half saturation, by weight, with distilled water. After the seedlings were up they were thinned to 10 plants in each pot and the moisture content kept at approximately one-half saturation with distilled water during the time the plants were making their growth.

Immediately after the crop of radishes was harvested soybeans were planted in each of the pots without further treatment of the soil. After the beans had reached the proper size they were thinned to 6 plants in each pot and allowed to grow until they were approaching maturity, the moisture content being meanwhile kept up to about one-half saturation with distilled water.

Plate 1, A, represents five pots from series No. 1. Pot No. 1 is one of the control pots and represents the productivity of the soil without treatment. Pot No. 2 shows the effect of the addition of appropriate amounts of phosphorus, potassium, calcium, magnesium, nitrogen and sulphur to the soil. This is one of the pots that received mineral nutrients

only and no manganese. Pot No. 3 received 5 parts per million of manganese in addition to the mineral nutrients. Pot No. 4 received 10 parts per million of manganese. Pot No. 5 received 50 parts per million of manganese. It is therefore readily apparent that 50 parts per million of manganese in the form of manganese sulphate are sufficient to produce toxicity in this soil. None of the seeds came up in the soil receiving this and greater concentrations of manganese sulphate. It is quite evident from Plate 1, A, that the maximum tolerance for manganese sulphate in this soil and with this particular plant lies between 10 and 50 parts per million.

Table II shows that the plants in the corresponding duplicate pots which are not shown made a similar growth.

There is no noticeable difference in the growth of the plants receiving the 5 and 10 parts per million of manganese, as compared with those that received no additional manganese. It is apparent that the small amount of manganese contained in the soil was sufficient for the requirements of the plants, under the conditions described.

The growth attained by the plants in the neutralized Volusia soil is shown in Plate 1, B, in which pot No. 1 is one of the control pots to which calcium carbonate was added. Comparison with the corresponding pot in Plate 1, A, shows that the plants made a slightly better growth in the neutralized soil than in the acid soil. Pot No. 2 received mineral nutrients and calcium carbonate and the plants did not make as good growth as did those in the corresponding pots with the acid soil. Pot No. 4 is one of two to which 10 parts per million of manganese was added. Neither of the two pots of soil that received 5 parts per million of manganese is shown in the photograph, but it will be seen in Table II that the plants that received 5 parts per million of manganese in series No. 2 made a slightly better growth than did the plants in the corresponding pots of soil in series No. 1. It will also be observed in Table II that the plants in the pots of soil that received 10 parts per million of manganese made a decidedly better growth in the neutralized soil than did the plants in the corresponding pots in the acid soil. Pot No. 5 shows the growth the plants attained in the neutralized soil to which 50 parts per million of manganese was added. It will be recalled that in the corresponding pots in the acid soil, the seeds did not germinate because of the toxic effect produced by an excess of manganese, while with the same concentration of manganese in the neutral soil the plants made a fair growth, though apparently retarded by an excess of this element. This experiment illustrates in a very striking way the effect of calcium carbonate in reducing the toxicity produced by an excess of manganese sulphate. In pot No. 6, to which was added 100 parts per million of manganese, no plants grew.

In Table II it will be observed that the applications of manganese to the acid soil reduced the yields, as shown by the green and dry weights of the plants, whereas with the same concentrations of manganese in the neutralized soil there was an appreciable gain.

TABLE II.—*Weights of radish and soybean plants grown in Volusia and Dunkirk soils, with and without nutrients, $MnSO_4$ and $CaCO_3$, duplicate pots*

		Acid reaction of soil.				Neutral reaction of soil.				
Crop.	Soil.	No treatment.	Minerals.	Minerals plus 5 parts per million Mn.	Minerals plus 10 parts per million Mn.	$CaCO_3$.	$CaCO_3$ plus minerals.	$CaCO_3$ plus minerals and 5 parts per million Mn.	$CaCO_3$ plus minerals and 10 parts per million Mn.	$CaCO_3$ plus minerals and 50 parts per million Mn.
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Green weight of radish tops and roots.	Volusia....	45.0	212.5	167.5	152.5	52.5	151.0	178.5	194.0	36.5
	Dunkirk..	92.5	240.5	205.0	101.0	95.0	129.0	185.0	222.5	146.5
Dry weight of radish tops and roots.	Volusia....	3.3	12.4	10.0	9.7	3.4	10.7	12.9	13.4	4.2
	Dunkirk..	7.3	15.8	13.2	8.8	7.8	7.7	12.3	13.8	10.1
Green weight of soybeans.	Volusia....	38.8	65.5	50.0	28.0	42.0	42.0	60.5	45.5	11.8
	Dunkirk..	30.3	67.8	41.3	20.5	27.5	27.5	50.8	44.3	48.5
Dry weight of soybeans.	Volusia....	4.4	12.5	9.6	6.0	9.3	9.9	12.5	10.1	2.6
	Dunkirk..	6.9	11.9	8.2	3.6	6.2	7.1	9.5	8.8	10.0

EXPERIMENTS WITH DUNKIRK CLAY LOAM SOIL

Two series of experiments similar to those just described with the Volusia soil were conducted at the same time and under the same conditions with the Dunkirk clay loam soil. Apparently this soil is slightly more productive than the Volusia silt loam soil. It has an acid reaction and responds to applications of certain plant nutrients.

A manganese determination on the Dunkirk soil gave 0.093 per cent of this element. The amount of water-soluble manganese found was 9.30 parts per million of the soil.

The radish plants which were grown on the Dunkirk soil under the same conditions as those previously described on the Volusia soil were harvested on the same day and treated in like manner for the green and dry weights, which are given in Table II.

No photographs were made of the plants grown in the Dunkirk soil; however, it will be seen from the green and dry weights of the plants, shown in Table II, that results somewhat comparable to those obtained with the Volusia soil were obtained under similar treatments with this soil. In series No. 1 the maximum yields occurred with the applications of mineral nutrients. Addition of manganese gave a diminution in yields as compared with the pots receiving mineral nutrients alone.

In series No. 2, application of calcium carbonate to the soil of pots No. 1 and 2 affected the growth of the plants but little as compared with the corresponding pots in series No. 1, in which the plants grew in acid soil. It thus appears that the application of calcium carbonate under these conditions had no effect as far as the growth of the plants was concerned. There is an increase which is apparently due to the applications of manganese in this series, as compared with the pots receiving mineral nutrients alone. This is in accord with the results obtained with applications of manganese to the neutralized Volusia soil.

Equal quantities of the radish tops grown in duplicate pots were combined to make a composite sample for chemical analysis, the results of which are given in Table III.

TABLE III.—*Analyses of composite samples of radish tops grown in Volusia and Dunkirk soils, with and without the addition of nutrients, manganese sulphate, and calcium carbonate (results calculated on moisture-free basis)*

		Acid reaction of soil.				Neutral reaction of soil.				
Element.	Soil.	No treatment.	Minerals	Minerals plus 5 parts per million Mn.	Minerals plus 10 parts per million Mn.	$CaCO_3$.	$CaCO_3$ plus minerals.	$CaCO_3$ plus minerals and 5 parts per million Mn.	$CaCO_3$ plus minerals and 10 parts per million Mn.	$CaCO_3$ plus minerals and 50 parts per million Mn.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Ash	Volusia	21.89	25.60	25.51	24.45	22.06	24.96	25.49	26.00	21.71
	Dunkirk	20.15	24.83	24.58	26.76	17.55	19.06	25.08	25.56	25.45
Fe	Volusia	.077	.059	.050	.091	.047	.095	.096	.071	.08
	Dunkirk	.073	.073	.073	.073	.074	.084	.095	.091	.11
Mn	Volusia	.046	.035	.058	.94	.054	.046	.070	.087	1.15
	Dunkirk	.024	.024	.056	.67	.019	.021	.034	.059	.84
Ca	Volusia	3.86	3.59	3.12	3.25	3.01	4.39	4.44	4.54	2.81
	Dunkirk	3.10	3.32	3.23	3.95	2.72	2.85	2.91	2.96	2.98
Mg	Volusia	.46	.45	.41	.38	.43	.38	.40	.44	.38
	Dunkirk	.43	.40	.42	.43	.45	.42	.41	.42	.34
P	Volusia	.25	.25	.78	.72	.24	.70	.70	.65	.68
	Dunkirk	.27	.56	.55	.50	.25	.59	.58	.61	.60
K	Volusia	4.56	4.72	6.67	7.47	6.94	4.33	5.55	6.52	6.35
	Dunkirk	4.56	4.56	7.69	7.99	7.31	3.52	3.16	7.83	7.24
N	Volusia	6.04	6.79	6.58	6.71	6.15	6.48	6.52	6.45	7.81
	Dunkirk	5.56	6.77	7.22	6.51	3.19	3.58	5.17	5.04	5.56

An examination of the results shown in Table III reveals some points of interest. The plants which grew in the untreated soil contained less mineral matter than did those to which mineral nutrients were added. The addition of manganese to the soil in series No. 1 did not increase the mineral content of the plants receiving it, whereas it appears to have done so in the neutralized soil, except in pots 9 and 10, in which toxicity was produced. About as much mineral matter was taken up by the plants grown in the soil to which calcium carbonate was added as where it was omitted in the corresponding control pots of soil in series No. 1. A little more calcium was taken up by the plants under neutral conditions of the Volusia soil than under acid conditions; however, a little less calcium was found in the plants that grew in the neutralized Dunkirk soil than in those that grew in the acid soil. A larger percentage of potassium was found in the plants that grew in the acid Volusia and Dunkirk soils to which manganese was added than in those plants that grew in the soil to which no manganese was added. There was an increase in the percentage of nitrogen in the plants to which manganese was added in the neutral Dunkirk soil, while the percentage of nitrogen was nearly constant in the other experiments.

It appears, therefore, that in the soils dealt with in the foregoing series of experiments with radish plants, there has been a slight gain in each of the soils where manganese was added to the neutral soil in connection with mineral nutrients. Under acid conditions there has been a diminution in the yield of the plants receiving manganese when compared with the pots receiving mineral nutrients alone. All the soils have responded vigorously to an application of mineral nutrients when compared with no treatment.

An inspection of the green and dry weights of the soybean plants in Table II indicates that a more favorable growth was obtained in the neutralized soil plus manganese than in the acid soil to which manganese

was added. The protective action of calcium carbonate against the toxic properties of an excess of manganese sulphate is demonstrated with soybeans. The plants receiving 50 parts per million of manganese in the neutral soil made a moderate growth, while in the corresponding pots of acid soil no plants were produced.

After the dry weights were determined the leaves and stems of the soybean plants in duplicate pots were combined to make a composite sample for chemical analysis. The results of the analyses are given in Table VI.

TABLE IV.—*Analyses of composite samples of soybean plants grown after radish plants in Volusia and Dunkirk soils, with and without the addition of nutrients, $MnSO_4$ and $CaCO_3$ (results calculated on moisture-free basis)*

		Acid reaction of soil.				Neutral reaction of soil.				
Crop.	Soil.	No treatment.	Minerals.	Minerals plus 5 parts per million Mn.	Minerals plus 10 parts per million Mn.	$CaCO_3$.	$CaCO_3$ plus minerals	$CaCO_3$ plus minerals and 5 parts per million Mn.	$CaCO_3$ plus minerals and 10 parts per million Mn.	$CaCO_3$ plus minerals and 50 parts per million Mn.
		Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Ash	Volusia	8.38	12.43	13.86	14.24	9.48	12.52	12.59	13.15	10.72
	Dunkirk	9.58	13.17	13.98	14.12	11.36	12.50	13.19	13.86	9.37
Fe	Volusia	.059	.078	.078	.069	.068	.059	.071	1.06	.114
	Dunkirk	.070	.070	.066	.051	.050	.052	.047	.052	.082
Mn	Volusia	.012	.012	.186	.303	.012	.014	.027	.042	.204
	Dunkirk	.013	.019	.162	.277	.069	.105	.346	.697	.186
Ca	Volusia	1.19	1.69	1.89	1.81	2.05	2.05	2.04	1.96	1.62
	Dunkirk	1.90	1.87	1.60	2.12	2.08	1.62	1.88	1.84	1.71
Mg	Volusia	.31	.29	.30	.28	.32	.30	.27	.28	.24
	Dunkirk	.33	.36	.31	.29	.31	.34	.28	.25	.36
P	Volusia	.13	.31	.35	.43	.18	.27	.24	.22	.36
	Dunkirk	.23	.45	.50	.55	.27	.48	.46	.40	.41
K	Volusia	1.43	3.40	3.62	3.92	1.40	3.12	3.05	3.05	3.03
	Dunkirk	1.34	2.80	3.39	2.20	3.51	3.71	3.51	3.34	3.21
N	Volusia	3.40	4.06	4.07	4.12	3.69	4.16	3.91	4.00	4.49
	Dunkirk	1.62	4.25	3.85	3.76	1.86	3.82	3.80	3.76	3.96

Table IV shows that the smallest percentage of ash was taken up by the plants grown in the untreated soils, while the largest percentage was found in the plants that had grown in the acid soil and to which 10 parts per million of manganese was added. Considerably more manganese than iron was taken up by the plants that grew in each of the acid soils to which manganese was added. Under neutral conditions more manganese than iron was absorbed by the plants that grew in the Dunkirk soil. The results for calcium vary to some extent between the two soils but apparently more calcium was taken up by the plants that grew in the neutralized soil. The plants that grew in the Dunkirk soil contained more phosphorus than those that grew in the Volusia soil. The amounts absorbed under acid and neutral conditions were nearly equal in the Dunkirk soil. Considerably more potassium was taken up by the plants receiving mineral nutrients and manganese than by either the untreated or the soils to which only calcium carbonate was added.

In the foregoing experiments with soils, the purpose was to determine the effect produced on the growth of plants in an acid and a neutral soil to which was added graduated amounts of manganese sulphate. It is readily apparent, therefore, that while such methods of experimentation are very important from an economic standpoint, they do not afford the desired proof to determine whether or not manganese is essential in the plant's economy.

The preparation of a medium that will, when the test is applied, show no manganese, requires certain procedures and precautions which, in so far as the writer has been able to discover, have not previously been taken into consideration in experiments planned to show the effect the absence of this element may have on the growth of plants. Tests for manganese in many different samples of chemical reagents which are used for plant nutrients show that manganese-free chemicals are rarely if ever found. So intimate is the association of manganese with iron that a manganese-free iron compound could not be purchased from dealers in chemicals and it was necessary to prepare it in the laboratory. Therefore in order to eliminate all possible sources of contamination with manganese it is necessary to test and purify all the reagents that enter the nutrient medium. Even though manganese has been eliminated from the nutrient medium it is necessary to grow plants until they approach maturity before any definite conclusions can be reached with respect to the effect of this element on their growth. The seeds of those plants with which the writer has carried on experiments apparently contained enough manganese to maintain a normal growth for the first six or eight weeks.

After having procured reagents that were proved by chemical tests to be free from manganese, a Knop's nutrient solution was made for water culture experiments.

Alaska garden pea seeds were germinated on moist cheesecloth in a porcelain-lined pan. When the seedlings were of the proper size they were transferred and held in place by means of plugs of cotton in holes made in squares cut from boards which were made to fit over the mouths of well-glazed one-half gallon earthenware jars. An attempt was made to waterproof the boards by keeping them submerged in paraffin kept near the boiling point for several hours. Although the squares of wood afford excellent means of support for the plants, they proved to be unsatisfactory in these and in previous experiments from the fact that the lower surface of the board, which is close to the top of the nutrient solution, affords favorable conditions for the growth of molds which, unless daily attention is given to washing them away, very soon attack the roots of the plants.

More recently supports have been made of well-glazed earthenware and no trouble from molds has been experienced.

The earthenware jars were filled nearly to the mouth with the Knop's solution and when the covers containing the seedlings were on, the roots were submerged in the solution. The pots containing the plants were kept at a suitable temperature for the growth of the peas in the greenhouse and the nutrient solutions were changed twice each week. The water lost by transpiration in the meantime was replaced with distilled water, thus keeping the concentration approximately constant.

Previous to starting the experiment new pots were obtained and tested for soluble manganese in the following manner: Three of the new pots were taken at random and filled to near the mouth with a mixture of equal parts of 1 : 1 nitric and hydrochloric acids and placed over holes on a water bath and kept at the temperature of boiling water for 48 hours. The mouths of the pots were covered with watchglasses during the digestion. The solutions were then transferred to porcelain dishes and brought to dryness. The residues were tested for the presence of manganese, and only a slight trace was found from any of the pots. The writer considers this a negligible source of manganese as it was thought

that even the trace found in the acid extraction would not be available to the plants growing in a Knop's solution.

Twelve Alaska pea seedlings were placed in each of four jars containing a Knop's solution. At the beginning of the experiment 1 part of manganese in the form of the sulphate to 1,000,000 parts of the solution was added to each of two of the pots. After the plants had been growing three or four weeks the concentration of manganese was increased to five parts per million of the Knop's solution. One cubic centimeter of a manganese-free ferric chlorid solution which contained 0.001 gm. iron was added to each of the pots at the time the solutions were changed.

During the first four or six weeks of growth there was little or no difference in the size or appearance of the plants. Therefore the plants which received no manganese except that contained in the seed began to show effects which can not be accounted for in any other way except for a lack of this element. The first effect to be observed was that the young buds as they unfolded were yellowish instead of a normal green color and later became flecked with small specks of brown, which was not observed at any time on the plants receiving manganese. The etiolated condition became more pronounced as the time progressed and finally resulted in the top branches dying back. The green and dry weights of the 12 plants that grew in each of the 4 pots were determined.

The average dry weights of the pea plants show an increase of 49.47 per cent in the total dry matter produced in the plants which were grown in the presence of manganese when compared with those plants that grew in a Knop's solution to which no manganese was added. There was also an increase of 24.63 per cent in the mineral nutrients assimilated, exclusive of nitrogen, in the plants that grew in the Knop's solution containing manganese. The plants which did not get any manganese contained a little more nitrogen than those to which manganese was added. The plants from which manganese was withheld contained only traces of this element, which was no more than could have been derived from the seeds from which the plants grew. It therefore appears that the trace of manganese derived from the seeds of the plants was not sufficient to maintain a normal metabolic process during the latter part of the time the plants were growing.

The duplicate sets of plants were combined to make a composite sample for chemical analysis and the results are given in Table V.

TABLE V.—*Analyses of pea, soybean, corn, and cowpea plants grown in media free from, and containing manganese (results expressed of the moisture-free material)*

	Ash (crude).	Iron (Fe).	Manganese (Mn).	Calcium (Ca).	Magnesium (Mg).	Potassium (K).	Phosphorus (P).	Nitrogen (N).
Alaska pea plants:	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Manganese	12.85	0.041	0.179	1.85	0.50	2.05	0.58	2.97
No manganese	15.41	.040	Trace.	2.41	.98	2.71	.68	4.71
Soybean leaves:								
Manganese	15.00	.051	.032	.80	.56	1.58	.62	3.42
No manganese	15.99	.094	Trace.	.82	.62	1.11	.48	3.85
Soybean stalks:								
Manganese	13.07	.036	.010	.57	.37	1.08	.45	1.69
No manganese	16.67	.033	None.	.68	.39	1.35	.46	2.21
Cowpea plants:								
Manganese	17.24	.046	.036	1.70	.54	5.56	.72	2.57
No manganese	20.14	.065	.003	1.32	.61	7.88	.51	4.51
Corn leaves:								
Manganese	8.71	.029	.040	.23	.31	3.80	.40	.22
No manganese	10.28	.034	.001	.24	.36	4.07	.35	1.47
Corn stalks:								
Manganese	6.37	.014	.003	.08	.13	3.02	.42	.77
No manganese	8.24	.011	Trace.	.10	.13	3.52	.37	.95

SAND CULTURE EXPERIMENTS

A quantity of quartz sand of medium sized grains was obtained for sand cultures. The sand, from external appearance, was good quality glass sand. Upon digesting 1,000 gm. in a mixture of 1:1 hydrochloric and nitric acids on a hot water bath for several hours, filtering out and washing the residue free of acids, it was found upon testing portions of the filtrate that the sand had contained small amounts of iron, manganese, zinc, arsenic, calcium, and magnesium. The residue of sand was snow white, except for a few black particles, which resisted further treatments of strong acids to dissolve them. A small amount of the black particles were separated, ground in an agate mortar, and fused with potassium bisulphate. The fused mass was extracted with dilute sulphuric acid and tested for manganese, but none was found.

Several hundred pounds of the sand were then washed with a mixture of nitric and hydrochloric acids and afterwards with distilled water until free of chlorids, as shown by a test with silver nitrate. The sand was then transferred to large, shallow, porcelain-lined agateware pans and dried. After the sand was dry 4,000 gm. portions were weighed into clean 1-gallon earthenware pots.

The sand prepared in this way possessed rather strong absorptive powers when wet with a Knop's nutrient solution. It, therefore, was necessary to saturate the absorptive capacity of the sand with respect to the plant nutrients before plants could be grown. This was accomplished by adding the following amounts of manganese-free mineral nutrients to each 1 gallon of dry sand (4,000 gm.); 25 gm. of $CaCO_3$, 10 gm. of K_2HPO_4, 10 gm. of $Ca(NO_3)_2$, 5 gm. of $MgSO_4$, 3 gm. of KCl and 3 gm. of Fe in the form of a suspension of $Fe(OH)_3$. These chemicals were all free from manganese and pots of sand receiving this treatment served as the controls. Equal numbers of other pots of the sand received 2 gm. each of manganese in the form of $MnCO_3$ and the above-mentioned nutrients. The mineral nutrients and the sand were thoroughly mixed by hand in a large porcelain-lined pan and returned to the respective pots. Equal numbers of plants of soybeans, cowpeas, and sweetcorn were grown in the purified sand, with and without manganese, until they approached maturity. They were then harvested and the dry weights determined and chemical analyses made of the plant material. The results are given in Tables V and VI.

TABLE VI.—*Dry weights of the plants*

Species of plant.	Manganese added.	No manganese added.	Increase due to manganese.
	Gm.	*Gm.*	*Per cent.*
Alaska peas (grown in water cultures)	28. 10	18. 80	49. 5
Soybeans	19. 80	12. 25	61. 6
Cowpeas	26. 50	11. 30	134. 5
Corn	72. 00	59. 00	22. 0

All the plants which grew in the pot cultures containing manganese made a very much better growth than did those from which manganese was withheld. The most striking result was obtained with cowpeas, in

which the increase in dry weight of the plants receiving manganese was 134.5 per cent. All the leguminous plants from which manganese was withheld made a normal growth for the first six or eight weeks; thereafter the young buds and leaves as they unfolded were etiolated and brown specks developed on the etiolated leaves later. After this condition had developed but very little growth was made, and the young and tender parts of the plants died back, while the plants to which manganese was added made a normal growth and no chlorosis developed during their growth. With corn, the only apparent effect produced on the growth of the plants was the failure in the production of dry organic matter. No chlorosis developed on the leaves of the corn, which was harvested after the plants had produced tassels. From these results it appears that leguminous plants are more sensitive towards the lack of manganese than are the nonlegumes.

In the cowpea and corn plants to which no manganese was added, enough of this element was present for a determination. In the other plants to which no manganese was added only a trace of manganese could be detected.

The stalk of the soybean plants which grew in the absence of manganese was the only material in which no manganese could be detected. The plants which grew in the pot cultures to which manganese was added contained a normal amount of this element.

More recent experiments with manganese-free sand cultures at the Kentucky Agricultural Experiment Station have shown the necessity of manganese in both nonleguminous and leguminous plants.

Plate 2, A, shows in a very striking way the beneficial influence of manganese on oats. The plants on the left had no manganese added to the sand in which they grew, while those on the right grew in the presence of manganese.

Plate 2, B, shows Canada field peas. The plants on the left received no manganese in the sand in which they grew, while to those on the right manganese was added, all other conditions being the same.

Sand cultures were carried out with the following species of plants: Wheat, oats, peas, cowpeas, beans, lettuce, tomatoes, onions, spinach, cabbage, carrots, radish, and clover, and similar effects were observed as those described and shown in Plate 2, A, B.

SUMMARY

(1) In the experiments with acid soil which contained approximately one-tenth of 1 per cent of manganese it was found that only small amounts of the total manganese were soluble in water. Applications of more manganese, in the form of the sulphate, to the acid soil caused a decrease in the yields of the crops, whereas like quantities of this compound, when applied to different portions of the same soil, after addition of calcium carbonate, caused an increase in the yields of other plants of the same species.

(2) The occurrence of soluble salts of manganese in an acid soil may be one of the causes of toxicity in such soils as exhibit toxic effects. An excess of manganese sulphate in a soil renders it sterile with respect to the growth of plants. Calcium carbonate in the soil causes a diminution in the toxic effects produced by an excess of manganese sulphate.

(3) In order to demonstrate whether or not manganese is essential for the normal growth of plants, it is necessary that great care should

be taken in the preparation of a manganese-free medium in which to grow plants.

(4) Manganese is intimately associated with compounds of iron, phosphorus, and calcium, and since very small amounts of this element are required for the growth of plants it is quite probable that the contamination of plant nutrients with manganese has hitherto been an unrecognized source of error in determining the necessity of this element in plant economy.

(5) Apparently leguminous plants are more sensitive to the lack of manganese than are the nonlegumes; however, further data obtained by growing nonleguminous plants for more than one generation in a manganese-free medium may be necessary to prove this point.

(6) A very small amount of manganese is required for the normal growth of plants.

(7) Seeds of the plants tested (radish, soybean, cowpea, field pea, and corn) do not contain enough manganese for the growth of the plant to maturity.

(8) The seeds of some plants contain enough manganese to maintain a normal development for the first four or six weeks of their growth; therefore experiments conducted for a shorter time in a manganese-free medium are not likely to give any indications as to the necessity of this element in the growth of plants.

(9) The lack of manganese affects the production of the dry matter in plants, thus indicating that it has some very important function in carbon assimilation.

(10) The etiolated condition of the young and tender leaves and buds obtained in well-controlled experiments indicates that manganese has a function in the photosynthetic process and the formation of chlorophyll.

(11) Manganese apparently is essential for the normal growth and development of plants.

(12) Further investigations on this subject are under way in which an attempt will be made to show the relation of manganese to other important phases of plant and animal life.

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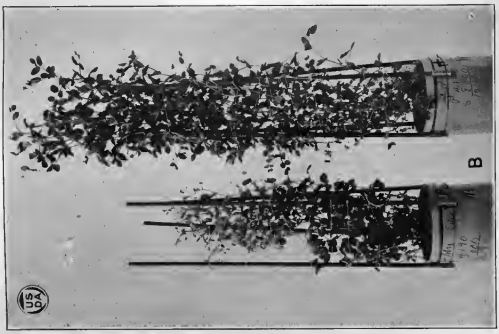
PLATE 1

A.—Radish plants in Volusia silt loam; $MnSO_4$ and plant nutrient added to No. 3, 4, and 5.

B.—Volusia silt loam with $CaCO_3$; $MnSO_4$ and plant nutrients added to No. 4, 5, and 6.


A
Nº1
Nº2
Nº3
Nº 4
Nº 5


B
Nº1
Nº2
Nº4
Nº5
Nº6


B

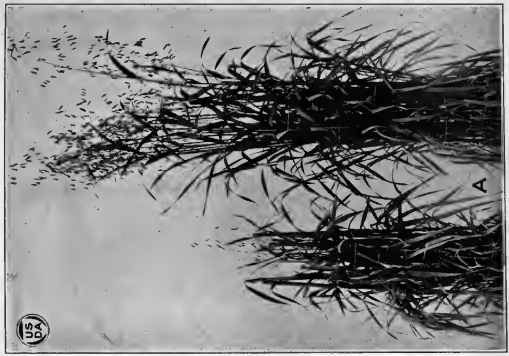

A

PLATE 2

A.—Oats in sand; left, no Mn added; right, Mn added.
B.—Canada field peas in sand; left, no Mn; right, Mn added.

SWEET CLOVER INVESTIGATIONS [1]

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COMPOSITION OF SWEET CLOVER HAY AND SILAGE

Sweet clover has become an important crop in Idaho, not only from the standpoint of feeding value, but also because it is finding a place in a proper rotation system of cropping which is so necessary for diversified farming. Sweet clover is a legume that is comparatively easy to grow in all sections of the Northwest. Its value as a soil improver is rapidly becoming known to progressive farmers both in the humid and semiarid regions. As a pasture crop, sweet clover is finding a much needed place on the farms of the Northwest. Its resistance to drought and its great productiveness under the most adverse conditions warrants a place on every farm. Recent indications are that sweet clover is an excellent crop for alkali districts when the alkali concentration is too heavy for the ordinary hay crops.

Since sweet clover is finding a place in a permanent agricultural system, the following investigation was planned to determine the feeding value at various stages of growth, and also to determine the possibility of utilizing the crop for silage whenever conditions did not warrant making hay. It appeared to the writers that owing to the heavy yields of sweet clover that can be obtained on lands that are not adaptable to the growing of corn or sunflowers, it would prove an excellent substitute for these crops for silage if it could be satisfactorily siloed in Idaho. Consequently the two varieties (*Melilotus alba*) or white, and (*Melilotus officinalis*), or yellow sweet clover, were studied. These two varieties were grown by the Agronomy Department in 1920, and were available for use in these investigations.

STAGES SELECTED

In studying the varieties, two stages were selected for the yellow sweet clover. The first stage was the time when the plant was in blossom. At this stage the plant was 37 inches in height. In all samplings the yield of a definite area was determined, and from this the acre yield was calculated. When the plant was partly in blossom and partly in seed it was cut, and the authors called this the second stage.

The samplings of the white sweet clover were made in three stages: First, before the plant was in blossom and when it was 49 inches in height; second, when the plant was beginning to blossom; and, third, in full blossom and when a few seeds were formed.

The samples were prepared for analysis and for silage by cutting in small pieces in a small silage cutter. A portion of the cut sample was air dried and then analyzed for protein, ether extract, fiber, ash, and nitrogen-free extract. The remaining portion of the sample was used for silage.

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PREPARATION OF THE SILAGE

Quart milk bottles were used for making the sweet clover silage, since these had been used on other types of silage experiments and found to be very well suited for such work.

The technic of silage making was as follows: A quart milk bottle was packed with the freshly-cut sweet clover and, if lacking in moisture, sufficient water was added to raise the moisture content to 75 or 80 per cent. A large rubber stopper containing a glass tube was placed in the bottle very tightly. This tube was bent at an angle so that its outer end was inserted into a beaker containing mercury. This arrangement allowed the fermentation gases, chiefly carbon dioxid, to escape from the bottle, but did not allow access of air to the silage. At the end of a few weeks, when the major fermentation had been completed, the bottles were opened and the silage examined. The acidity of the silage was determined, also the protein, ether extract, fiber, ash, and nitrogen-free extract.

METHODS USED

In determining the composition of the sweet clovers at various stages of growth, the methods described by the Association of Official Agricultural Chemists were used.[2] The results on the composition are given for the various stages in Table I.

[2] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. Revised to Nov. 1, 1919. xii, 417 p., 18 fig. Washington, D. C. 1920. Bibliographies at end of chapters.

TABLE I.—*Composition of sweet clover and sweet clover silage*

Variety.	Stage of cutting hay.	Date.	Height.	Acre yield.	Moisture.	Wet basis.					Anhydrous basis.				
						Crude protein.	Ether extract.	Crude fiber.	Ash.	Nitrogen-free extract.	Crude protein.	Ether extract.	Crude fiber.	Ash.	Nitrogen-free extract.
			In.	*Tons.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Yellow	In blossom	July 2, 1920	37	11.7	73.5	5.56	0.85	6.8	2.99	10.3	21.09	3.21	25.66	11.28	38.86
	In blossom and partly in seed	July 13, 1920	37	10.9	69.5	5.06	.63	9.99	2.90	11.92	16.59	2.07	32.78	9.49	39.07
	(Silage made from the above two stages of yellow sweet clover):														
	First stage				75.6	4.50	.63	7.48	2.60	9.19	18.44	2.59	30.65	10.61	27.71
	Second stage				72.4	4.38	.49	9.31	2.78	10.64	15.87	1.78	33.71	10.06	38.58
White	Before blossoming	July 2, 1920	49	21	75.4	5.38	.45	7.59	2.65	8.53	21.87	1.83	30.83	10.75	34.72
Original	Beginning to blossom	July 14, 1920	60	14	73.4	5.06	.55	9.01	2.16	9.82	19.02	2.06	33.83	8.12	37.97
Hay	In full blossom	July 22, 1920	60	16.8	69.4	4.25	.57	11.64	2.34	11.80	13.99	1.87	38.02	7.64	38.48
	(Silage made from the above stages of white sweet clover):														
	First stage				77.16	3.75	.51	7.83	2.09	8.66	16.42	2.23	34.20	9.16	37.99
	Second stage				75.32	3.69	.23	8.52	1.99	10.25	14.95	.93	34.50	8.05	41.57
	Third stage				70.64	4.06	.47	10.75	2.16	11.92	15.67	1.61	36.57	7.36	38.79

ACIDITY OF SWEET CLOVER SILAGE

METHODS OF OBTAINING SAMPLES AND DETERMINING ACIDITY

The silage was removed from the containers and 50 gm. were immediately weighed out and dried at 100° C. to determine the moisture content. The remaining silage was placed in a hydraulic press and the juice pressed out. Then 100 gm. of the juice was placed in a vacuum steam distilling flask, together with 5 cm. of normal sulphuric acid. The volatile acids were separated from the nonvolatile acids by distilling in a current of steam in a partial vacuum. Four liters of the distillate were collected, which contained all the volatile acids. The distillate was neutralized with one-tenth normal barium hydroxid and evaporated to a small volume. The volatile acids were then freed from the barium salts by the addition of the theoretical amount of sulphuric acid. After filtering the barium sulphate, the solution was made up to volume and the volatile acids were quantitatively determined, using the methods described by one of the authors in a former publication.

Total acidity was determined by titrating 10 gm. of expressed juice with one-tenth normal barium hydroxide. Nonvolatile acid was determined by subtracting the centimeters of volatile acids found in 100 gm. of silage juice from the total acidity determination. The difference was calculated as lactic, or nonvolatile acid. The results of the volatile and nonvolatile acids are given in Table II, on the silage both with and without moisture.

TABLE II.—*Acidity of sweet clover silage*

Variety.	Stage of cutting.	Height of sweet clover.	Acre yield.	Amount of acids in 100 gm. wet silage.			
				Acetic.	Propionic.	Lactic.	Total.
		In.	*Tons.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Yellow	In blossom	37	11.7	0.371	0.011	1.399	1.781
Do	In blossom and partly in seed	37	10.9	.694	.054	.853	1.601
White	Before blossoming	49	21	.515	.013	1.353	1.881
Do	Beginning to blossom	60	14	.333	.021	1.250	1.604
Do	In full blossom	60	16.8	.332	.023	1.642	1.997

Variety.	Stage of cutting.	Height of sweet clover.	Acre yield.	Amount of acids in 100 gm. dry silage.			
				Acetic.	Propionic.	Lactic.	Total.
		In.	*Tons.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Yellow	In blossom	37	11.7	1.520	0.043	5.728	7.291
Do	In blossom and partly in seed	37	10.9	2.516	.020	3.091	5.627
White	Before blossoming	49	21	2.167	.06	5.699	7.926
Do	Beginning to blossom	60	14	1.348	.09	4.063	5.501
Do	In full blossom	60	16.8	1.133	.08	5.599	6.812

DISCUSSION

A comparison between the white and yellow sweet clover shows that the former is the higher yielding variety. It must be borne in mind that both varieties were grown side by side, hence were subjected to the same climatic and soil conditions. The white sweet clover produced a taller and bushier plant than the yellow, which accounts for its greater yield.

In both varieties the first stage contained the larger percentage of water, as was expected. The later cuttings were higher in dry matter. The only significant fact shown (Table I) is that the nitrogen or crude protein decreased and the crude fiber increased, the longer the sweet clover was allowed to grow. This is true for both varieties and clearly demonstrates that if the sweet clover is to be used for hay the early cutting should be chosen rather than the later stage. For silage purposes this conclusion is equally true, for there is slightly less crude fiber and a slightly higher percentage of protein in the earlier stages of cutting.

The acidity determinations show that a normal silage fermentation takes place when sweet clover is siloed. The silage samples all had the characteristic silage acids and odor and were classed as excellent silage.

It is not the purpose of this paper to advise the growing of sweet clover for silage rather than corn or sunflowers, but under certain conditions it is thought that sweet clover can be so used to advantage. In its use as a pasture crop one of the chief difficulties encountered is to pasture sufficient stock upon the sweet clover to keep down the growth. In many cases the sweet clover grows so rapidly that the growth exceeds the pasture requirements and a portion of the sweet clover attains a considerable height. It is thought that in such conditions, where the crop may not make the best hay, it can well be used for silage, since the stalks, if they have been allowed to become too mature, will soften up materially in the silo. Two purposes have been accomplished when a sweet clover crop from pasture land has been used in this manner. First, the food value of the excess growth has been saved and, second, the pasture has been improved by removing the excess growth, and new tender shoots will appear.

The fact that sweet clover is a legume and acts as a soil improver and gives a comparatively high yield of green material under the most adverse conditions of drought, tends to make it a promising crop for the silo in many parts of the Northwest.

GROWTH AND COMPOSITION OF ORANGE TREES IN SAND AND SOIL CULTURES[1]

By H. S. REED, *Professor of Plant Physiology*, and A. R. C. HAAS, *Assistant Professor of Plant Physiology, College of Agriculture, University of California*

The limited control over the factors of nutrition operating in the field (*10*)[2] (*4*) has, perhaps, contributed in a large measure to the heterogeneous array of causes assigned to specific effects. Before any explanation of so-called nutritional disturbances of citrus trees can be accepted, they must be produced experimentally under conditions admitting of scientific analysis. The method subsequently described for growing citrus trees under controlled conditions should make such an analysis possible. The present paper deals with a comparison of the growth and composition of trees when grown in sand and in soil cultures.

It is important to determine, first, whether citrus trees can be grown successfully for several years in sand cultures receiving only inorganic salt solutions; second, whether the growth obtained in sand receiving a nutrient solution (which has been shown to be well suited to the growth of barley) compares favorably with that obtained in soil receiving occasional additions of the same nutrients; and, third, how the composition of the trees grown in sand compares with that of the trees grown in soil.

The importance of the method and the successful results thus far obtained seem to justify a brief description of the way in which these cultures were installed and cared for. The procedure to be described was adopted only after considerable study and experimentation.

As experiments upon trees are usually of long duration, adequate protection and cultural attention are quite essential. A screened enclosure with suitable windbreaks provided the necessary protection against animals and destructive winds. The experiments were conducted in sheet-iron tanks and in large galvanized iron cans. The former were sunk in the ground; the latter were placed in trenches for protection from the direct rays of the sun.

The trenches were 28 inches to 30 inches wide, about 3 feet deep, and were lined on both sides with boards. In the bottom of each trench were two 2-inch by 4-inch pieces of redwood extending the entire length of the trench, which served as supports for the cans and prevented rusting. Thus far it has not been found necessary to provide wooden covers for the trenches. The necessary protection against rain has been obtained by placing frames of roofing paper on wooden trapezes swung by wires from the roof of the enclosure.

Each of the galvanized iron cans (Pl. 1, A) is about 20 inches in diameter and about 26 inches in depth. In order to have a means of weighing the cans, three wrought-iron lugs ¼ inch by 1 inch, each bearing a three-eighths inch hole at the top, were fastened by bolts to the inside of the heavy upper rim. The lugs were equally spaced around the rim and protruded through holes cut in the lid (Pl. 1, B).

[1] Accepted for publication Nov. 6, 1922. Paper No. 77, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Reference is made by number (italic) to "Literature cited," p. 813–814.

A 4-inch hole was cut in the center of each lid to accommodate the tree. About midway between this center hole and the lid margin, four equally spaced 1¼-inch holes were cut, through which solutions could be poured. These holes were corked when not in use. The inside surface of the can was given a heavy coat of asphalt paint.

The sand used in the cans upon mechanical analysis showed the following percentage composition:

Size by screen (meshes per inch):	Per cent.
Larger than 20 mesh	0
20 to 40	0.33
40 to 60	7.85
60 to 80	54.11
80 to 100	21.63
Smaller than 100	16.08

Chemical analysis of the sand showed 99 per cent silica, 0.2 per cent volatile matter, and 0.8 per cent total water-soluble solids (other than silica or volatile matter), much of which was iron.

A 1-inch pipe, inserted through the lid and extending to the base of the can, provided a means for removing drainage water. About it and to a depth of 4 to 6 inches was placed a well-compacted layer of pure quartz rock crushed to about one-fourth to one-half inch in diameter, to prevent the sand from blocking the drainage system. The sand was added gradually and thoroughly moistened and settled before further additions, care being taken to avoid the use of excessive amounts of water. When the can was filled with sand to within about 2 inches from the top, the excess of water used in filling the can was at once removed through the outlet pipe.

The drainage water was removed by means of powerful air-suction (7) (Gardner-Rix vacuum pump run by one-half horsepower motor, with a capacity of 9 cubic feet per minute), six to eight cans being usually drained in one operation.

A slender iron tube (Pl. 1, B) was inserted through the drainage pipe to the bottom of the can whenever it was necessary to remove drainage water. As a means of excluding sand the lower end of the drainage tube was covered with a piece of fine tin screen. Excellent results over a period of years are readily obtained if the drainage water is removed as rapidly as it enters the drainage pipe. Plate 1, B, shows one of the drainage bottles removed from its wooden rack and raised so as to show the general scheme used in removing the drainage water.

The air outlet from each drainage bottle is provided with a gas cock by means of which the rate of flow into each bottle can be regulated.

A 2-year-old Valencia orange tree was planted in each can after the soil was thoroughly removed from the roots by repeated washing. The root system was pruned until only a small part remained. The upper portion of the tree was also pruned (Pl. 1, A); the lateral shoots and the leaves were removed and the cut surfaces covered with asphalt paint. Where it was not desired to exclude calcium from the culture, the trees were given a coat of whitewash. Where whitewash was not applied, a cylinder of wire netting was placed about the tree and the east and south sides were protected with cheesecloth attached to the wire. When the trunk was shaded by new shoots, the cloth protector was removed, preferably during cloudy or foggy weather. After the tree was planted in the sand and the lid was placed on the can a piece of white oilcloth, with a hole in the center, was slipped down over the tree trunk and was fastened to the lid with asphalt paint. A thin wad of cotton was placed

between the oilcloth and the tree. A cylinder of heavy brown wrapping paper was placed about the base of each trunk to give added protection from sunburn.

Where soil was employed the cans were prepared as stated before, which made possible the removal of salts while the tree was in the soil. Wilting-point determinations were made on the soils and they were then brought to a suitable water content prior to being gently compacted in the cans.

In the case of sand, additions of carbon-treated distilled water were continued until the first indications of drainage water appeared in the drainage pipe. Air suction was then applied. Nutrient solutions (6 to 9 liters or more) made up with carbon-treated distilled water were then added and the drainage continued until complete. If drainage water appeared later in the bottom of the cans, the air-suction was again applied until all the free solution had been removed. The problem of drainage was subsequently simplified by providing a means for gravity drainage. The cans were placed on supports over the trenches and a short iron pipe was inserted into the bottom of each can. As drainage water percolated to the bottom of the can, it dripped through the short pipe into a bottle standing in the trench. This arrangement is decidedly advantageous because it precludes the accumulation of water in the bottom of the can and also materially lessens the amount of labor required in caring for the cultures. The cans were shielded from the direct rays of the sun by housing them in a wooden box. The treatments were applied every one to three weeks as conditions required. During hot weather a small tree growing in sand (about 225 pounds of moist sand) may require from 24 to 30 liters of distilled water each 20 days. A Barnstead still capable of giving 5 gallons of distilled water per hour, with a block-tin-coated tank capable of storing 2,000–2,500 liters of distilled water, and 24 large carboys for treating the distilled water with carbon black together with filtering facilities, have made it possible to maintain in operation 136 trees in cans as well as several trees in tanks of sand.

In the case of cans of soil when drainage water may not be desired, the weighing device patterned after that of Briggs and Shantz (*2*) is used (Pl. 1, C). A windlass with wire rope and pulleys is mounted on a frame work of 2-inch galvanized-iron piping to which wide swivel castors are attached. Two spring scales with circular disks, 500 and 600 pounds capacity, respectively, are used for bringing the cans to constant weight. As the trees develop in cans of soil, it is necessary to make allowance for the increased weight of the tree in order to maintain suitable moisture content of the soil. The criteria used for this purpose are, appearance of the tree, presence or absence of drainage water, and appearance of the surface of the soil upon slightly raising the lid, or preferably by examining a sample of the soil. It is a good practice occasionally to raise the lids of the cans of soil to ascertain whether channels have been formed in the surface layers. It is best to weigh the cans frequently, since if large additions of solution are required, channels may appear throughout the soil and drainage water may appear without much of the soil being appreciably above the wilting point.

In order to carry on experiments with trees for a longer period of years, containers of a larger capacity than that of the cans are necessary. Tanks of two sizes are being used. The smaller are 3 feet 8 inches in diameter by 4 feet deep (Pl. 2, A), the larger are 7 feet 8 inches in diam-

eter by 4 feet 4 inches deep (Pl. 3, A). The bottom of each tank tapers toward the center, which is 6 inches deeper than the circumference.

The center of the bottom of each tank bears a perforated brass plate, beneath which is attached a 4-inch elbow that connects with 2-inch galvanized-iron piping leading to a trench. Each tank has its individual drainage outlet. Crushed quartz rock was placed in the bottom of each tank above which is placed sand or soil, as the case may be.

The nutrient solution employed in the present experiments was that used by Hoagland (5) in the culture of barley. Filtered carbon-treated distilled water was used whenever water was required. The stock solutions were made up as follows:

Solution A.	Gm.
KNO_3	1,200
$MgSO_4+7H_2O$	1,800
NaCl	55
H_2O to give volume of 18 liters.	
Solution B.	
$Ca(NO_3)_2+4H_2O$	2,600
H_2O to give volume of 18 liters.	
Solution C.	
KH_2PO_4	900
H_2O to give volume of 18 liters.	
Solution G.	
$MnSO_4+4\ H_2O$	0.406
H_2O to give volume of 2 liters.	

The nutrient solution was prepared by using the following amounts of the stock solutions: 55 cc. of solution A, 65 cc. of solution B, 30 cc. of solution C, and 20 cc. of solution G, made up to 10 liters with distilled water.

The nutrient solution as thus made had a P_H of 5.2 and an osmotic pressure of 0.728 atmospheres. The composition of the nutrient solution was as follows:

Parts per million.

NO_3	SO_4	K	Ca	PO_4	Mg	Cl	Na	Fe	Mn	Total
718	216	185	159	105	54	10	7	1	0.1	1455

The iron was not added to the nutrient solutions until they were ready to be added to the cultures. As a rule 5 to 10 parts per million of Fe were used in the nutrient solution, the plan being to have sufficient Fe present, as indicated largely by the appearance of the tree. Small quantities of ferric tartrate were subsequently added whenever the cultures were irrigated.

The trees were planted on May 21, 1920, and were removed September 20, 1921. Trees 1 and 2 were typical of the sand cultures (1–5), and tree 85 was typical of the soil cultures (84–88). The tree in can 2 was much smaller than the others in its series at the start, although it made excellent growth.

Plate 2 B, is a photograph of trees 1, 2 and 3 taken August 20, 1921. The photograph indicates that citrus trees can be grown in pure sand to which no organic matter has been added, except the small amount as iron tartrate. Plate 4 shows the leaves of tree No. 3 which were typical of the series, being dark green in color and giving no indications of malnutrition.

This is of interest in connection with the results (1) of field studies in which it was found that low humus content of the soil was associated with the mottling of orange trees and also that applications of organic

fertilizers tended to reduce the amount of mottling. Although the authors have grown 23 orange trees in sand cultures and 5 in soil receiving various concentrations of an inorganic nutrient solution, no typical mottle-leaf has been found as yet on any of the trees.

A word of caution is here necessary as regards the choice of orange trees for sand cultures. It seems best to use trees which are free from mottle-leaf, although trees which previously bore mottled leaves may present no difficulties after their behavior is once known. When mottled trees have been used in sand cultures receiving Hoaglands solution, and the leaves, shoots, rootlets, etc., removed as before stated, the first cycle of growth may bear a few mottled leaves most of which may recover, but the subsequent cycles have thus far been free from any evidence of mottling.

In order to observe the effect of the sand on the P_H of the solution, some of the nutrient solution was placed in liter Erlenmeyer flasks closed with rubber stoppers. Sand was added to one flask, shaken, and allowed to stand from December 29, 1920, to January 24, 1921. The P_H of the nutrient without sand had not changed, while that in contact with sand had increased from 5.2 to 5.9. Other culture solutions treated in this manner gave similar results. Shive (*9*) has reported that the reaction of his nutrient solution was not markedly altered by contact with unwashed sand. He finds that sand has an initial adsorptive effect but this is soon satisfied in sand cultures where the nutrient solution is renewed frequently. The increase in the P_H of the solution in contact with the sand was probably due to chemical reactions and not to adsorption.

The adsorptive property of unwashed sand probably was due to the very finely divided colloidal or semicolloidal material which was removed from the sand in the process of washing. The P_H values of percolates from the cans were determined from time to time to learn whether material changes ensued after prolonged contact with the sand and tree roots. The results of several determinations on percolates from cans 1 and 2 are given in Table I. The first two determinations showed the increase when a nutrient solution having an initial P_H of 5.2 was allowed to percolate through the sand of one of the cans. The other determinations show the changes in P_H when distilled water was allowed to percolate through the sand. These determinations are more variable, owing in part to the fact that the distilled water washed out varying quantities of the residual nutrient solution. In every case the P_H of the percolate tends to move in the direction of neutrality.

TABLE I.—*P_H values of nutrient solution and percolate from trees grown in sand cultures*

Date.	Application.	Amount of percolate collected.	Tree No.	P_H of percolate.
1920:		*Cc.*		
Nov. 2	Nutrient solution	100	1	6.8
Do	do	100	2	6.7
Nov. 29	Distilled water	500	1	7.25
Do	do	500	2	6.6
1921:				
Mar. 28	do	500	1	5.4
Do	do	500	2	6.5
Apr. 20	do	10	1	5.9
Do	do	10	2	6.7

The osmotic pressure of the sap of leaves from trees in the sand cultures was 20.80 atmospheres and that of leaves from the soil cultures was 21.03 atmospheres. The P_H of the former was 6.00 and that of the latter 5.80. We can not regard these differences as very significant.

Can 85, as well as the other cans of the series 84–88, inclusive, contained soil obtained from an uncultivated hillside near Riverside, Calif.

The hygroscopic moisture content of the soil was determined and the soil was brought to a suitable moisture content.

The cans of soil were brought to a given weight at frequent intervals by the addition of distilled water or nutrient. During the experimental period (May 21, 1920, to September 20, 1921) can 85 received the following additions of salts.

Salts.	Amounts added.
	Gm.
KNO_3	9.89
$MgSO_4+7H_2O$	14.85
NaCl	0.46
$Ca(NO_3)_2+4H_2O$	25.27
KH_2PO_4	4.05
$Fe_2(C_4H_4O_6)_3$	0.17
$MnSO_4+4H_2O$	0.0108

Plate 3, B, shows a typical tree in the soil series (84–88). The growth in sand compared very favorably with that in soil.

In November, 1920, after the addition of distilled water to cans 1 and 2, the first 500 cc. of percolate obtained from both cans were analyzed for the PO_4 and NO_3 content. The results given below indicate that an excess of both ions was present.

Parts per million.

	PO_4	NO_3
Can 1	55.7	1,329
Can 2	65.5	886

The soil in can 85 received only distilled water from May, 1920, to May, 1921, after which tap water was used. Hoagland's nutrient was occasionally added. Soil samples were taken from can 85 on October 8, 1920, and also in September, 1921. A partial analysis of a 1 to 5 aqueous extract of the dried soil gave the following results when expressed in terms of the dried soil:

Soil samples from can 85, October 8, 1920.

	Parts per million.		Parts per million.
Ca	22	Cl	18
Mg	9	HCO_3	76
Na+K (calculated as Na)	3	SO_4	15
Total solids as sulfates	128	NO_3	40
SiO_2	19	P_H of extract (colorimetric)	6.6

Soil samples from can 85, September, 1921.

	Parts per million.		Parts per million.
Ca	18	Cl	31
Mg	8	HCO_3	61
Na	4	SO_4	44
K	27	NO_3	22
Total solids	313	P_H of extract (colorimetric)	6.6
SiO_2	36		

The trees were removed from cans 1, 2, and 85 on September 20, 1921. Tree 85 was free from mottle-leaf, as were all the trees in the soil series, and was making excellent growth. Plate 5, A, shows the air-dried root systems of trees 1 and 85, grown in sand and in soil respectively. It is evident that starting with trees of nearly similar size, as good growth may be obtained in sand as in soil cultures. The root systems obtained in the sand cultures were somewhat coarser than those obtained in the soil cultures, the root system in the soil cultures being extremely fibrous (Pl. 5, B).

The initial operation in removing a tree from the can was to remove and count the leaves, which were then cleaned with a dry cloth. The shoots were removed at the trunk and after being cut up finely were placed in paper bags. The lid of the can was then removed and soil samples taken. The root system was obtained intact by washing the sand or soil from the tilted can with a strong jet of water. After carefully removing the adhering sand or soil from the rootlets with tap water, they were washed with distilled water. The upper portion of the tree axis was sawed off close to the first lateral root and was designated as the trunk. In cases where the trunk had been previously coated with whitewash it was cleaned with a soft scrubbing brush and tap water and then rinsed with distilled water.

When air dry the trunk was further cleaned with a soft wire brush. All new root laterals formed subsequent to the planting of the tree were removed, placed in bags, and were designated as "rootlets." The root axis, together with any pruned laterals which were present when the tree was first planted in the can, were designated as "root." The root was thoroughly cleaned with the use of a soft wire brush.

The several portions of each tree were dried to constant weight at 60° to 70° C. The rootlets were then shaken in the upper compartment of a set of soil sieves, to remove as much adhering matter as possible. In spite of the care taken in cleaning the rootlets the amount of silica still adhering to them was sufficient to require the calculation of analytical results to a silica-free basis. In the case of trees grown in soil the task of obtaining clean rootlets was so difficult that determinations of ash constituents were omitted from the analyses.

The dry weight, number of leaves, and water transpired for each of the three trees are given in Table II.

TABLE II.—*The dry weight of various portions of the trees, the number of leaves, and the water transpired*

Tree No.	Number of leaves on tree.	Dry weight (60° to 70° C.).						Total nutrient added.	Total distilled water added.	Total drainage water.	Transpiration.	Total transpiration per total dry weight of trees.
		Leaves.	Shoots.	Trunk.	Root.	Rootlets.	Total.					
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Liters.*	*Liters.*	*Liters.*	*Liters.*	
1	996	237.1	122.5	194	212	136	901.6	243	125	139.5	228	0.253
2	302	76.5	20.7	126	64	57	344.2	187	83	173	97	0.282
85	1,042	205.9	96	140.5	135	129.5	706.9	[a] 27	[b] 29	0	166	0.235

[a] After May, 1921. [b] May, 1920, to May, 1921. Tap water after May, 1921, 110.2 liters.

When the trees were removed from the cans, their appearance was noted. Tree 1 was an excellent tree with large top and splendid foliage. A few leaves tended to split along the midribs. The root system was large, well developed, and filled practically the entire can.

Tree 2 was the smallest in its series, but was smallest when planted. The leaves were excellent. The lowermost portion of the root axis had died, but a large mass of healthy laterals developed directly above the dead portion. Tree 85 was splendidly developed and was typical of its series. The rootlets were much more finely divided than in the sand series (Pl. 5, A, B).

The dry leaves, shoots, and rootlets were passed through a large hand mill, and any pieces remaining underground were removed. The trunk and root portions were sawed into thin sections, the sawdust being used to represent the trunk and root, respectively.

When ready for analysis the samples were thoroughly mixed and so far as the material available permitted duplicate determinations were run. The methods of analysis employed were essentially those used by Kelley and Cummins (*6*) in their analyses of citrus material. Manganese was determined colorimetrically by the persulphate method, which is very satisfactory for small amounts (*11*). Iron was also determined colorimetrically. A few sodium and potassium determinations were made, using the filtrate after the Fe, Al, PO_4, Ca and Mg were removed, but the analyses indicated that low results were obtained. Subsequently more satisfactory results were obtained by using the filtrate from the sulphate determination.

The analytical results for the several portions of each tree are presented in Tables III to VII, inclusive. The data have been calculated as percentage of dry matter and as percentage of ash. The figures given are the averages of two closely agreeing duplicates. It is seen that the total percentage of the ash approximates 100 in most cases, but in none reaches it. Those who are familiar with the details of analytical work of this kind appreciate its difficulties. If the reader be one who insists upon total percentages equaling 100 the authors can merely say, in common with other workers in this field, "peccavimus."

TABLE III.—*Analyses of leaves*

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
N	3.24	3.14	2.15	.....	.....	.....
S	0.31	0.30	0.29	.....	.....	.....
P	0.19	0.20	0.15	.....	.....	.....
Ash	14.37	14.32	13.72	.....	.....	.....
K	3.54	3.56	1.44	24.59	24.91	10.51
Na	0.13	0.21	0.16	0.90	1.43	1.16
Ca	2.71	2.66	3.94	18.85	18.57	28.66
Mg	0.31	0.26	0.37	2.14	1.75	2.65
Mn	0.004	0.004	0.002	0.03	0.03	0.02
Fe	0.025	0.04	0.03	0.17	0.26	0.23
Al	0.17	0.14	0.08	1.14	0.97	0.59
Cl	0.04	0.05	0.02	0.26	0.31	0.15
CO_3	.....	.....	.....	40.70	40.93	42.64
SO_4	.....	.....	.....	3.18	3.17	3.91
SiO_3	0.39	0.43	0.47	2.75	3.02	3.43
PO_4	.....	.....	.....	3.92	4.02	3.23
Total	.....	.....	.....	98.63	99.37	97.18

TABLE IV.—*Analyses of shoots*

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
N	1. 55	1. 76	0. 79	.....	.....	.....
S	.....	.....	.....	.....	.....	.....
P	0. 21	0. 23	0. 14	.....	.....	.....
Ash	6. 81	8. 30	5. 69	.....	.....	.....
K	1. 15	1. 22	0. 55	16. 85	14. 47	9. 79
Na	0. 13	0. 27	0. 08	1. 88	3. 23	1. 47
Ca	1. 46	1. 88	1. 58	21. 34	22. 62	27. 71
Mg	0. 21	0. 22	0. 18	3. 06	2. 67	3. 19
Mn	0. 001	0. 002	Trace.	0. 02	0. 02	Trace.
Fe	0. 02	0. 02	0. 05	0. 32	0. 23	0. 89
Al	0. 10	0. 10	0. 07	1. 46	1. 15	1. 15
Cl	0. 01	0. 01	0. 01	0. 14	0. 11	0. 21
CO_3	.....	.....	.....	35. 90	39. 91	40. 95
SO_4	.....	.....	.....	3. 13	2. 38	3. 01
SiO_3	0. 11	0. 12	0. 16	1. 53	1. 48	2. 75
PO_4	.....	.....	.....	8. 58	7. 64	6. 45
Total	.....	.....	.....	94. 21	95. 91	97. 57

TABLE V.—*Analyses of trunks*

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
N	0. 05	0. 15	0. 40	.....	.....	.....
S	0. 03	0. 07	0. 03	.....	.....	.....
P	0. 09	0. 11	0. 07	.....	.....	.....
Ash	2. 85	3. 22	2. 08	.....	.....	.....
K	0. 39	0. 31	0. 22	13. 57	9. 40	10. 57
Na	0. 18	0. 24	0. 11	6. 03	7. 45	4. 86
Ca	0. 68	0. 80	0. 51	23. 91	24. 77	24. 20
Mg	0. 09	0. 09	0. 07	3. 12	2. 77	3. 09
Mn	Trace.	Trace.	Trace.	.....	.....	.....
Fe	0. 003	0. 02	0. 01	0. 11	0. 40	0. 51
Al	0. 09	0. 16	0. 09	3. 06	4. 92	4. 49
Cl	Trace.	0. 001	0. 006	.....	0. 04	0. 28
CO_3	.....	.....	.....	38. 02	34. 18	33. 45
SO_4	.....	.....	.....	1. 96	3. 46	4. 51
SiO_3	0. 04	0. 11	0. 06	1. 31	3. 15	2. 94
PO_4	.....	.....	.....	5. 11	3. 70	3. 97
Total	.....	.....	.....	96. 20	94. 24	92. 87

TABLE VI.—*Analyses of roots*

Element.	Expressed as dry matter.			Expressed as ash.		
	Tree 1.	Tree 2.	Tree 85.	Tree 1.	Tree 2.	Tree 85.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
N	1. 01	0. 98	0. 82	.....	.....	.....
S	0. 01	0. 13	0. 06	.....	.....	.....
Ash	3. 03	3. 08	2. 77	.....	.....	.....
K	0. 36	0. 33	0. 28	11. 74	10. 55	10. 05
Na	0. 14	0. 10	0. 07	4. 33	3. 15	2. 42
Ca	0. 72	0. 71	0. 70	23. 77	23. 06	25. 26
Mg	0. 08	0. 09	0. 05	2. 69	2. 87	1. 74
Mn	Trace.	Trace.	Trace.	.....	.....	.....
Fe	0. 02	0. 02	0. 02	0. 50	0. 49	0. 63
Al	0. 08	0. 13	0. 07	2. 41	4. 10	2. 35
Cl	0. 003	0. 006	0. 016	0. 09	0. 17	0. 59
CO_3	.....	.....	.....	38. 28	33. 15	41. 17
SO_4	.....	.....	.....	2. 51	4. 47	2. 38
SiO_3	0. 08	0. 14	0. 15	2. 75	4. 68	5. 35
PO_4	.....	.....	.....	7. 74	9. 43	5. 55
Total	.....	.....	.....	96. 81	96. 12	97. 49

TABLE VII.—*Analyses of rootlets calculated to a silica free basis*

Element.	Expressed as dry matter.		Expressed as ash.	
	Tree 1.	Tree 2.	Tree 1.	Tree 2.
N	2. 26	2. 22	.....	.....
S	0. 29	0. 27	.....	.....
P	0. 69	0. 56	.....	.....
Ash	11. 93	11. 19	.....	.....
K	2. 04	2. 38	17. 14	21. 24
Na	0. 12	0. 09	1. 02	0. 82
Ca	2. 36	2. 01	19. 78	17. 98
Mg	0. 36	0. 34	3. 06	2. 98
Mn	0. 02	0. 02	0. 16	0. 19
Fe	0. 04	0. 04	0. 30	0. 28
Al	0. 36	0. 36	3. 03	3. 22
Cl	0. 12	0. 13	0. 89	1. 12
CO_3	.....	.....	22. 25	24. 00
SO_4	.....	.....	8. 89	8. 46
PO_4	.....	.....	19. 65	14. 62
Total	.....	.....	96. 17	94. 91

In the analyses reported by Kelley and Cummins (*6*) the carbonates of the ash were not determined. At their suggestion these determinations have been included in the present studies. With rootlets it is practically impossible to remove all of the adhering sand, and consequently the results obtained for the rootlets (Table VII) have been calculated as percentages on a silica-free basis. The rootlets of tree 85 (soil culture) could not be sufficiently freed from adhering soil to permit of satisfactory analyses.

The precautions necessary in distinguishing between cause and effect, when interpreting analytical data of plant tissue, have already been emphasized (*6*). In the dry matter there is a progressive decrease in the percentage of total nitrogen in the sand and soil cultures as we pass from the leaves toward the root, where there may be a slight increase which becomes augmented in the rootlets, whose percentage is considerably below that found in the leaves but greater than that found in the shoots.

The percentage of total sulphur was approximately the same for the corresponding parts of the trees in both sand and soil, the shoots and root in every case containing but a trace.

The percentage of total phosphorus was usually somewhat greater for the sand than for the soil cultures. The leaves and shoots contained approximately the same percentage of total phosphorus; while the trunk and roots contained considerably less, with an appreciable increase in the rootlets above that of the roots.

We find the total ash content of the parts examined to be greater in each case for the sand than for the soil culture. The ash of the leaves was approximately double that of the shoots which in turn was more than double that of the trunk and root.

No significant difference as regards total ash existed between the trunk and root. It is of interest to note that the leaves and rootlets contain in their dry matter the greatest percentage of total ash and of nitrogen, which no doubt indicates that here the assimilatory processes are most active.

The percentage of CO_3 constitutes approximately four-tenths of the total ash, except in the rootlets, where it is only about two-tenths. The large percentage of CO_3 found in the ash of various parts of the citrus tree may indicate that not only the leaves, but the other portions of the tree as well, contain large quantities of organic acids.

The percentage of SiO_3 in the ash varies widely in the various parts.

The percentage content of potassium (K) in the ash of citrus leaves obtained in the field (Kelley and Cummins (*6*)) was approximately 20 per cent when 1 week old, 13 per cent when 6 weeks old, 6 per cent when 6 months to 2 years old, and 2 per cent when 3 or more years old. In the sand cultures the potassium constituted approximately 25 per cent of the total ash of the leaves, about 17 per cent of that of the shoots and about 11 per cent of that of the trunk and roots, respectively. In the soil culture, the potassium in the ash of all parts of the tree examined was approximately 10 per cent. The leaves collected from trees in these cultures probably ranged from 3 to 17 months of age and therefore represent a mixed sample so far as age is concerned. The data for potassium indicate that not only the age of the leaf but also the composition of the medium in which the tree grew may influence the percentage content of potassium in the various parts. The data do not necessarily conflict therefore with those obtained by Kelley and Cummins.

The sodium (Na) content of the ash of the leaves and shoots is relatively small compared with that of potassium. The difficulty of accurately determining a small amount of Na in presence of considerable amounts of K will be appreciated by those familiar with analytical work. The data show that the trunk and root contain a much larger percentage of sodium in the ash than either the leaves or shoots. As the location of the sodium storage in citrus trees is of considerable interest and the data thus far obtained are too meager to justify any far-reaching assumptions,

we will reserve further discussion of the matter until the results of experiments in sand, in which the culture solution contained large amounts of sodium chlorid, have been reported.

The percentages of calcium (Ca) in the ash of the leaves and shoots of the soil culture were higher than for the sand cultures; the trunk and roots contained, approximately, the same percentages respectively in all three cultures. The calcium in the total ash varied from 18 to 30 per cent in the various parts in both sand and soil cultures. The percentage content of calcium in the leaves of the sand cultures was considerably lower than that of the soil culture, while the potassium relation was the reverse of this. These as well as previous analyses have shown that citrus trees have a large capacity for absorbing calcium. Coville with the aid of Breazeale (*3*) has reported the percentage of calcium oxid in the ash of other trees as compared with that of citrus. They have found that the percentage of CaO in dried freshly fallen leaves ranged from 1.73 per cent in Red Oak (*Quercus rubra*), to 6.77 per cent in orange (*Citrus aurantium*) with 10 other species of forest trees having intermediate values, basswood (*Tilia Americana*) attaining the second highest percentage, or 4.50 per cent.

It is of interest to note the consistently low percentage of magnesium (Mg) in the ash of the various parts of the trees in the sand and soil cultures.

The percentage of aluminum (Al) in the ash of the trunk, root, and rootlets is greater than that of the leaves or shoots. The small percentage of aluminum in the ash of the leaves and shoots indicates that these parts of the trees were thoroughly cleaned from adhering dust. As the nutrient medium used in the sand cultures contained no aluminum, we would expect the trees in these cultures to contain but little.

The percentage of iron (Fe) in the ash of the various parts of the three trees ranged from 0.2 to 0.9 per cent. The percentage of manganese (Mn) was usually very low. The percentage of chlorin (Cl) in the ash ranged usually below 1.2 per cent. The percentage of sulphate (SO_4) present in the ash was considerably higher than that of chlorin, being 2 to 5 per cent, although the rootlets in the sand culture showed over 8 per cent.

The percentage of phosphate (PO_4) in the ash of the shoots was considerably higher in both the sand and soil cultures than that of the leaves, with a decrease in the trunks and an increase in the roots. The ash of the rootlets in the sand cultures contained about 17 per cent phosphate as compared with 8 per cent in the ash of the shoots and 4 per cent in that of the leaves.

It is of interest to note the high percentage content of K, Ca, and PO_4 in the ash of the rootlets in the sand culture, constituting over 50 per cent of the ash of the rootlets, with a marked reduction in the percentage of CO_3. The percentage of silica-free ash in the rootlets approaches that in the leaves and is much greater than that in the shoots, trunk, or root.

The percentage of PO_4 in the ash of rootlets was greater than for any other portion of the tree. The largest percentages of mineral constituents appear to be deposited in those parts of the tree in which the assimilatory processes are most active.

The sap of citrus leaves possesses the power to neutralize considerable base. Kelley and Cummins (*6*) have shown that 10 cc. of normal leaf sap required 3 cc. of N/10 alkali for neutralization, while mottle-leaf

sap required 7.05 cc. of N/10 alkali. Protein formation or decomposition may bring about the formation of organic acids (*8, p. 237–249*). Since a large portion of the calcium in many plants is used for the neutralization of acids, there should be more calcium in plants producing large amounts of acid. The analyses indicate that this may be the case with orange trees, namely, that one of the chief functions of calcium in the tree may be the neutralization of the organic acids. Opportunity to test out this hypothesis further will be afforded in cultures now growing.

SUMMARY

(1) Data have been presented to show that the nutrition of young trees may be studied in a quantitative way under controlled conditions by the procedure described.

(2) The reaction of the nutrient solution used shifted from P_H 5.2 to 5.9 when the solution was in contact with sand, and further toward the neutral point when in contact with sand containing citrus rootlets.

(3) The relative amounts of new growth produced by the trees grown in sand or soil cultures were quite similar when one estimates the ratio between total dry weight and the dry weight of corresponding portions. The ratio of total transpiration to total dry weight of tree was approximately the same in both cases. The rootlets of the trees grown in soil were much more finely divided than those grown in sand.

(4) Analyses of the trees showed both a high calcium (Ca) content and a high Ca/N ratio.

(5) The percentages of total phosphorus in leaves and shoots were approximately equal.

(6) The leaves and rootlets contain in their dry matter the greatest percentage of total ash and of nitrogen.

(7) The ash obtained from all parts of the tree contained large amounts of carbonate, although the rootlets contained less than the other portions.

(8) The ash of all parts of the trees was markedly rich in potassium and relatively poor in sodium. The former was relatively uniform in the various parts of the tree, but the distribution of the latter was more variable.

(9) The various parts of the trees likewise contained large amounts of calcium and relatively small amounts of magnesium. Both constituents show fairly uniform distribution throughout the tree.

(10) The distribution of sulphate in the ash of the various parts of the tree is quite uniform with the exception that the rootlets contained nearly three times as much as the other portions of the tree.

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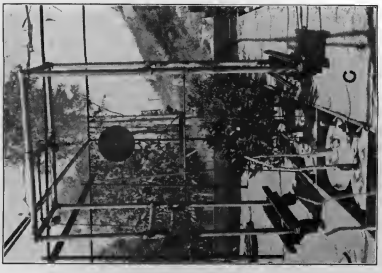
PLATE 1

A.—A typical trench with cans of sand containing orange trees just beginning growth.

B.—Showing attachment of air-suction hose to one of a series of drainage bottles. The drainage tube is removed from the drainage pipe. Can and bottle are raised considerably for photographic purposes.

C.—Device for weighing cans.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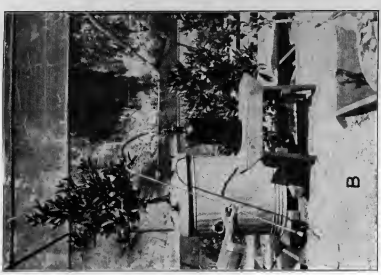

B


A


B
No. 364


A
No. 365

PLATE 2

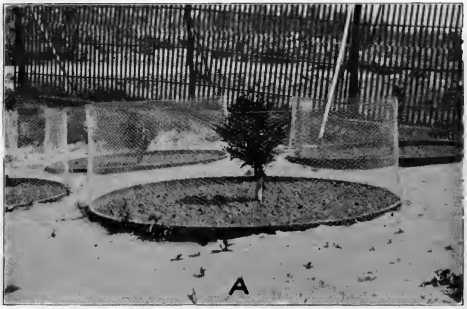
A.—Walnut tree after six months in covered tank of sand that receives a nutrient solution made up with distilled water.

B.—Valencia orange trees after 15 months in cans of sand to which Hoagland's nutrient has been added.

PLATE 3

A.—Valencia orange tree in large tank of soil. Seven feet 8 inches in diameter and 4 feet 4 inches in depth.

B.—Growth typical of trees in soil series 84-88 receiving Hoagland's nutrient at intervals.


A


B
USDA


1
2
3
4
5
6
7

PLATE 4

Orange leaves typical of the sand series. The lighter areas are due to reflection of the artificial light. Scale of inches at bottom of photograph.

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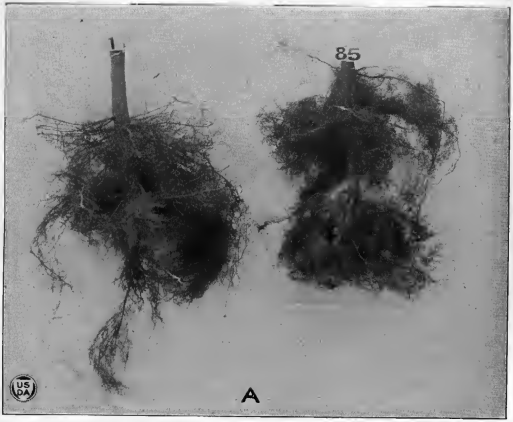
PLATE 5

A.—Air-dried root systems of trees 1 and 85, in sand and soil, respectively, receiving Hoagland's nutrient solution.

B.—Root systems of trees 3 and 86, in sand and soil, respectively, receiving Hoagland's nutrient solution. Note the more finely divided root systems in the soil culture.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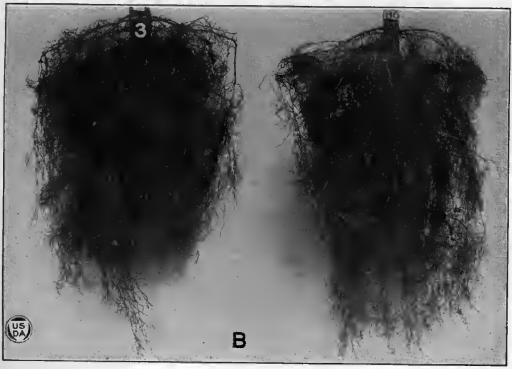
85
A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CONTENTS

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FURTHER STUDIES OF THE INHERITANCE OF "ROGUE" TYPE IN GARDEN PEAS (PISUM SATIVUM L.)[1]

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INTRODUCTION

Since the rediscovery of Mendel's classical paper on heredity, characters of the garden pea (*Pisum sativum*) have been used to illustrate typical Mendelian inheritance. In 1915 Bateson and Pellew (*1*) [2] published an account of the genetics of types of peas known as "rogues"—types in which heredity has thus far appeared to be non-Mendelian. This paper was followed by later publications (*2*, *3*) dealing especially with variations in the proportion of rogues obtained from seeds from different parts of the same intergrading intermediate plant. Briefly stated, the facts reported were as follows:

(*a*) Certain varieties of the garden pea, such as Early Giant, Ne Plus Ultra, and Duke of Albany, are characterized by broad, wavy stipules and leaflets, both having rounded, emarginate apices and broad, straight pods. Occasionally there occur in pure-lines of such varieties plants called "rogues" that are described as "wild" or "vetch-like." The rogues differ from the parent form mainly in the reduction in width of the foliar parts (stipules and leaflets) and of the pods. The stipules of the rogues are narrow and pointed; the pods are narrow and curved along the upper suture. Rogues are not produced with any definite regularity or in such numbers that a characteristic ratio can be established between the rogues and parent form.

(*b*) Between the parent types and the vetch-like rogue are found intermediate forms, especially in the variety Early Giant. The intermediates are of several classes and may grade almost insensibly into types or rogues. The most usual form is characterized by broad foliage in the early stages of development, but as the plant matures the stipules become pointed and more roguelike. Such a plant is called by Bateson and Pellew an intergrading intermediate. Genetically there are two kinds of intermediates: (1) Those whose progeny consists of many rogues and few intermediates; and (2) those whose progeny consists of few rogues and many intermediates. The rogues from both kinds of families breed true, while the intermediates tend to produce rogues and intermediates in proportions comparable to those of their parents, i. e., there are high and low rogue-producing strains. Nevertheless, no regular ratio of rogues to intermediates can be found in successive generations.

[1] Accepted for publication Jan. 18, 1923. This contribution records cooperative work between the Bureau of Plant Industry and the University of Michigan which was carried on at the Department of Botany, University of Michigan.

[2] Reference is made by number (italic) to "Literature cited," p. 852.

(*c*) Any zygote derived from the fusion of a type gamete with a rogue gamete, whichever way the cross is made, produces a rogue; an intermediate gamete on union with a type gamete produces an intermediate or nonrogue form.

(*d*) Rogues are apparently produced more often from the upper (roguelike) portion of the intermediate plants than from the lower (typelike) part. It is also found that pollen grains produce a greater ratio of rogue gametes to nonrogue gametes than do the ovules, and that the proportion of rogue gametes produced by the pollen grains increases more rapidly, in going from the lower to upper flowers, than the proportion of rogue gametes produced by ovules.

(*e*) Intergrading intermediates of strains not throwing many rogues were found to produce rogues no more often from pods of the upper parts of the plant than from the lower nodes.

(*f*) Rogues with narrow, pointed stipules and narrow, curved pods, from whatever source, breed true.

(*g*) The cross rogue × type, no matter how made, produces only rogues in the F_1 and succeeding generations. In the seedling stage of the F_1 hybrid until the seventh or eighth node is developed, many of the plants are typelike in the character of the stipules and leaflets. These plants at maturity, with few exceptions, are all rogues. The plants in the F_2 generation are recognizable as rogues at all stages of development. There is apparently no segregation in the F_1 hybrids, at sporogenesis, of the rogue and type factors.

(*h*) Intervarietal crosses of rogue with type plants of varieties producing rogues give results similar to crosses of rogue with the variety from which the rogue originated as far as the rogue characters are concerned. Side by side with the apparently non-Mendelian behavior of the rogue characters is found the expected Mendelian segregation of such characters as pod shape, color of seed, shape of seed, and height of plant.

Bateson and Pellew (1) have suggested in explanation of the anomalous genetic behavior of the rogue characters that they are due to somatic segregation of the type "elements" in the F_1 hybrid so that the type "elements" are missing from the germ plasm at the time of gamete formation. Intergrading intermediates are assumed to be mosaics of type and rogue tissue, rogue gametes being formed from rogue tissue and type gametes from type tissue.

The present paper is in part a confirmation of the above results. However, a different interpretation of the results is advanced. In addition, data are submitted of the F_1, F_2, and F_3 generations of intervarietal crosses of rogue and type plants with type plants of varieties that apparently do not produce rogues. These crosses have given entirely new results, from which important conclusions have been drawn.

EXPERIMENTAL METHOD

The experimental methods used in the work here reported were the same as those described in a previous paper (*4*). At that time the fact of cross-pollination of rogue flowers by bumblebees was noted. In growing large cultures of peas it is impossible to protect all the individual flowers with paper or cloth coverings. In 1921 an attempt was made to grow peas under wire mosquito netting. This method is too expensive to be used on a large scale, so that in the future it seems advisable to carry on as much as possible of the breeding work with rogues in the greenhouse during the winter.

The results of 1919 and 1920 may be used to illustrate the amount of volunteer crossing that may be expected in the field in crosses between Gradus and Rogue. The number of volunteer hybrids is sufficiently great to make necessary some sort of protection of the rogue plants.

In 1919, 2,932 F_2 plants of the crosses Gradus rogue with Gradus (Table II) were grown. Of the 2,932 plants, 65, or 2.3 per cent, were volunteer crosses made by insects in 1918. The out-crosses were detected by the appearance of such dominant Mendelian characters as smooth seed, blunt pods, and colored flowers in F_2 cultures that should have contained only recessives for the three characters mentioned. Any crosses inter se, or out-crosses with recessive strains, could not be detected, but, apart from a few plants of Early Giant and of Peter Pan, no varieties which did not exhibit one of the three dominant characters were planted in the experimental garden in 1918. In 1920, 3,475 F_2 and F_3 plants of the cross rogue with type (Tables II, III and IV) were raised. Among them were 42 plants of obviously foreign origin, or about 1.2 per cent, showing that the amount of volunteer crossing was considerably less in 1919 than in 1918.

Occasional volunteer crosses have been found among the rogues and intermediate rogues, but no count has been kept. However, only one case of cross-pollination by insects has been noted among the thousands of typical Gradus plants grown.

Plants used in crosses have been previously inbred for at least one generation. The rogues used were either picked from commercial plantings of Gradus or arose de novo in pure cultures of Gradus plants. In either case the rogue strains were identical and gave the same results in crosses.

Uncontrolled cross-pollination in Pisum on the scale reported here was unlooked for at the beginning of these experiments and no steps were taken to insure self-pollination. By the time it was realized that rogues were an exception to the general rule that self-pollination is invariable in Pisum it was too late to protect the hybrid rogue plants in 1919. Aside from the writer's own experience with self-pollinating varieties of peas, his garden technic was of course influenced by the general conclusion of geneticists and practical horticulturists that peas are normally self-pollinated. White (*15*) found no cross with peas having yellow cotyledons among 10,000 seeds with green cotyledons grown at the Brooklyn botanic garden.

BEHAVIOR OF GRADUS ROGUES WHEN CROSSED WITHIN THE GRADUS STRAIN

F_1 GENERATION OF CROSSES BETWEEN GRADUS AND GRADUS ROGUE

In a former publication (*4*) the results in the F_1 generation of crossing typical Gradus with Gradus rogue [3] are reported.

The F_1 seedlings were variable in regard to stipule shape, showing gradations from typelike to roguelike plants. At maturity with one exception the F_1 plants were all rogues.

In addition to the hybrids between rogue and type previously listed by the author (*4*), 134 F_1 plants from 37 other crosses were grown, making a total from all sources of 282 F_1 plants of the cross Gradus × Gradus rogue, and reciprocal. Of the 282 plants all but three were classified as rogues at maturity.

[3] The name "Gradus rogue" or "Rogue" as used throughout this paper refers to the same type of mutation as that previously referred to (*4*) as a "Rabbit-ear" Rogue.

Two of the exceptional plants occurred in a single F_1 culture,[4] of which two plants were thorough rogues, while two others were intergrading intermediates, i. e., they were typelike at the lower nodes, but at upper nodes the stipules were pointed but much broader than in typical rogues. In the F_2 generation the progeny of the intergrading intermediates consisted of 32 and 33 rogues, respectively.

The third exceptional plant among the F_1 of rogue × type was described in 1919 (*4*). Its offspring in 1919 consisted of 19 intergrading intermediates and 15 type plants. The same cross and its reciprocal produced 7 other F_1 plants, described as rogues at maturity. From the character of the F_2 progenies (Table I) it is probable that the F_1 plants were in reality extreme intergrading intermediates, as the F_2 is characteristic of the progeny of intergrading intermediates. The plants behaved as intergrading intermediates of the sort described by Bateson and Pellew (*1*) as producing types, intergrading intermediates, and few or no rogues.

TABLE I.—*F_1 and F_2 generation of atypical hybrids of Gradus rogue × Gradus type and reciprocal*

1919 culture No.	Pedigree.	F_2		
		Rogues.	Intergrading intermediates.	Types.
9.1207	(G13–1–6–1t×G19–1–3r)—1	0	27	0
9.1208	(G13–1–6–1t×G19–1–3r)—2	0	23	0
9.1209	(G13–1–6–1t×G19–1–3r)—3	0	9	2
9.1210	(G13–1–6–1t×G19–1–3r)—4	0	0	23
9.1216	(G19–1–3r×G13–1–6–1t)—1	2	8	3
9.1217	(G19–1–3r×G13–1–6–1t)—2	0	27	0
9.1218	(G19–1–3r×G13–1–6–1t)—3	0	0	4
9.1219	(G19–1–3r×G13–1–6–1t)—4..Type foliage	0	19	15

F_2 AND F_3 GENERATIONS OF CROSSES BETWEEN GRADUS AND GRADUS ROGUE

From 184 F_1 hybrids of Gradus × Gradus rogue and reciprocal 4,319 F_2 plants were raised in 1919 and 1920. In the seedling stage the greater portion were easily recognizable as rogues; others could be termed intermediates, but none showed the typelike appearance found among the F_1 seedlings. (Among the 4,319 plants shown in Table II, 81, or 1.8 per cent, were from foreign pollination, as explained elsewhere in this paper. The volunteer out-crosses were generally distinguished in the seedling stage as plants with typelike stipules.) At maturity the plants were either rogues or various sorts of intermediates. Thirty-nine F_2 rogues gave in the F_3 generation 575 plants, all rogues except five plants (0.8 per cent). The products of volunteer out-crossing in 1919 are shown in Table III.

[4] The intergrading intermediates were plants No. 3 and No. 4 of cross (G10–1–12–1t×G23–1–1–16r) in the F_1 generation.

TABLE II [a].—*Gradus rogue × Gradus type and reciprocal in the F_2 generation*

Culture No.	Rogues.	Crosses.
1919:		
9.1200	10	
9.1201	3	
9.1202	13	
9.1203	16	
9.1204	4	
9.1205	10	
9.1206	3	
9.1211	29	
9.1212	39	
9.1213	36	
9.1214	35	
9.1215	54	2
9.1220	4	
9.1221	4	
9.1222	2	
9.1223	2	
9.1226	12	
9.1227	14	
9.1251	80	
9.1252	82	
9.1253	86	
9.1254	10	
9.1255	208	
9.1265	37	3
9.1266	27	
9.1267	24	
9.1268	51	1
9.1269	15	1
9.1270	37	
9.1271	39	
9.1272	47	
9.1273	28	
9.1274	27	3
9.1275	34	4
9.1276	57	3
9.1277	54	
9.1278	43	1
9.1279	6	
9.1280	19	
9.1284	118	4
9.1285	73	1
9.1286	36	
9.1287	49	1
9.1288	85	2
9.1289	22	1
9.1293	6	1
9.1294	52	4
9.1295	73	12
9.1296	54	
9.1297	19	
9.1298	17	2
9.1299	43	1
9.1300	32	
9.1301	95	
9.1302	77	7
9.1304	45	1
9.1303	15	
9.1305	71	1
9.1306	46	
1919—Continued.		
9.1307	29	
9.1308	18	
9.1309	25	
9.1316	58	2
9.1317	13	3
9.1318	44	
9.1319	73	
9.1320	108	4
9.1321	31	
9.1322	43	
9.1323	4	
9.1324	21	
9.1325	25	
9.1326	46	
Total	2,867	65
1920:		
0.89	15	2
0.90	17	1
0.91	12	
0.92	37	1
0.93	12	
0.94	7	
0.95	15	1
0.96	7	
0.97	30	
0.98	48	1
0.99	10	
0.100	6	
0.101	14	
0.102	7	
0.103	15	
0.104	14	1
0.105	14	
0.106	12	
0.107	30	
0.108	26	
0.109	10	
0.110	9	
0.112	21	2
0.113	20	1
0.114	13	
0.115	8	
0.116	18	
0.117	18	
0.118	15	
0.119	20	
0.120	22	
0.121	21	
0.122	18	
0.123	31	
0.124	12	
0.125	25	
0.126	14	
0.127	25	
0.128	22	
0.129	16	
0.130	15	

[a] In Tables II, III, and IV, unless otherwise noted, the parent plants were rogues in each instance. The column headed "Crosses" shows the number of volunteer hybrids occurring in the various cultures.

TABLE II.—*Gradus rogue X Gradus type and reciprocal in the F_2 generation*—Continued.

Culture No.	Rogues.	Crosses.	Culture No.	Rogues.	Crosses.
1920—Continued.			1920—Continued.		
0.131	36	.....	0.199	9	1
0.184	27	.....	0.200	32	.....
0.185	20	.....	0.202	6	.....
0.186	23	.....	0.203	15	.....
0.187	38	.....	0.204	18	.....
0.188	37	.....	0.205	30	.....
0.189	34	.....	0.206	37	2
0.190	8	.....	0.207	3	.....
0.191	26	2	0.208	14	.....
0.192	10	.....	0.209	39	.....
0.193	26	.....	0.210	36	.....
0.194	21	.....	0.211	7	.....
0.195	31	.....	0.212	2	.....
0.196	29	.....	0.213	32	.....
0.197	7	.....			
0.198	21	1	Total (1919–20).	4,238	81

TABLE III.—*Gradus rogue × Gradus type and reciprocal in the F_3 generation*

Culture No.	Rogues.	Crosses.	Culture No.	Rogues.	Crosses.
1920:			1920—Continued.		
0.371	22	.....	0.391	10	.....
0.372	10	.....	0.392	15	.....
0.373	23	.....	0.393	12	.....
0.374	12	.....	0.394	14	.....
0.375	11	.....	0.395	16	.....
0.376	13	.....	0.396	24	2
0.377	16	.....	0.397	3	.....
0.378	13	.....	0.398	25	.....
0.379	3	.....	0.399	17	1
0.380	6	.....	0.400	21	.....
0.381	6	.....	0.401	18	.....
0.382	15	.....	0.402	11	.....
0.383	10	.....	0.403	22	.....
0.384	20	.....	0.404	17	.....
0.385	12	.....	0.405	18	.....
0.386	13	.....	0.406	23	.....
0.387	17	.....	0.407	23	2
0.388	17	.....	0.408	18	.....
0.389	15	.....			
0.390	14	.....	Total	575	5

F_1 AND F_2 GENERATIONS OF CROSSES BETWEEN GRADUS TYPE AND THE F_1 OF GRADUS TYPE WITH GRADUS ROGUE

The cross type × (type × rogue) gave an F_1 of 88 plants which showed a wide range of variation from typelike to roguelike plants in the seedling stage, but which at maturity were all rogues. Sixty-six F_1 plants of the sesquireciprocal crosses gave an F_2 progeny (Table IV) of 1,477 rogues, 6 intermediate types, and 22 volunteer hybrids (1.4 per cent).

The exceptional intermediate types occurred in the F_2 culture 0.322, which also contained 23 rogues and 1 out-crossed plant. Seven other families from the original cross which produced culture 0.322 gave only rogues in the F_2 generation.

TABLE IV.—*Back-crosses Gradus type×(Gradus type×Gradus rogue) in F_2 generation*

Culture No.	Rogues.	Others.	Crosses.	Culture No.	Rogues.	Others.	Crosses.
1920:				1920—Contd.			
0.216	3	.....	.....	0.325	23	.....	.....
0.217	2	.....	.....	0.326	16	.....	1
0.292	36	.....	2	0.327	22	.....	.....
0.293	25	.....	.....	0.328	17	.....	.....
0.294	23	.....	.....	0.329	40	.....	.....
0.295	23	.....	.....	0.330	31	.....	.....
0.296	2	.....	.....	0.331	41	.....	.....
0.297	31	.....	.....	0.333	18	.....	.....
0.298	18	.....	.....	0.334	54	.....	1
0.299	11	.....	.....	0.335	22	.....	.....
0.300	11	.....	.....	0.336	36	.....	.....
0.301	38	.....	.....	0.338	73	.....	.....
0.302	12	.....	.....	0.341	18	.....	1
0.303	27	.....	.....	0.342	21	.....	.....
0.304	1	.....	.....	0.343	20	.....	2
0.305	6	.....	.....	0.344	6	.....	.....
0.306	27	.....	.....	0.345	28	.....	.....
0.307	17	.....	.....	0.346	20	.....	.....
0.308	20	.....	1	0.347	15	.....	.....
0.309	20	.....	.....	0.348	23	.....	.....
0.310	16	.....	.....	0.349	18	.....	.....
0.311	44	.....	4	0.350	23	.....	.....
0.312	33	.....	.....	0.351	15	.....	.....
0.313	17	.....	.....	0.352	30	.....	.....
0.314	10	.....	.....	0.353	26	.....	.....
0.315	44	.....	.....	0.355	7	.....	.....
0.316	45	.....	2	0.356	13	.....	.....
0.317	38	.....	2	0.357	18	.....	5
0.318	7	.....	.....	0.358	27	.....	.....
0.319	16	.....	.....	0.368	14	.....	.....
0.320	31	.....	.....	0.369	22	.....	.....
0.321	3	.....	.....	0.370	15	.....	.....
0.322	23	6	1				
0.324	35	.....	.....	Total...	1,487	6	22

STATISTICAL STUDY OF THE INHERITANCE OF STIPULE SHAPE IN GRADUS ROGUE AND GRADUS TYPE

In order to get a more exact comparison of the rogues and types, on the one hand, and the F_1 and F_2 generations of hybrids between the two, measurements of the length and width of the stipules from the fourth to the ninth node were made in the various categories of plants. The ratio of width to length of stipule was then calculated. Because of disease and mechanical injury the same number of stipules could not be measured at each node in each group of plants. The class frequency distributions (Table V) are, therefore, expressed as percentages of the total number of variates. In this manner 49 pure type plants, 43 pure rogues, 69 F_1 hybrids, and 104 F_2 plants were measured.

The mean, standard deviation, and coefficient of variation of the ratios at each node and for the plant as a whole were calculated from the data in Table V. These statistical constants are given in Table VI. Figure 1 shows the graphs obtained by plotting the means for each node in each

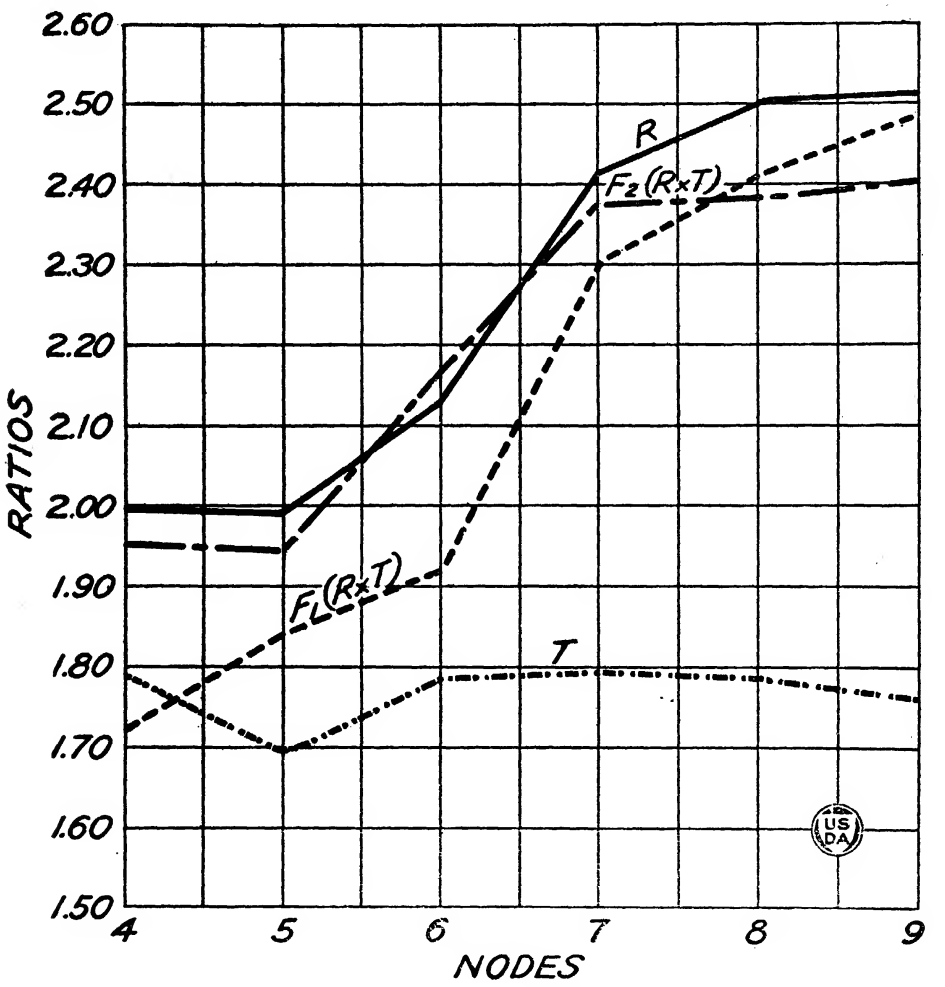

FIG. 1.—Graphs obtained by plotting the mean ratio of $\frac{\text{length of stipule}}{\text{width of stipule}}$ at the fourth to ninth nodes of Gradus type (T), Rogue (R), and the F_1 and F_2 hybrids of crosses between Gradus and Gradus rogue, based on data from Table VI.

group of plants. The similarity of the F_1 and F_2 generations to the rogue parent is easily seen by inspection of Figure 1 and Tables V and VI. The F_1 hybrids up to the seventh node are like the type, or intermediate between the type and rogue; above the seventh node the plants become rapidly more roguelike. At all nodes the F_1 plants appear more variable than Gradus and are like the rogues in this respect.

TABLE V.—*Frequency distribution of ratio* $\left(\frac{\text{length of stipule}}{\text{width of stipule}}\right)$ *at the fourth to the ninth nodes in Gradus rogue, Gradus, F_1 and F_2 hybrid plants. As the number of stipules measured at each node was not always the same, the ratios in any one class are expressed in percentages*

Ratio.	Nodes.						
	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	Total.
Gradus rogue:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1.25	.....	.....	.....	.....	.....	.....	.....
1.35	.....	.....	3.02	3.02	.....	.....	0.45
1.45	2.66	1.20	.....	0	.....	.....	.67
1.55	5.32	2.40	1.16	0	.....	.....	1.56
1.65	10.64	6.00	1.16	1.51	.....	.....	3.36
1.75	13.30	8.40	3.48	0	.....	3.56	4.93
1.85	9.31	18.00	9.28	0	3.75	3.56	7.84
1.95	5.32	12.00	10.44	3.02	0	1.78	5.79
2.05	11.97	25.20	24.36	4.53	2.50	0	12.55
2.15	10.64	8.40	10.16	4.53	2.50	0	6.73
2.25	15.96	8.40	16.24	10.35	6.25	5.34	10.76
2.35	6.65	4.80	10.16	12.08	16.25	5.34	9.64
2.45	5.32	3.60	.48	21.14	12.50	1.24	9.42
2.55	1.33	1.20	4.64	16.61	21.25	28.44	11.21
2.65	0	.....	1.16	19.63	16.25	19.58	8.52
2.75	1.33	.....	0	3.02	10.00	8.90	3.58
2.85	.....	.....	1.16	.....	0	5.34	.89
2.95	.....	.....	.....	.....	6.25	1.78	1.34
3.05	.....	.....	.....	.....	1.25	1.78	.45
3.15	.....	.....	.....	.....	1.25	.....	.22
	[a] 75	[a] 83	[a] 86	[a] 66	[a] 80	[a] 56	.....
Gradus:							
1.25	.....	.....	.....	.....	.....	.....	.....
1.35	5.40	1.08	2.24	.....	.....	.....	1.59
1.45	6.48	3.24	3.36	6.18	1.08	.....	3.78
1.55	10.80	19.44	8.96	10.30	7.56	9.52	11.35
1.65	11.88	24.84	11.20	13.39	15.12	21.42	15.93
1.75	19.44	23.76	30.24	19.57	29.16	33.32	25.30
1.85	35.64	18.36	20.16	23.69	27.00	19.04	24.70
1.95	3.24	7.56	13.44	13.39	16.20	10.90	10.95
2.05	4.32	0	6.72	7.21	2.16	4.76	4.18
2.15	0	1.08	3.36	6.18	0	0	.20
2.25	0	0	0	0	0	0	0
2.35	1.08	.....	.....	.....	.....	.....	.02
2.45	.....	.....	.....	.....	.....	.....	.....
	[a] 91	[a] 92	[a] 89	[a] 97	[a] 91	[a] 42	.....
F_1 hybrid:							
1.25	.85	.....	1.46	.....	.....	.....	.41
1.35	.85	.75	0	.77	.....	.....	.41
1.45	5.10	1.50	1.46	0	.....	.....	1.38
1.55	16.15	10.50	1.46	.77	.....	.....	4.98
1.65	30.60	16.50	10.22	.77	.....	.....	10.11
1.75	22.10	16.50	10.95	.77	1.60	.....	9.14
1.85	22.10	19.50	20.44	4.62	1.60	.....	12.18
1.95	4.25	10.50	13.14	5.39	1.60	.....	6.37
2.05	3.40	18.75	12.41	10.01	8.80	1.35	8.44
2.15	0	12.00	9.49	11.55	3.20	1.35	5.67
2.25	.85	9.09	10.95	13.86	12.00	10.80	8.72
2.35	1.70	.75	3.65	17.71	12.00	20.25	8.44
2.45	.....	0	.73	8.47	17.60	18.90	6.65
2.55	.....	0	2.19	14.63	19.20	24.30	8.86
2.65	.....	.75	.73	6.16	10.40	14.85	4.71
2.75	.....	.....	.....	2.31	7.20	5.40	2.21
2.85	.....	.....	.....	1.54	.80	1.35	.55
2.95	.....	.....	.....	.....	3.20	1.35	.69
	[a] 117	[a] 132	[a] 136	[a] 129	[a] 124	[a] 74	.....

TABLE V.—*Frequency distribution of ratio* $\left(\frac{\text{length of stipule}}{\text{width of stipule}}\right)$ *at the fourth to the ninth nodes in Gradus rogue, Gradus* F_1 *and* F_2*, hybrid plants. As the number of stipules measured at each node was not always the same, the ratios in any one class are expressed in percentages*—Continued.

Ratio.	Nodes.						
	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	Total.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
F_2 hybrid:							
1.05	.49	.....	.....	.....	.....	.....	.08
1.15	.96	.49	.....	.....	.....	.....	.24
1.25	.96	0	.....	.....	.....	.....	.16
1.35	.96	.49	.....	.....	.....	.....	.24
1.45	.48	.49	.....	.....	.....	.....	.16
1.55	1.45	.98	.....	.....	.....	.....	.41
1.65	6.26	3.42	.....	.....	.....	.....	1.62
1.75	11.20	14.20	.96	.....	.....	.....	4.37
1.85	15.80	22.42	4.33	1.44	.49	.49	7.52
1.95	14.50	20.42	3.85	1.44	.49	.49	6.87
2.05	26.10	21.40	27.40	4.82	4.90	2.94	14.63
2.15	8.20	6.34	21.61	8.18	8.78	5.88	9.70
2.25	8.67	3.42	11.52	16.33	17.55	15.70	13.90
2.35	1.45	3.90	5.30	26.40	20.00	25.00	14.70
2.45	.96	.49	2.88	14.90	17.55	22.10	10.20
2.55	.48	.49	.48	18.20	21.92	13.20	9.55
2.65	.96	0	.....	4.81	4.39	10.30	3.48
2.75	.48	.49	.....	2.40	3.42	2.40	1.53
2.85	.....	.....	.....	.96	.49	1.47	.48
	[a] 207	[a] 205	[a] 208	[a] 208	[a] 205	[a] 204	.....

[a] The actual number of measurements from which the frequency distributions (expressed in percentages) were derived.

TABLE VI.—*Constants for stipule shape calculated for each node separately and for the plant as a whole, based on data in Table V*

	Nodes.						
	Fourth.	Fifth.	Sixth.	Seventh.	Eighth.	Ninth.	All.
Type:							
M	1.705	1.706	1.782	1.797	1.782	1.766	1.765
σ	.205	.155	.170	.177	.130	.130	1.53
C. V.	12.05	8.11	9.55	9.88	7.30	7.32	8.66
Rogue:							
M	2.001	1.994	2.133	2.411	2.500	2.515	2.259
σ	.294	.223	.223	.288	.257	.266	.339
C. V.	14.70	11.20	10.46	11.53	10.28	10.59	15.00
F_1 hybrid:							
M	1.725	1.846	1.926	2.308	2.410	2.485	2.118
σ	.174	.217	.275	.266	.261	.172	.377
C. V.	10.11	11.79	14.32	11.56	10.82	6.93	17.79
F_2 hybrid:							
M	1.957	1.941	2.173	2.370	2.384	2.405	2.205
σ	.256	.206	.148	.184	.170	.171	.275
C. V.	13.08	10.61	6.81	7.75	7.13	7.71	12.47

The F_2 hybrids differ from the F_1 generation in being remarkably like the pure rogues at all stages in their development. The stipules at the fourth, fifth, and sixth nodes of the former are especially different from those of the F_1 plants. The measurements serve to emphasize what is plainly seen by inspection of the cultures, namely, that the F_2 seedlings resemble the pure rogues of the same age rather than the young F_1 plants which so generally exhibit a typelike character. To the eye, neither of the hybrid generations, at maturity, differs in any appreciable way from the rogue parent.

Besides measuring the stipules at the fourth to the ninth nodes in the case of the rogue, type, and F_1 and F_2 hybrids, measurements were made of four stipules per plant from about the eighth to the fourteenth node of mature individuals in each category. The mature plants measured included those used in the comparison by nodes of the four groups, and, in the case of all but the F_1 hybrids, additional plants were measured.

In Table VII are shown the frequency distributions and statistical constants calculated for each of the four categories of plants. The range of variation is strikingly significant, the range of the type not overlapping that of the rogue. The F_1 and F_2 hybrids have a range of variation equal in extent to that of the rogue parent. The F_2 generation differs from the pure rogue and the F_1 in that it is less variable as indicated by the coefficients of variation (Coefficient Variation): F_2 generation, 5.84 ± 0.2677; rogue, 7.06 ± 0.2912; F_1 generation, 7.09 ± 0.3662, respectively. The probable errors of the differences between the coefficients of variation of the pure rogue and of the F_2, and of the F_2 and F_1, are practically three times the difference, in each case indicating that the differences are real. However, the differences in variability of the three groups as shown by the stipule measurements of the mature plant were not apparent on inspection of the cultures.

The resemblance of the F_1 and F_2 hybrids to the pure rogues is further accentuated by a comparison of the mean stipule ratio of the three. The mean for the rogue parent is 2.339 ± 0.0096; for the F_1, 2.383 ± 0.0196; and for the F_2 it is 2.360 ± 0.0089. There is no significant difference between any of the three means.

Thus in regard to variability, range of variation, and mean stipule ratio, the two hybrid generations resemble only the rogue parent. The influence of the type parent, in crosses between type and rogue is apparent only in the F_1 plant and at only the lower nodes.

CROSSES BETWEEN THE GRADUS STRAIN AND MUMMY, A NONROGUE-PRODUCING VARIETY.

In 1918 the English "Mummy" pea was used in crosses with Gradus rogue and Gradus type. Seed of the Mummy variety was first obtained from O. E. White of the Brooklyn Botanical Garden (White's No. P1–3–4–2), and only this strain has been used in the crosses. In Mummy, fasciation of the main stem is characteristic, but does not appear until the plant is well developed. With the appearance of the fasciated condition the stipules become narrower and more pointed than those at the lower and middle nodes of the same plant. Plate 2, A, B, C is of a mature plant of Mummy. Although having stipules much narrower than in Gradus type, no rogue comparable to those found in Gradus have ever been observed by the writer among the several thousand

TABLE VII.—*Frequency distributions and statistical constants of ratio of* $\frac{\text{length of stipule}}{\text{width of stipule}}$ *for Gradus type, Gradus rogue, P 16, and of the* F_1 *and* F_2 *generations of various hybrids and hybrid segregates from crosses between the three parent types. The frequency distributions and statistical constants for the* F_2 *generation of Gradus rogue×Mummy are based on data in Table XIV.*

Types, hybrids and segregates.	1.35	1.45	1.55	1.65	1.75	1.85	1.95	2.05	2.15	2.25	2.35	2.45	2.55	2.65	2.75	2.85	2.95	3.05	Total.	M	σ	Coefficient variation.
Gradus type (P_1)	1	0	16	64	33	18	3	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	135	1.693±0.0107	0.098±0.0075	5.78±0.2381
Gradus rogue (P_1)	.....	.....	.....	.....	.....	.....	.....	7	21	33	30	22	10	8	4	.....	.....	.....	135	2.339± .0096	.165± .0068	7.06± .2912
Gradus type×Gradus rogue (F_1)	.....	.....	.....	.....	.....	.....	.....	6	4	18	23	12	11	10	2	.....	.....	.....	86	2.383± .0196	.169± .0087	7.09± .3662
Gradus type×Gradus rogue (F_2)	.....	.....	.....	.....	.....	.....	.....	4	3	33	30	23	10	5	1	.....	.....	.....	109	2.360± .0089	.138± .0063	5.84± .2677
Mummy (P_1)	.....	.....	.....	.....	.....	3	29	68	56	15	5	.....	.....	.....	.....	.....	.....	.....	176	2.088± .0050	.100± .0073	4.77± .1719
Gradus type×Mummy (F_2), all	.....	1	1	7	21	19	24	8	2	1	2	.....	.....	.....	.....	.....	.....	.....	87	1.868± .0115	.159± .0081	8.51± .4382
AA and *Aa* segregates (colored)	.....	.....	.....	3	12	15	11	8	2	0	2	.....	.....	.....	.....	.....	.....	.....	53	1.905± .0142	.154± .0101	8.08± .5394
A, B, C, segregates (purple)	.....	.....	.....	2	9	10	8	6	2	0	2	.....	.....	.....	.....	.....	.....	.....	39	1.909± .0175	.162± .0124	8.48± .6523
A, B, segregates (pink)	.....	.....	.....	1	3	5	3	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	14	1.864± .1950	.112± .0136	6.00± .7415
aa, segregates (white)	.....	1	1	4	6	3	6	0	0	1	.....	.....	.....	.....	.....	.....	.....	.....	22	1.710± .0260	.181± .0184	10.58± 1.0873
Gradus rogue×Mummy (F_2) all	.....	.....	1	11	37	86	143	167	122	136	187	231	169	131	58	39	12	4	1,534	2.307± .0049	.288± .0035	12.48± .1542
AA, Aa, segregates (colored)	.....	.....	.....	4	18	52	107	133	88	100	152	181	142	107	46	33	11	4	1,178	2.326± .0016	.281± .0011	12.08± .0475
A, B, C, segregates (purple)	.....	.....	.....	4	12	40	79	95	66	78	118	132	106	79	29	24	9	2	873	2.325± .0063	.278± .0045	11.95± .1805
A, B, c, segregates (pink)	.....	.....	.....	.....	6	12	28	38	22	22	34	49	36	28	17	9	2	2	305	2.336± .0110	.286± .0078	12.24± .3392
aa, segregates (white)	.....	.....	1	7	19	34	36	34	34	36	35	50	27	24	12	6	1	.....	356	2.230± .0106	.297± .0075	13.31± .3338
Gradus rogue×Mummy (F_2) all (omitting Nos. 0.1059 and 0.1093)	.....	.....	1	8	30	72	102	122	110	133	183	230	169	131	58	39	12	4	1,404	2.333± .0050	.280± .0036	12.00± .1549
AA and *Aa*, segregates (colored)	.....	.....	.....	3	16	45	74	94	77	97	148	180	142	107	46	33	11	4	1,077	2.355± .0056	.275± .0039	11.67± .1720
A, B, C, segregates (purple)	.....	.....	.....	3	12	37	58	64	60	75	115	132	106	79	29	24	9	2	805	2.349± .0064	.271± .0045	11.53± .1964
A, B, c, segregates (pink)	.....	.....	.....	.....	4	8	16	30	17	22	33	48	36	28	17	9	2	2	272	2.375± .0110	.269± .0078	11.32± .3427
aa, segregates (white)	.....	.....	1	5	14	27	28	28	33	36	35	50	27	24	12	6	1	.....	327	2.258± .0110	.294± .0077	13.02± .3491
Gradus rogue×Mummy (F_2) all (Nos. 0.1059 and 0.1093)	.....	.....	.....	3	7	14	41	45	12	3	4	1	.....	.....	.....	.....	.....	.....	130	1.998± .0082	.139± .0058	6.95± .2912
AA and *Aa* segregates (colored)	.....	.....	.....	1	2	7	33	39	11	3	4	1	.....	.....	.....	.....	.....	.....	101	2.027± .0087	.127± .0060	6.26± .2982
A, B, C, segregates (purple)	.....	.....	.....	1	0	3	21	31	6	3	3	.....	.....	.....	.....	.....	.....	.....	68	2.038± .0095	.116± .0067	5.69± .3290
A, B, c, segregates (pink)	.....	.....	.....	.....	2	4	12	8	5	0	1	1	.....	.....	.....	.....	.....	.....	33	2.008± .0170	.145± .0120	7.22± .6025
aa, segregates (white)	.....	.....	.....	2	5	7	8	6	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	29	1.899± .0159	.127± .0112	6.68± .5899

plants of Mummy grown in the last three years. The Mummy variety differs from Gradus in several Mendelian characters.

NOMENCLATURE.—The factors concerned in the crosses of Mummy and Gradus and the symbols used to designate the factors are as follows:

A. Factor for flower color. A must be present to have any color develop. Alone A produces light purple flowers; a produces white flowers. Dominance is complete. Tedin, (*11*).
B. Interacts with A to produce pink flowers; b has no visible effect. Tedin, (*11*).
C. Interacts with A to produce violet flowers. Interacts with AB to produce dark purple; c has no visible effect on A or B. Tedin, (*11*).
N. Factor for normal stem as opposed to n for fasciated. Mendel, (*8*); White, (*14*).
X. Factor for mean stipule ratio of approximately 2.35. The allelomorph x in Gradus determines a ratio of about 1.70. (Described here.)
x′. Factor for stipule shape in Mummy allelomorphic to X in Gradus rogue and x in Gradus.
Y. Factor responsible in Mummy for mean stipule ratio of approximately 2.05. Its allelomorphy in Gradus determines a stipule ratio of about 1.70. (Described here.)

It is tentatively assumed that the change which takes place when Gradus produces Rogue is a point mutation of factor x to X.

To find the mean stipule ratios for which it is assumed Xx and Yy are responsible, the average ratio of width to length was obtained for four stipules from the middle nodes of each plant measured. In this manner the mean stipule ratio per plant was obtained for 135 Gradus types, 135 Gradus rogues, and 176 plants of the Mummy variety. Frequency distributions of the three groups of mean ratios were made (Table VII) and the mean, standard deviation, and coefficient of variation calculated for each category of plants. The mean stipule ratios were found to be: Gradus type 1.693 ± 0.0107; Gradus rogue, 2.339 ± 0.0096; and Mummy, 2.088 ± 0.0050.

In regard to the factors here considered, the three strains have the following genetic constitution:

Gradus type, aaBBCCxxyyNN.
Gradus rogue, aaBBCCXXyyNN.
Mummy, AABBccx′x′YYnn.

GRADUS TYPE × MUMMY

RELATION OF THE X AND X′ FACTORS FOR STIPULE WIDTH

From the data at hand, factors x and x′ are considered to have practically the same somatic expression in regard to stipule shape. The difference is in the ability of x to mutate to X, a quality lacking in x′, at least in the x′x′ or xx′ combination. In other respects the two allelomorphic factors x and x′ can be viewed as essentially indentical, as far as visible expression is concerned.

RELATION OF COLOR FACTORS A AND a

Considering the allelomorphic pair A and a in the F_2 generation of crosses of Gradus type with Mummy, the colored flowered plants (AA and Aa) and noncolored (aa) are found to be in close agreement with the expected 75 and 25 per cent (Table VIII).

TABLE VIII.—*Gradus×Mummy and Gradus rogue×Mummy in the F_2 generation (aa×AA)*

TYPE×MUMMY

	AA, Aa.	aa.	Total.
Observed	563	179	[a] 742
Percentage found	75.88	24.12	.....
Percentage expected	75.00	25.00	.....
Difference (D)	0.88	0.88	.....
Standard error (S. E.)	1.17	.....	.....
D/S. E	0.75	.....	.....

ROGUE×MUMMY

Observed	1178	356	1,534
Percentage found	76.79	23.21	.....
Percentage expected	75.00	25.00	.....
Difference (D)	1.79	1.79	.....
Standard error (S. E.)	.74	.....	.....
D/S. E	2.41	.....	.....

[a] Data from 25 F_2 families including 2 families (34 plants) classified as to color segregation and measured for ratio of width to length of stipule but not classified as to stipule shape, i. e., as types or intermediates.

RELATION OF COLOR FACTORS A AND C

The F_1 plants of Gradus type×Mummy were all purple-flowered. The F_2 generation gave the expected recombinations in approximately the proportion 56.25 per cent purple (with factors A, B, and C), 18.75 per cent pink (with factors A and B), and 25.00 per cent white (with factors aa), the observed number in each class being in sufficient agreement with that theoretically expected when two factors are independently inherited (Table IX).

TABLE IX.—*Segregation of factors for flower color in F_2 generation of cross Gradus with Mummy and of Gradus rogue with Mummy (aaBBCC×AABBcc)*

GRADUS×MUMMY

	Purple ABC.	Pink ABc.	White aBc, aBC.	Total.
Observed	406	157	179	742
Percentage found	54.72	21.16	24.12	.....
Percentage expected	56.25	18.75	25.00	.....
Difference	1.53	2.41	.88	.....
χ^2=2.8854.				
P=.3405.				

GRADUS ROGUE×MUMMY

Observed	873	305	356	1,534
Percentage found	56.91	19.88	23.21	.....
Percentage expected	56.25	18.75	25.00	.....
Difference	.66	1.13	1.79	.....
χ^2=3.0225.				
P=0.2214.				

From seeds of 25 F_1 hybrids, 742 F_2 hybrids were matured in 1920. A total of 708 individuals, belonging to 23 families, were classified according to stipule shape as Graduslike types (yy) and intermediates (YY or yy), the latter class being made up of plants resembling Mummy in stipule character. The remaining families, consisting of 34 plants, were not directly classified in the same way, but the mean stipule ratio per plant was calculated from measurements of the width and length of the stipules. A record of the flower color was kept for all the 742 F_2 plants (Tables VIII and IX).

Of the 708 plants classified according to stipule shape, 540 were grouped as intermediates resembling Mummy and 168 as Graduslike types (Table X), indicating a single factor difference, in regard to stipule shape, between Gradus type and Mummy. The expected percentage in the two groups is 75 per cent intermediates and 25 per cent types; the observed percentages, 76.27 per cent intermediates and 23.73 per cent Graduslike types, in the F_2, are well within the limits of the standard error. The standard error as used here is calculated according to the formula of Yule (*16*).

TABLE X.—*Segregation of stipule shape in F_2 generation of Gradus×Mummy* (yy×YY)

GRADUS×MUMMY

	YY, Yy	yy	Total.
Observed	540	168	708
Percentage found	76.27	23.73	.....
Percentage expected	75.00	25.00	.....
Difference (D)	1.27	1.27	.....
Standard error (S. E.)	1.09	.....	.....
D/S.E.	1.16	.....	.....

RELATION OF Y AND y, FACTORS FOR STIPULE SHAPE

In all, 37F_1 plants were grown in 1919 from the cross Gradus type with Mummy. In size and shape the stipules of the F_1 plants were intermediate between Mummy and Gradus type. The upper part of a branch of a mature F_1 plant is shown in Plate 3, A.

LINKAGE RELATION OF Yy AND Aa

The relation of the factor pairs Yy and Aa was determined from a count of 708 F_2 plants of the cross Gradus type with Mummy (aayy×AAYY). In Table XI are tabulated the observed numbers of the F_2 recombinations. The actual ratios of the four classes AY, aY, Ay, and ay indicate that factors AY and ay are linked. The calculated zygotic series is approximately 66 AY : 9 Ay : 9 aY : 16 ay; the gametic series approximately 4 : 1 : : 1 : 4; and the percentage of crossing-over for all the F_2 families is 20.41 ± 1.28 per cent, using Haldane's method (*6*) for the determining of zygotic and gametic series from observed F_2 frequencies.

TABLE XI.—*Linkage relation of* Aa *and* Yy *from F_2 generation of Mummy×Gradus*

	aayy×AAYY.				
	AY.	Ay.	aY.	ay.	Total.
Observed	476	63	64	105	708
Calculated	467	64	64	113	.....
Difference	9	1	0	8	.....
$\chi^2 = 0.7554$	.....	.....	.....	.....	.....

Plates 4 A, B, and 5 A, B, are of the upper parts of a Graduslike type and of an intermediate segregate, respectively, from the F_2 of Gradus × Mummy. The intermediate shown has stipules wider than the stipules of the F_1 plant of the same cross shown in Plate 3, A. The difference is largely due to the fact that the stipules of the F_1 plant are on a branch while those of the F_2 intermediate are on the main stem. Stipules on a branch are smaller and narrower than those of the main stem in nearly all instances, irrespective of the variety or strain.

It should be pointed out here that Y and y are considered as the factors chiefly responsible for the difference for stipule shape between Mummy and Gradus. It is highly probable, from the increased variability of the F_2 generation over that of the parents, that a number of modifying factors interact with Y and y to affect stipule shape.

RELATION OF FACTORS Aa FOR FLOWER COLOR AND Nn FOR CHARACTER OF STEM

It has been said before that stipules from the fasciated portion of the stem of Mummy are narrower than those of the apparently nonfasciated part of the same plant or from a normal plant. The F_1 plants of the crosses of Mummy with Gradus were normal stemmed and had narrow, intermediate, or Mummylike stipules. No accurate counts were made of the fasciated F_2 segregates, but apparently the expected 3 : 1 ratio of normal to fasciated plants occurred.

Since fasciated plants appeared, on the whole, to have narrower stipules than normal plants, it might be suggested that linkage existed between n and A, and N and a, so that the existence of a factor pair Yy would not have to be assumed. That such is not the case is shown by the results of White (*14*), who found factors Aa and Nn to be independently inherited. The two cases are comparable as the AAnn parent of the hybrids studied by White was from the same strain as that used by the present writer.

STATISTICAL EVIDENCE OF LINKAGE BETWEEN FACTORS AY AND ay

In addition to the stipule measurements of the 34 plants mentioned in the preceding section, stipules of 41 other plants were measured from the F_2 generation of Gradus × Mummy. Of the 75 plants measured (Table VII), 53 were A segregates (39 purples and 14 pinks) and 23 were a segregates (whites). The mean stipule ratio of the F_2 plants as a whole was found to be 1.868 ± 0.0126, for the A's 1.905 ± 0.0142, and for the a's 1.719 ± 0.0260. Considering a difference between the means of at least three times the probable error of the difference as being significant, it is found that the difference between the means of the A and a segregates is significant, since it is five times the standard error.

The same relation holds between the mean ratio of the colored and white F_2 segregates, whether the A plants be considered collectively, or separately as purples (with the factors A and B) and as pinks (with the factors A and b), the mean of the purples being 1.909 ± 0.0175 and of the pinks 1.864 ± 0.0142 as compared with 1.905 ± 0.0142 for the A segregate as a whole (Table VII).

No essential difference exists between the means of Gradus and the white-flowered (aa) F_2 segregates from the cross Mummy with Gradus. However, the hybrid whites show a greater variability than do the pure whites, the coefficient of variation of the former being 10.58 ± 1.0870 compared with 5.78 ± 0.2381 for the latter.

The increased variation of the F_2 aa plants is probably due for the most part to crossing-over of the factors Yy for stipule shape and partly to the recombination of modifying factors affecting the same character.

The mean ratio of the A segregates, 1.905 ± 0.0142, is significantly less than that of the Mummy parent, 2.088 ± 0.0050, and the variability of the former is greater than that of the pure AA plants. The increased variability of the colored-flowering segregates, besides being accounted for largely by crossing-over, could, in addition, be due to a dissimilarity in stipule shape of AA and Aa plants. The heterozygous plants being in excess would tend to lower the mean of the population. Actually the F_1 plants did appear to have stipules intermediate in shape between those of the two parents, but no measurements are available for comparison.

It is realized that the number of F_2 individuals measured is small but, taken alone, the statistical results would offer fairly good evidence of linkage between the factor or factors for stipule shape and the A factor for flower color. In connection with the F_2 classification based on stipule shape and flower color, the fact of linkage between the Aa and Yy factors is well established.

GRADUS ROGUE × MUMMY

The Gradus rogues used in the series of crosses with Mummy were all descended from a rogue plant, S15, selected in 1916 from a commercial planting of Gradus. Progeny of the same rogue, S15, when crossed with Gradus type behaved as typical rogues in inheritance (cultures 9.1270–9.1275, 9.1320, and 9.1321, Table II).

The F_1 plants (Table XII) of Gradus rogue × Mummy, and reciprocal had, with one exception, rogue stipules, and in this respect appeared similar to the pure rogues. As volunteer crossing occurred in the F_1 of Gradus rogue × Gradus, it might be expected to take place among the F_1 hybrids of rogue and Mummy. Since the F_1 plants in the latter cross exhibited most of the dominant Mendelian factors present in any of the strains growing in the experimental gardens in 1919, it would be difficult to detect volunteer hybrids in the F_2 generation (F_1 out-crosses). However, as the F_1 hybrids were very late in flowering, cross-pollination by insects, if not entirely absent was cut down to a minimum.

TABLE XII.—*Pedigrees of F_1 cultures of Gradus rogue × Mummy (P16) and reciprocal*

Culture.	Pedigree.	Mature plants.
9.720	(S15–2–5r x P16–10)—1 to 6	Rogues.
9.721	(S15–2–12r x P16–10)—1 to 9	Do.
9.722	(S15–2–3r x P16–10)—1 to 7	Do.
9.723	(S15–2–19r x P16–10)—1 to 6	Do.
9.725	(S15–1–6r x P16–10)—1 to 3	Do.
9.726	(S15–1–9r x P16–10)—1 to 8	Do.
9.727	(S15–3–11r x P16–10)—1 to 8	All rogues but No. 6, which resembled F_1 of P 16 x type.
9.728	(S15–3–4r x P16–10)—1 to 3	Rogues.
9.729	(S15–3–5r x P16–10)—1 to 8	Do.
9.730	(P16–12 x S15–4–5r)—1 to 11	Do.
	Total 69	

As some uncontrolled pollinations may have occurred, there is possibly an experimental error in the results which should be kept in mind when interpreting the F_2 data. On this account the crosses are being repeated, and it is planned to grow the F_1 generation under glass for protection. It is believed, however, that the data here presented are of sufficient interest to warrant publication at this time and that the errors from out-crossing will prove minimal.

RELATION OF THE A AND A FACTORS FOR FLOWER COLOR

The F_1 plants of the crosses between rogue and Mummy had colored (purple) flowers. In Table VIII is shown the percentage of colored-flowered (AA + Aa), segregates to white-flowered (aa), segregates in the F_2 generation of rogue × Mummy, and reciprocal. The observed percentages, 76.79 per cent AA and Aa plants and 23.21 per cent aa segregates conform to expectation.

RELATION OF A AND C, FACTORS FOR FLOWER COLOR

Gradus rogue × Mummy in the F_2 generation gave approximately the expected 56.25 per cent purples (with factors A, B, and C); 18.75 per cent pinks (with factors A and B); and 25.00 per cent whites (with factors aa), the observed percentages being 56.91 per cent, 19.88 per cent and 23.21 per cent, respectively, the deviations being well within the limits of error due to random sampling (Table IX).

Although the dovetailing of observation and theory is good for the three classes of color segregates as a whole, there is in the F_2 in both the case of Mummy × Gradus and Mummy × Gradus rogue, a deficiency of white segregates. The deficiency of aa plants can be explained by differences in disease resistance as well as by variations in time of maturing between colored and noncolored F_2 segregates. On the whole, plants with colored flowers are more resistant to disease than are the white flowered forms, so that proportionately more of the latter would be lost before they reached maturity. For unavoidable reasons the F_2 data were collected late in the season after a number of the plants had matured. Plants whose stipules were too dried to measure were not classified as to flower color but their occurrence was noted. Tschermak (*12*), Hoshino (7), and others have noted a correlation between the presence of the A factor and the habit of late flowering. In all probability the same correlation existed in these cultures. On this account, relatively more of the white segregates than of the colored ones were too mature to be used in this study at the time the stipule measurements were made.

RELATION OF N AND n FACTORS FOR NORMAL AND FASCIATED STEM, RESPECTIVELY

The 69 F_1 plants of Gradus rogue × Mummy had normal stems. No F_2 counts were made of the normal and fasciated plants, but apparently there occurred the expected Mendelian segregation.

RELATION OF Xx′ AND Yy FACTORS FOR STIPULE SHAPE

In 1919, 69 F_1 hybrids (Table XII) were grown from the seed of 13 pollinations made the preceding year. The young plants resembled Mummy in regard to stipule shape and were uniform in appearance.

With the exception of one plant (9.727–6), the hybrids were all classified as rogues at maturity. Plate 3, B, is of the upper part of a branch of a mature F_1 plant from Mummy × Gradus rogue.

The exceptional plant (9.727–6) resembled the F_1 of Mummy × Gradus, although 7 other plants from the same cross had rogue stipules. The atypical F_1 hybrid (9.727–6) produced 61 F_2 plants in 1920 (Culture No. 0.1084). At the time the notes were taken 14 individuals were too dried to classify. Of the 47 remaining plants, 23 were grouped as intermediates, with stipules intermediate in shape between those of Mummy and those of Gradus, and 24 were classed as Graduslike types. Apparently there were no rogues among the F_2 offspring.

Besides the F_2 family (0.1084) derived from the atypical F_1 plant, five other F_2 cultures were classified by inspection as rogues and nonrogues, the latter being subdivided into broads and intermediates (Tables XIII and XIV). The number of individuals in each of the three categories is shown in Table XIV. The ratio of rogues to nonrogues indicates a single factor difference between the two parents as being primarily responsible for the difference in stipule shape. The observed ratio of 77.12 per cent rogues to 22.88 per cent nonrogues agrees with that theoretically expected being within the error due to the limitations of random sampling.

On the assumption of a single factor pair, X and x′, primarily responsible for a difference in mean stipule ratio of 2.05 in Mummy and as compared with 2.35 in Gradus rogue, one would expect the F_2 to segregate only into rogues and Mummylike plants. Actually three classes based on stipule shape were distinguished, namely, rogues, intermediates (Mummylike), and broads (Table XIV), the latter having stipules comparable to those of Gradus.

TABLE XIII.—*Classification into rogues and nonrogues of five F_2 families from the cross Gradus rogue × Mummy*

Parent.	Culture No.	Rogues.	Nonrogues.		Unclassified.	Total.
			Intermediate.	Broad.		
1919:	1920:					
9.726–5.....	0.1076.....	79	19	3	8	109
9.726–6.....	0.1077.....	68	15	2	1	86
9.726–7.....	0.1078.....	8	4	2	2	16
9.726–8.....	0.1079.....	11	4	0	4	19
9.727–8.....	0.1085.....	16	4	1	6	27
		182	46	8	21	257

TABLE XIV.—*Relation of Xx′ and Yy in F_2 generation of cross Mummy×Gradus rogue (x′x′YY × XXyy), data from Table XIII.*

	XY or Xy rogues.	x′Y or x′y nonrogues.		Total.
		Intermediate.	Broads.	
Observed, 12:3:1 ratio	182	46	8	236
Percentage found	77.12	19.49	3.39	.....
Percentage expected	75.00	18.75	6.25	.....
Difference (D)	2.12	.74	2.86	.....
χ^2=2.8587				
P=.2231				
Observed, 3:1 ratio	182	54	.....	236
Percentage found	77.12	22.88	.....	.....
Percentage expected	75.00	25.00	.....	.....
Difference (D)	2.12	2.12	.....	.....
Standard error (S. E.)	1.89	.....	.....	.....
D/S. E.	1.12	.....	.....	.....

The presence of the broads in the F_2 generation of the cross Gradus rogue with Mummy is accounted for by assuming the presence of two factors y and x for stipule width in Gradus, factor y being allelomorphic to Y in Mummy and factor x allelomorphic to x′ found in Mummy. Ordinarily neither x nor x′ has any visible effect on stipule shape in either variety. Occasionally the factor x in Gradus mutates to X, which has for its somatic expression a mean stipule ratio of about 2.35. Factor X interacts with its allelomorph x as found in Gradus and is dominant to x′ of Mummy. It also masks either member of the factor pair Yy. Any F_2 segregate that is heterozygous or homozygous for the factor X has rogue stipules, irrespective of whether Y or y is present. An individual in which X is absent has intermediate or broad stipules, depending on the presence or absence, respectively, of the factor Y. In the F_2 generation the plants segregate into approximately 12 rogues; 3 intermediates; 1 broad, according to the following scheme:

P_1 ..... Gradus rogue, XXvy×Mummy, XX x′ x′
F_1 ..... Rogue, Xx′ Yy
F_2 .....

	Rogues:			Intermediates:		Broads:
	Xy	Xy		x′Y		x′y
Percentage	56.25	18.75		18.75		6.25
Ratio	12		:	3	:	1

	Rogues (X):		Non-rogues (X′):
Percentage	75		25
Ratio	3	:	1

	Rogues and Intermediates:		Broads:
Percentage	93.75		6.25
Ratio	15	:	1

Returning again to the results tabulated in Table XIV, the observed number in any one category of plants is expressed in percentages of the total number of plants classified. For the rogues, intermediates, and types the percentages are 77.12, 19.49, and 3.39, respectively, compared with the theoretically expected 75.00, 18.75, and 6.25 for the respective classes, the deviations of the observed percentages from the calculated are not statistically significant (P having a value of 0.2331). The greatest deviation is caused by the exceptionally low number of board-stipuled plants found. The deficiency in the broad class, however,

may be partially explained by assuming a differential mortality rate among the F_2 segregates as explained in the paragraph dealing with the relation of the Aa and Cc factors in the F_2 generation of Gradus rogue × Mummy.

The occurrence of the early flowering segregates offers a partial explanation of the deviation in the number of broad-stipuled plants observed from that theoretically expected, as there is in all likelihood a correlation in Gradus and Mummy between the shape of the stipules and time of maturing. As has been shown, the Graduslike character of the broad-stipuled F_2 plants was inherited through the rogue parent, which also carried the factors for early flowering. The results with Gradus × Mummy show that linkage exists in Gradus between the factors y for stipule shape and a for flower color. Hoshino (7) has shown the factor a to be linked with a factor for early flowering. Necessarily linkage exists in some degree between the factor y for stipule shape and the factor for early flowering. One is, therefore, justified in assuming that many of the white, broad-stipuled segregates would be early in maturing and the correlation would account for a part of the deviation of the observed number of broad F_2 types from the number theoretically expected.

STATISTICAL STUDY OF THE F_2 GENERATION OF THE CROSSES GRADUS ROGUE AND MUMMY

In 1920 were grown 51 F_2 families of the cross Gradus rogue with Mummy. The average ratio of stipule width to length in the mature F_2 plants was calculated in the same manner as for the P_1 generation. The F_2 segregates were also classified according to flower color but not described as broads, intermediates, or rogues.

RELATION OF COLOR FACTORS A AND a TO STIPULATE SHAPE IN TWO ATYPICAL FAMILIES

With the exception of the plant already mentioned,[5] all of the F_1 hybrids were rogues and appeared similar in regard to stipule shape. Although somatically alike the F_2 frequency distributions of the progeny of two apparently normal F_1 rogues are different from those of the remaining 49 F_2 families in both their range of variation and mean stipule ratio (Table XV). The means of the two families are 1.999 ± 0.9106 and 1.905 ± 0.0126 and for the two together the mean is 1.998 ± 0.0082 as compared with 2.333 ± 0.0050 for the other 49 F_2 cultures (Table XVI).

Because of their similarity both in frequency distribution and in the value of the mean stipule ratios, the two anomalous cultures have been lumped together for a statistical study of the color segregates. The means of the three color classes are: For the whites, 1.899 ± 0.0159; for the pinks, 2.046 ± 0.0167; for the purples, 2.038 ± 0.0095; and for colored plants as a whole, 2.027 ± 0.0085. The difference in means of the AA and aa as compared with aa plants is 6.7 times the probable error. The frequency distribution of the a plants resembles more that of Gradus than it does that of Mummy or Gradus rogue. The frequency distribution of the A population resembles that of Mummy rather than that of either Gradus or Gradus rogue. The difference in the mean stipule ratios of the two color groups, as well as the difference in their ranges

[5] The two exceptional F_2 families were cultures numbered 0.1059 and 0.1093 (Table XV), the progeny of F_1 plants 9.722–1 and 9.729–5, respectively (Table XII).

TABLE XV.—*Gradus rogue×Mummy, and reciprocal. Frequency distributions of ratio of* $\left(\frac{\text{length of stipule}}{\text{width of stipule}}\right)$ *of* F_2 *families*

Culture No.	1. 55	1. 65	1. 75	1. 85	1. 95	2. 05	2. 15	2. 25	2. 35	2. 45	2. 55	2. 65	2. 75	2. 85	2. 95	3. 05	Total.	M.	Coefficient variation.
0.1045	.....	1	0	1	4	4	3	1	5	7	2	3	2	2	.....	.....	35	2. 325±0. 0329	12. 43±0. 9848
0.1046	.....	1	1	1	0	0	3	4	3	6	3	1	.....	.....	.....	.....	23	2. 245± . 0374	11. 85±1. 1949
0 1047	.....	.....	.....	.....	1	1	0	0	3	3	1	1	0	0	0	1	11	2. 359± . 0390	8. 14±1. 6217
0.1048	.....	.....	1	1	1	3	4	4	7	7	11	12	3	3	1	1	59	2. 466± . 0231	10. 75± . 6751
0.1049	.....	.....	1	0	2	5	4	5	2	7	6	8	3	0	0	1	44	2. 396± . 0850	11. 48± . 0850
0.1050	.....	.....	1	1	4	1	2	5	9	9	2	3	2	1	.....	.....	40	2. 340± . 0264	10. 59± . 8075
0.1051	.....	.....	1	5	2	6	6	5	10	8	4	3	2	1	.....	.....	53	2. 283± . 0245	11. 56± . 7674
0.1052	.....	.....	.....	.....	2	2	0	1	1	4	1	2	1	.....	.....	.....	14	2. 358± . 0467	10. 98±1. 4190
0.1053	.....	.....	1	1	2	1	0	1	0	1	.....	.....	.....	.....	.....	.....	7	2. 036± . 0568	10. 95±1. 9973
0.1054	.....	1	1	3	4	3	3	5	6	5	11	9	2	4	3	.....	60	2. 410± . 0209	9. 95±1. 8113
0.1055	.....	.....	.....	1	2	3	3	4	4	5	4	1	2	.....	.....	.....	29	2. 326± . 0295	10. 14± . 9072
0.1056	.....	1	1	5	3	7	9	6	6	17	6	8	1	2	.....	.....	72	2. 312± . 0215	11. 72± . 6580
0.1057	.....	.....	1	1	1	9	5	1	2	5	1	0	2	.....	.....	.....	28	2. 215± . 0312	11. 06±1. 0033
0.1058	.....	.....	.....	.....	.....	2	0	2	2	2	1	1	.....	.....	.....	.....	10	2. 340± . 0397	7. 94±1. 2142
0.1059 a	.....	1	3	9	23	24	6	3	2	.....	.....	.....	.....	.....	.....	.....	71	1. 999± . 0106	6. 65± . 3781
0.1060	.....	.....	.....	.....	.....	1	0	0	2	1	1	.....	.....	.....	.....	.....	5	2. 350± . 0494	6. 97±1. 5003
0.1061	.....	.....	2	0	3	3	1	0	1	.....	.....	.....	.....	.....	.....	.....	10	2. 000± . 0358	8. 40±1. 2758
0.1062	.....	.....	1	2	1	1	2	1	3	4	6	1	1	2	.....	.....	25	2. 370± . 0362	11. 35±1. 0966
0.1063	.....	.....	1	1	0	1	1	1	2	4	1	.....	.....	.....	.....	.....	12	2. 160± . 0522	12. 41±1. 7348
0.1064	.....	.....	.....	1	1	0	2	4	4	.....	.....	.....	.....	.....	.....	.....	12	2. 208± . 0302	7. 02± . 9684
0.1065	1	0	1	5	5	1	7	9	7	7	4	2	0	3	.....	.....	52	2. 255± . 0265	12. 59± . 8459
0.1066	.....	.....	1	1	3	1	1	3	4	3	3	3	1	.....	.....	.....	24	2. 312± . 0370	11. 63±1. 1492
0.1067	.....	.....	1	1	5	5	6	7	3	7	9	8	5	.....	.....	.....	57	2. 357± . 0256	12. 17± . 7798
0.1068	.....	1	1	2	3	2	4	1	2	4	10	7	1	2	.....	.....	40	2. 357± . 0322	12. 81± . 9817
0.1069	.....	.....	2	3	4	6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	15	2. 215± . 0313	12. 59±1. 0165
0.1070	.....	.....	.....	1	1	0	2	2	1	1	.....	.....	.....	.....	.....	.....	8	2. 175± . 0436	8. 41±1. 4282
0.1071	.....	.....	.....	2	0	2	3	1	4	0	4	1	2	.....	.....	.....	19	2. 328± . 0414	11. 51±1. 2760
0.1072	.....	.....	.....	.....	.....	.....	1	2	2	4	0	0	1	.....	.....	.....	10	2. 390± . 0334	6. 65± . 9998
0.1073	.....	.....	.....	.....	1	0	0	0	2	2	.....	.....	.....	.....	.....	.....	5	2. 310± . 0552	7. 92±1. 6998
0.1074	.....	.....	1	2	3	6	2	6	2	12	4	2	1	.....	.....	.....	41	2. 309± . 0248	10. 22± . 7691
0.1075	.....	.....	.....	.....	.....	4	0	1	1	4	2	0	2	.....	.....	.....	14	2. 372± . 0386	9. 02±1. 1563
0.1080	.....	.....	.....	1	0	0	2	1	4	2	0	1	0	1	1	.....	13	2. 397± . 0525	11. 72±1. 5715
0.1081	.....	.....	.....	.....	.....	.....	.....	2	3	1	3	2	1	0	2	.....	14	2. 543± . 0398	8. 69±1. 1160
0.1082	.....	.....	.....	.....	3	4	1	2	4	11	5	8	2	2	.....	.....	42	2. 434± . 0251	9. 90± . 7356
0.1083	.....	.....	.....	1	1	2	1	2	6	8	7	7	3	1	0	1	40	2. 470± . 0254	9. 63± . 7329
0.1086	.....	.....	.....	1	0	2	0	1	1	1	1	2	.....	.....	.....	.....	9	2. 317± . 0607	11. 65±1. 8772
0.1087	.....	.....	2	0	0	2	3	1	3	1	1	3	2	1	.....	.....	19	2. 350± . 0479	13. 19±1. 4682
0.1088	.....	.....	.....	.....	.....	3	0	0	4	4	2	3	1	1	1	.....	19	2. 472± . 0385	10. 07±1. 1050
0.1089	.....	.....	2	2	6	2	5	6	7	11	10	3	3	2	1	.....	60	2. 350± . 0245	11. 91±1. 4496
0.1090	.....	.....	.....	3	5	1	1	4	6	5	6	2	0	1	.....	.....	34	2. 303± . 0304	11. 41± . 9453
0.1091	.....	.....	1	0	2	1	2	1	5	1	1	1	.....	.....	.....	.....	15	2. 250± . 0406	10. 35±1. 2893
0.1092	.....	.....	.....	1	0	1	1	0	1	1	.....	.....	.....	.....	.....	.....	5	2. 170± . 0642	9. 81±2. 1125

o. 1093 [a]	.....	2	4	5	18	21	6	0	2	1	.....	.....	.....	.....	.....	.....	59	1. 905± .0129	7. 72± .4822
o. 1094	.....	2	2	11	15	5	3	0	4	2	4	4	1	.....	.....	.....	53	2. 094± .0224	11. 55± .7667
o. 1095	.....	1	0	3	4	6	4	13	12	18	5	3	1	1	.....	.....	71	2. 306± .0173	9. 36± .5338
o. 1096	.....	.....	.....	.....	.....	2	0	0	0	2	.....	.....	.....	.....	.....	.....	4	2. 250± .0951	12. 53±3. 0347
o. 1097	.....	.....	2	7	6	8	6	14	14	16	19	9	8	7	3	.....	119	2. 385± .0181	12. 88± .5725
o. 1098	.....	.....	.....	1	2	1	0	1	0	0	0	1	.....	.....	.....	.....	6	2. 117± .0738	12. 65±2. 5023
o. 1099	.....	.....	.....	.....	.....	.....	1	1	4	2	2	3	0	1	.....	.....	14	2. 471± .0326	7. 32±1. 6792
o. 1100	.....	.....	.....	.....	.....	1	0	0	0	1	0	1	1	.....	.....	.....	4	2. 475± .0904	10. 82±2. 6185
o. 1101	.....	.....	1	0	0	1	2	0	2	0	1	0	0	1	.....	.....	8	2. 275± .0741	13. 67±2. 3478
Total	1	11	37	86	143	167	122	136	187	231	169	131	58	39	12	4	1, 534	2. 307± .0048	12. 48± .1542

[a] Atypical cultures o. 1059 and o. 1093.

of variation, indicates linkage in the two parents between the factors Yy for stipule width and the Aa factors for flower color. Recombinations in the F_2 of cross-over and noncross-over gametes would account for differences in the range of variation and in the means between the F_2 A and a segregates and the A and a parents.

However, as the combined frequency distributions of the two crosses of Mummy with rogue cultures under discussion (0.1059 and 0.1093) show considerable variation from the F_2 frequency distribution of Gradus as type × Mummy, especially in the position of the respective modes and means, it is unlikely that the factor allelomorphic to x′ in the F_1 hybrid is the x of Gradus type; it is apparently a mutation of X to some other factor for stipule shape, the mutation being analogous to the genetic change occurring when intergrading intermediates are produced from Early Giant types (*1*). In this case the intermediates are of the sort producing few or no rogues among their progeny.

Although the ranges of variation of the two atypical F_2 progenies (0.1059 and 0.1093) overlaps the range of Gradus rogue, no rogues were present in the cultures judging from the character of the F_3 generation. Six F_2 plants of culture 0.1093 from classes 2.05, 2.15, and 2.45 of the variation curve gave only broad and intermediate F_3 segregates. It would appear that the rogue factors which entered the F_1 zygote failed to function as rogue factors at gametogenesis, having mutated to something different from X.

RELATION OF COLOR FACTORS A AND C TO STIPULE SHAPE IN THE TYPICAL F_2 FAMILIES OF THE CROSSES OF MUMMY WITH GRADUS ROGUE

The stipules of 1,404 plants, progeny of the remaining F_2 families (omitting cultures 0.1059 and 0.1093), were measured. The plants represented 49 families, of which the frequency distributions and the derived statistical constants are given in Table XV.

Of the 1,404 individuals, 356 were white (of constitution aa); 305 pink (with A and B factors); and 873 were purple (with A, B, and C factors). The mean stipule ratio of the white segregates was found to be 2.258 ± 0.0110 as compared with 2.336 ± 0.0110 for the pinks and 2.325 ± 0.0063 for the purples (Table VII).

As no essential difference exists between the means of the pink and purple F_2 plants, and as they each contain the A factor, the two color populations may be combined for comparison with the aa segregates. The mean of the AA and Aa plants is 2.335 ± 0.0056 as contrasted with 2.258 ± 0.0110 for the aa segregates, a difference in the means of over 6 times the probable error of the difference.

For a comparison inter se of the frequency distributions of the AA and Aa, and aa segregates and of the F_2 as a whole the number of variates in each class was expressed in percentages of the total number of variates in that particular category of plants. Figure 3 shows the curves obtained by plotting the percentage of variates in each class for the AA and Aa, and aa segregates separately, and for all the F_2 plants irrespective of the color factors present. The graphs for Gradus, Gradus rogue, and Mummy are shown in figure 2, for comparison with the graphs of the F_2 generation.

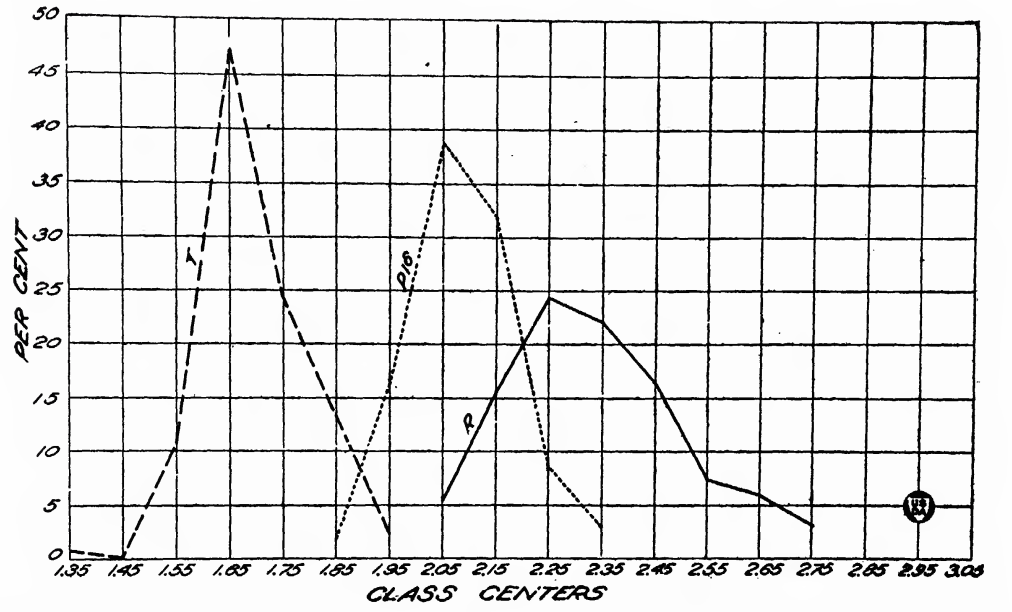

FIG. 2.—Graphs obtained by plotting the frequency distributions, expressed in percentages, of ratio of $\frac{\text{length of stipule}}{\text{width of stipule}}$ for Gradus (T), Mummy (M), and Gradus rogue (R), based on data in Table VIII.

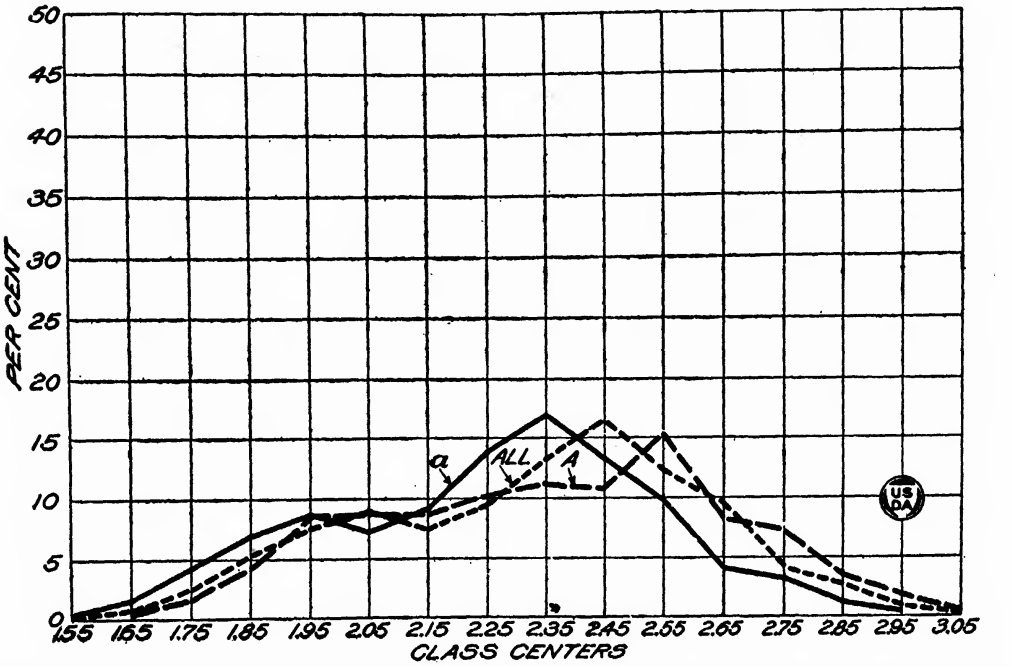

FIG. 3.—Graphs obtained by plotting the frequency distributions, expressed in percentages, of ratio of $\frac{\text{length of stipule}}{\text{width of stipule}}$ for all the F_2 variates of Mummy × Gradus rogue and for the AA and Aa segregates (A) and aa segregates (a) taken separately, based on data in Table VIII.

The majority of variates in a population of Gradus types have a mean stipule ratio between 1.50 and 1.79 and none have been observed with a stipule ratio above 1.99. For Gradus rogue no plants have been found with a stipule ratio of less than 2.00 and by far the greatest number occur between the limits 2.10 and 2.49. The mean stipule range of Mummy extends over portions of the ranges of both Gradus and Gradus rogue, but the majority of individuals occur in classes 1.95 to 2.15, inclusive. Consequently, if linkage existed between the factor for rogue stipules and the a factor for white flowers, one would expect proportionately more of the aa variates to lie close to the mean of the rogue parent, 2.339, and a greater concentration of the variates with the A factor around the mean of Mummy, 2.088, than would be found close to the mean of Gradus rogue. The modes of the frequency distributions of both the colored (AA and Aa) and white (aa) segregates are actually at 2.45, or very near the mean of the rogue parent, however. It is evident, therefore, that no linkage exists between the factor for rogue stipules and the a factor.

The difference in means of the two populations is caused in a large measure by the linkage of the AY and ay factors, so that among the nonrogue segregates the AA and Aa variates tend to have a stipule ratio approaching that of Mummy and the mean of the aa segregates approaches that of the Gradus parent. The effect of linkage is to lower the mean of the aa plants and to increase the mean of the AA and Aa segregates. For this reason more aa segregates than AA and Aa segregates are found in classes 1.55 to 1.95, inclusive. Linkage of the two factors also accounts for the tendency to bimodality that both curves exhibit. (Fig. 3.)

GRADUS ROGUE X MUMMY, AND RECIPROCAL IN THE F_3 GENERATION; CLASSIFICATION OF F_3 FAMILIES

On the hypothesis that Gradus rogues are produced from Gradus by mutation of a single factor, x to X, which is allelomorphic to x′ in Mummy, the expectation in regard to the F_2 rogues of Gradus rogue × Mummy is that approximately 66 per cent will show segregation in the F_3 generation into rogues and nonrogues, while the remaining 33 per cent should breed true. The F_2 broads and intermediates (nonrogues) should not contain rogues among their progeny except as the product of primary mutation.

No record was kept of the F_2 plants saved for seed as to their general appearance, i. e., whether they were rogues, intermediates, or broads. Instead, stipule measurements were made and the stipule ratio was used for a criterion as to which of the three categories, rogue, intermediate, or broad, that the plant belonged. Altogether 184 plants were chosen from a number of F_2 frequency classes (Tables XVI to XVIII). As expected, the parent plants of families containing no rogues came for the most part from classes 1.65 to 2.25. The parent plants of progenies breeding true to the rogue character or segregating into rogues and nonrogues were largely from classes 2.35 to 2.95. This and a classification of seregating F_3 families of Gradus rogue × Mummy and reciprocal are shown in Table XVI.

TABLE XVI.—*Families in which no rogues appeared. Generic constitution of F_2 parents was* x′x′YY, x′x′Yy, *or* x′x′yy

Culture No.	Stipule ratio of F_2 parent.	Broads.	Broads and intermediates.	Intermediates.	Total.
0.279	.....	9	.....	.....	9
0.281	1.65	29	.....	.....	29
0.283	.....	.....	31	.....	31
0.285	.....	.....	.....	13	.....
0.289	.....	.....	16	.....	16
0.291	.....	27	.....	.....	27
0.293	.....	.....	25	.....	25
0.294	.....	.....	16	.....	16
0.296	.....	.....	44	.....	44
0.297	.....	.....	35	.....	35
0.298	1.75	.....	21	.....	21
0.299	.....	18	.....	.....	18
0.300	.....	.....	24	.....	24
0.301	.....	.....	61	.....	61
0.302	.....	.....	49	.....	49
0.303	.....	.....	33	.....	33
0.304	.....	.....	.....	38	38
0.305	.....	.....	.....	37	37
0.306	.....	.....	35	.....	35
0.308	.....	.....	12	.....	12
0.309	.....	.....	37	.....	37
0.310	.....	13	.....	.....	13
0.311	.....	46	.....	.....	46
0.314	.....	21	.....	.....	21
0.315	.....	.....	23	.....	23
0.316	.....	.....	73	.....	73
0.317	1.85	.....	44	.....	44
0.318	.....	.....	21	.....	21
0.319	.....	.....	25	.....	25
0.321	.....	.....	38	.....	38
0.322	.....	.....	91	.....	91
0.323	.....	.....	62	.....	62
0.324	.....	.....	70	.....	70
0.325	.....	.....	20	.....	20
0.326	.....	.....	38	.....	38
0.327	.....	.....	74	.....	74
0.328	.....	.....	11	.....	11
0.330	.....	.....	47	.....	47
0.331	.....	.....	20	.....	20
0.332	.....	.....	22	.....	22
0.340	.....	.....	71	.....	71
0.341	2.05	58	.....	.....	58
0.344	.....	.....	30	.....	30
0.345	.....	.....	47	.....	47
0.347	.....	.....	25	.....	25
0.348	.....	.....	23	.....	23
0.428	.....	.....	75	.....	75
0.429	.....	.....	31	.....	31
0.432	2.25	.....	42	.....	42
0.433	.....	.....	110	.....	110
0.454	2.35	.....	99	.....	99
0.466	2.45	.....	42	.....	42

TABLE XVII.—*Families segregating into rogues and nonrogues. Genetic constitution of F_2 parents was $Xx'YY$, $Xx'Yy$, or $Xx'yy$*

Culture No.	Stipule ratio of F_2 parent.	Rogues.	Nonrogues.	Total.
0.307	1.85	31	11	42
0.329	.....	18	8	26
0.333	.....	?	?	70
0.418	.....	?	?	38
0.420	.....	18	2	20
0.421	2.25	16	3	19
0.422	.....	27	3	30
0.425	.....	31	2	33
0.426	.....	69	4	73
0.431	.....	34	4	38
0.436	.....	18	2	20
0.440	.....	24	3	27
0.441	.....	21	2	23
0.443	.....	19	3	22
0.446	.....	31	6	37
0.450	.....	33	2	35
0.451	2.35	34	3	37
0.453	.....	32	2	34
0.457	.....	57	3	60
0.459	.....	36	2	38
0.460	.....	117	13	130
0.461	.....	64	5	69
0.462	.....	33	4	37
0.465	.....	88	6	94
0.466	.....	25	4	29
0.470	.....	23	5	28
0.471	.....	25	3	28
0.472	.....	14	9	23
0.473	.....	29	3	32
0.474	.....	12	1	13
0.477	.....	13	4	17
0.482	2.45	15	8	23
0.484	.....	29	4	33
0.486	.....	22	6	28
0.489	.....	28	7	35
0.491	.....	24	7	31
0.492	.....	37	9	46
0.493	.....	14	2	16
0.497	.....	19	4	23
0.500	.....	18	2	20
0.502	.....	6	5	11
0.503	.....	16	5	21
0.2419	.....	66	9	75
0.2420	.....	36	5	41
0.2423	.....	28	14	42
0.2424	.....	58	3	61
0.2427	.....	46	3	49
0.2428	.....	25	6	31
0.2429	2.55	26	3	29
0.2430	.....	11	4	15
0.2434	.....	51	2	53
0.2439	.....	?	?	36
0.2441	.....	?	?	11
0.2442	.....	?	?	51
0.2443	.....	6	3	9
0.2444	.....	6	1	7
0.2447	.....	25	2	27
0.2448	.....	18	6	24
0.2449	.....	57	3	60

TABLE XVII.—*Families segregating into rogues and nonrogues. Generic constitution of F_2 parents was Xx′YY, Xx′Yy, Xx′yy*—Continued

Culture No.	Stipule ratio of F_2 parent.	Rogues.	Nonrogues.	Total.
0.2451	.....	49	12	61
0.2452	.....	29	2	31
0.2458	.....	35	2	37
0.2462	2.65	10	2	12
0.2463	.....	15	3	18
0.2464	.....	20	1	21
0.2466	.....	6	2	8
0.2467	.....	9	2	11
0.2468	.....	19	4	23
0.2473	.....	5	4	9
0.2475	.....	34	3	37
0.2476	2.75	31	5	36
0.2477	.....	40	8	48
0.2484	2.85	19	7	26
0.2489	.....	18	1	19
0.2490	2.95	39	5	44
0.2491	.....	25	2	27
Total	.....	2,097	308	.....
0.423 [a]	.....	7	57	64
0.424 [a]	2.25	4	19	23
0.430 [a]	.....	7	23	30
0.439 [a]	2.35	4	33	37
0.455 [a]	.....	3	67	70
0.469 [a]	2.45	4	54	58
0.574 [a]	.....	6	8	14
0.2445 [a]	2.65	13	19	32
0.2470 [a]	.....	12	17	29
0.2482 [a]	2.75	10	14	24
Total	.....	70	311	.....
Grand total	.....	2,167	619	.....

[a] Cultures containing an excess of nonrogues over rogues. The nonrogues were comparable to the intergrading intermediates described by Bateson and Pellew (*1*).

Of the 184 F_2 plants, 52 had progenies containing (1) only broads, (2) both intermediates and broads, or (3) intermediates only; i. e., they were recessive for the X factor but may have contained the Y or y factor. There were 86 progenies which segregated into rogues and nonrogues; while the progenies of 46 plants were all rogues (Tables XVI to XVIII. The observed number of XX : Xx′ F_2 segregates is very close to that theoretically expected (Table XIX).

Inspection showed segregating families to be of two kinds: First, those with an excess of rogues over nonrogues, and second, those with an excess of nonrogues over rogues. In the latter case the nonrogues were often comparable in appearance to the intergrading intermediates described by Bateson and Pellew (*2*). Except in cultures containing a very few plants, one would not expect such an excess of recessives as was found in these families.

TABLE XVIII.—*Families in which only rogues appeared. Genetic constitution of F_2 parents was XXYY, XXYy, or XXyy*

Culture No.	Stipule ratio of F_2 parent.	Rogues.	Culture No.	Stipule ratio of F_2 parent.	Rogues.
0.419	.....	55	0.2425	.....	42
0.427	2.25	49	0.2426	2.55	38
0.434	.....	50	0.2435	.....	32
0.442	.....	25	0.2436	.....	61
0.444	2.35	23	0.2437	.....	44
0.445	.....	52	0.2440	.....	41
0.456	.....	30	0.2418	.....	34
0.463	.....	18	0.2446	.....	36
0.476	.....	16	0.2450	.....	16
0.478	.....	29	0.2454	.....	27
0.480	.....	15	0.2455	.....	20
0.481	.....	21	0.2456	.....	33
0.483	.....	18	0.2457	2.65	28
0.485	.....	26	0.2459	.....	31
0.487	2.45	24	0.2460	.....	19
0.488	.....	17	0.2465	.....	51
0.495	.....	20	0.2469	.....	17
0.496	.....	13	0.2471	.....	23
0.498	.....	42	0.2472	.....	18
0.499	.....	39	0.2479	2.75	34
0.502	.....	14	0.2483	.....	44
0.2421	.....	34	0.2486	2.85	15
0.2422	.....	35	0.2487	.....	17

TABLE XIX.—*Ratio of XX : Xx′ plants among 132 F_2 segregates of Gradus.rogue × Mummy, as determined from analysis of the F_3 generation, based on data in Table XVII. The expectation is two segregating and one nonsegregating F_3 families*

	Nonsegregating.[a] XX.[b]	Segregating.[a] Xx′[b]	Total.
Observed	46	86	132
Calculated	44	88	132
Difference (D)	2	2	.....
Standard error (S. E.)	3.5	3.5	.....
D/S. E.	.5	.5	.....

[a] Character of F_3 family. [b] Constitution of F_2 parent.

The total number of rogues (2,097) in the cultures containing few nonrogues is obviously far in excess of the total number of nonrogues (308) that would be expected if normal Mendelian segregation occurred in the F_2 plants heterozygous for factors X and x′ (Tables XVI, XVII, XVIII). In the families producing many nonrogues and few rogues the situation is reversed, the cultures containing in all 70 rogues and 311 nonrogues, plainly not in accord with the theoretical, monohybrid 3 : 1 ratio shown in the same tables.

Disregarding the two types of F_3 families and considering all the segregating families as a whole, the observed numbers are: 2,167 rogues (XX and Xx′) and 619 nonrogues (x′x′). The numbers actually found in the two groups show an excess of 78 individuals in the rogue class, a deviation of nearly 4 times the probable error of the difference between the observed and calculated number of variates (Table XX).

TABLE XX.—*Ratio of rogue (XX and Xx′) segregates to nonrogue (x′x′) segregates in all F_3 cultures segregating into rogues and nonrogues, based on data in Table XVII*

	XX and Xx′.	x′x′.	Total.
Observed	2167	619	2786
Calculated	2089.5	696.5	2786
Difference (D)	77.5	77.5	.....
Standard error (S. E.)	22.85	.....	.....
D/S. E	3.4	.....	.....

STATISTICAL STUDY OF STIPULE SHAPE IN THE F_3 FAMILIES OF GRADUS ROGUE X MUMMY, AND RECIPROCAL

Besides classifying the F_3 cultures on the basis of stipule character a number of families were chosen for a statistical study of the stipule shape. A record was kept of whether the families consisted of nonrogues only, rogues, or of both rogues and nonrogues. Table XX shows the frequency distributions and means of the cultures measured. In general the mean stipule ratio of each family tends toward the mean of the category to which the F_2 parent belonged (Table XXI). Thus the means of the x′x′ families tend to approach the mean of x′x′ parent (Mummy) while the Xx′ and XX families have means close to that of the XX parent (Gradus rogue). This tendency in the means of the three types of families is greater if the frequency distributions of the several families belonging to each group be combined. In Table XXII are shown the total frequency distributions from Table XXI of 28 segregating families, of 8 families breeding true to the rogue character, and of 15 families in which no rogues occurred. The mean of the rogue families is 2.503 ± 0.0093 as compared with 2.339 ± 0.0096 for Gradus rogue. The increased narrowness of the stipules of the rogue segregates over that of the rogue parent may be due to the accumulative effect of factors modifying stipule shape such as the Y and n factors responsible for stipule shape and stem fasciation, respectively, in Mummy. The mean of the segregating cultures is 2.259 ± 0.0062 or very near the mean, 2.333 ± 0.0050, of all the F_2 cultures of Gradus rogue × Mummy. For the families in which no rogues appeared the mean is 1.899 ± 0.0053 or intermediate between the means of Gradus type and Mummy, 1.693 ± 0.0107 and 2.088 ± 0.0050, respectively. The mean of the x′x′ families is especially significant as indicating a segregation into Graduslike plants and Mummylike plants among the F_3 plants lacking the X factor. Such a segregation is to be expected in the progeny of F_2 plants of the composition x′x′Yy, and from the effect of lumping together into one frequency distribution the F_3 families with formulae x′x′YY and x′x′yy.

The evidence from the statistical study of stipule shape in the F_3 families points to the homozygous nature of the broad or intermediate F_2 segregates in regard to the x′ factor. In addition the occurrence of F_2 rogues homozygous and heterozygous for the X factor is also demonstrated by measurement of the stipules of their F_3 progenies.

TABLE XXI.—*Frequency distributions and statistical constants of ratio of* $\frac{\text{length of stipule}}{\text{width of stipule}}$ *for the F_3 families of Gradus rogue×Mummy. The rogue families (indicated by R), the nonrogue families (indicated by N), and the families segregating into rogues and nonrogues (indicated by S), are marked in the extreme right-hand column.*

Culture No.	Characters of F_2 parent.	1.45	1.55	16.5	1.75	1.85	1.95	2.05	2.15	2.25	2.35	2.45	2.55	2.65	2.75	2.85	2.95	3.05	3.15	3.25	3.35	Total.	M.	Coefficient variation.	Classification of F_3 family.
1.279	1.65	...	...	...	1	1	4	2	1	...	...	...	...	...	...	...	...	...	...	...	...	9	1.961±0.0245	5.60±0.8931	N
1.281		...	2	6	6	10	5	....	...	...	...	...	...	...	...	...	...	...	...	...	...	29	1.792± .0136	6.08± .5332	N
1.283		...	...	1	11	9	7	3	....	...	...	...	...	...	...	...	...	...	...	...	...	31	1.850± .0126	5.62± .4829	N
1.285	1.75	...	...	...	3	4	6	....	...	...	...	...	...	...	...	...	...	...	...	...	...	13	1.873± .0072	2.08± .2649	N
1.289		...	...	...	2	3	4	7	....	...	...	...	...	...	...	...	...	...	...	...	...	16	1.950± .0178	5.43± .6493	N
1.293		...	...	3	0	3	11	4	4	....	...	...	...	...	...	...	...	...	...	...	...	25	1.950± .0188	7.18± .6884	N
1.294		...	...	...	2	3	2	3	4	1	....	...	...	...	...	...	...	...	...	...	...	15	2.056± .0315	9.09±1.0817	N
1.301		1	2	21	16	11	7	1	0	1	....	...	...	...	...	...	...	...	...	...	...	60	1.759± .0117	7.65± .5069	N
1.304		...	...	2	5	15	4	3	1	2	0	1	2	....	...	...	...	...	...	...	...	35	1.941± .0247	11.17± .9036	S
1.306	1.85	...	...	1	1	2	6	13	7	2	....	...	...	...	...	...	...	...	...	...	...	32	2.050± .0137	5.65± .4778	N
1.307		...	...	...	...	5	6	4	7	5	3	7	2	3	...	...	...	...	...	...	...	42	2.231± .0247	10.67± .7941	S
1.309		1	1	0	0	0	11	12	7	2	1	2	....	...	...	...	...	...	...	...	...	37	2.050± .0184	8.09± .6393	S
1.314		...	...	1	7	7	6	....	...	...	...	...	...	...	...	...	...	...	...	...	...	21	1.864± .0035	1.28±1.3325	N
1.329		...	...	...	2	4	4	6	3	2	3	0	1	0	1	...	...	...	...	...	...	26	2.089± .0349	12.63±1.2000	S
1.308		...	...	...	2	3	2	0	3	2	....	...	...	...	...	...	...	...	...	...	...	12	1.991± .0348	8.99±1.2491	N
1.325	1.95	...	...	2	6	6	10	7	4	....	...	...	...	...	...	...	...	...	...	...	...	35	1.925± .0133	6.07± .4854	N
1.341		...	...	2	6	16	11	7	1	....	...	...	...	...	...	...	...	...	...	...	...	43	1.917± .0114	5.79± .4225	N
1.342		...	...	...	2	2	3	2	2	7	10	10	0	3	1	2	...	...	...	...	...	44	2.308± .0262	11.17± .8131	S
1.343		...	...	...	...	...	...	1	2	4	3	4	2	....	...	...	...	...	...	...	...	16	2.331± .0239	6.09± .7179	R
1.346		...	...	2	2	10	14	8	1	3	2	1	0	2	1	...	...	...	...	...	...	46	2.124± .0239	11.34± .8076	S
1.347		...	...	...	1	7	7	5	2	2	1	....	...	...	...	...	...	...	...	...	...	25	1.990± .0199	7.43± .7127	N
1.333	2.15	...	...	...	3	0	0	5	11	12	16	10	5	5	1	2	...	...	...	...	...	70	2.336± .0179	9.54± .5487	S
1.418	2.25	...	...	...	3	3	8	4	4	6	5	3	1	....	...	...	...	...	...	...	...	37	2.117± .0242	10.01± .7919	S
1.419		...	...	...	...	...	...	1	7	7	10	14	10	4	1	1	...	...	...	...	...	35	2.427± .0140	6.34± .4093	R
1.423		...	...	...	3	4	9	9	11	10	7	3	5	1	2	....	...	...	...	...	...	64	2.071± .0196	11.26± .6786	S
1.425		...	...	...	1	1	1	4	5	4	3	7	3	2	1	1	...	...	...	...	...	33	2.207± .0328	12.68± .0695	S
1.428		...	...	...	...	2	9	12	1	1	....	...	...	...	...	...	...	...	...	...	...	25	2.010± .0066	2.43± .2319	N
1.430		...	...	...	3	5	8	4	1	0	1	0	2	1	....	...	...	...	...	...	...	25	2.022± .0308	11.32±1.0930	S
1.445	2.35	...	...	1	6	6	9	9	1	3	1	4	2	0	2	0	0	1	...	...	...	45	2.132± .0297	13.88±1.0057	S
1.446		...	...	1	2	7	2	3	5	9	3	3	1	0	1	....	...	...	...	...	...	37	2.068± .0248	10.69± .8477	S
1.450		...	...	...	3	3	5	9	4	3	2	3	2	1	....	...	...	...	...	...	...	35	2.138± .0261	10.71± .8741	S
1.458		...	...	...	...	6	7	8	12	7	6	1	0	2	....	...	...	...	...	...	...	49	2.128± .0168	8.22± .5638	S
1.467	2.45	...	...	1	1	2	8	2	7	4	7	3	3	....	...	...	...	...	...	...	...	38	2.165± .0267	10.77± .8429	S
1.2437	2.55	...	...	...	...	...	...	...	1	5	9	2	6	10	3	5	2	1	...	...	...	44	2.606± .0220	8.32± .6023	R
1.2438		...	...	...	...	...	2	0	1	3	3	2	3	1	1	....	...	...	...	...	...	16	2.362± .0364	9.99±1.2041	S
1.2440		...	...	...	...	...	1	0	1	5	6	7	18	3	0	0	1	...	...	...	...	42	2.462± .0147	5.76± .4253	R
1.2441		...	...	...	1	1	3	1	1	3	0	3	1	....	...	...	...	...	...	...	...	14	2.164± .0440	11.27±1.4548	S
1.2442		...	...	2	3	9	3	5	7	8	10	2	1	....	...	...	...	...	...	...	...	50	2.126± .0294	14.58±1.0041	S

I.2449	2.65	....	....	....	1	0	2	4	4	2	7	8	9	9	3	1	....	....	....	....	....	50	2.418± .0231	10.04± .6812	S
I.2451		....	....	2	2	3	6	7	5	12	7	6	4	2	1	0	1	....	....	....	....	58	2.229± .0231	11.71± .7433	S
I.2452		....	....	....	....	1	1	2	1	3	4	4	4	2	2	1	....	....	....	....	....	25	2.394± .0337	10.41±1.0067	S
I.2454		....	....	....	....	....	....	....	4	2	3	7	6	1	2	0	2	....	....	....	....	27	2.469± .0259	8.10± .7483	R
I.2455		....	....	....	....	....	....	2	1	2	4	2	5	1	2	1	....	....	....	....	....	20	2.440± .0322	8.77± .9442	R
I.2456		....	....	....	....	....	....	....	....	2	2	5	9	5	4	3	2	0	2	....	....	34	2.562± .0270	9.13± .0191	R
I.2458		....	....	....	....	....	1	2	4	0	2	3	6	6	3	5	0	1	....	....	....	33	2.462± .0321	11.29± .9495	S
I.2470		....	....	....	2	0	2	1	0	3	1	5	3	4	0	1	....	....	....	....	....	22	2.359± .0451	13.31±1.3772	S
I.2472		....	....	....	....	....	....	....	1	0	3	0	1	3	4	2	5	2	....	....	....	21	2.727± .0348	9.05± .9442	R
I.2476		....	....	....	....	....	1	0	4	4	10	8	4	2	1	1	....	....	....	....	....	35	2.392± .0203	7.44± .6031	S
I.2477	2.75	....	....	....	....	....	....	....	3	3	1	4	6	6	6	6	2	4	3	0	2	46	2.709± .0303	11.25± .8010	S
I.2483	2.85	....	....	....	....	....	1	2	0	2	3	4	5	6	10	4	5	1	0	1	1	45	2.652± .0282	10.59± .7614	S
I.2490	2.95	....	....	....	1	0	0	5	2	3	2	2	2	6	5	4	4	1	0	0	1	38	2.556± .0392	13.26±1.0438	S

TABLE XXII.—*The combined frequency distributions and derived statistical constants of ratio of* $\left\{\begin{matrix}\text{length of stipule}\\ \text{width of stipule}\end{matrix}\right\}$ *for the rogue (R), segregating (S), and the nonrogue (N) families of the F_3 generation from cross Gradus rogue×Mummy based on data in Table XXI.*

Cultures. No.	Classification.	1.45	1.55	1.65	1.75	1.85	1.95	2.05	2.15	2.25	2.35	2.45	2.55	2.65	2.75	2.85	2.95	3.05	3.15	3.25	3.35	Total.	M.	s.	Coefficient variation.
8	R	....	....	....	....	....	1	4	17	27	40	41	57	27	16	12	12	3	1	....	....	258	2.503±.0093	0.2230±.0066	8.89±.2655
28	S	1	1	13	47	84	120	130	119	119	121	108	73	61	41	29	12	8	3	1	4	1,095	2.259±.0062	.306±.0044	13.54±.1911
15	N	1	4	37	64	87	97	64	27	9	1	....	....	....	....	....	....	....	....	....	....	391	1.899±.0053	.154±.0037	8.81±.2141
Total		2	5	50	111	171	218	198	163	155	162	149	130	88	57	41	24	11	5	1	4	1,745			

DISCUSSION OF RESULTS.

True-breeding hybrids have been found in other genera than Pisum, notably in *Oenothera*, and have been interpreted by Muller (*10*) as due to the action of balanced lethal factors. In self-fertilized Gradus plants there is no evidence of zygotic lethals since under proper growing conditions all the ovules in a pod develop. Although an occasional sterile rogue is met with, all ovules generally form viable seed. In over 60 instances crosses between Gradus and Gradus rogue gave an average of 4.5 seeds per pod out of a possible 7 or 9. About 4 seeds per pollination is the number generally obtained from artificial crosses of Gradus with other varieties. However, it has been customary to pollinate two or three stigmas with the pollen of one flower, the result being one well filled pod and one or two partially filled, as a consequence of deficient pollination.

A microscopical examination of the pollen of Gradus and Gradus rogue showed well-developed pollen grains in both forms. Their pollen is about 100 per cent perfect. There was no morphological evidence of defective pollen which might be interpreted as due to the action of gametic lethals.

It is, therefore, apparently out of the question to explain the anomalous behavior of the rogues in crosses with Gradus types as due to the presence of zygotic or gametic lethals.

As previously stated, the nonappearance of Gradus segregates in the F_2 generation of the cross Gradus with Gradus rogue is explained by Bateson and Pellew (*1*) as probably due to somatic segregation of the type and rogue "elements" in the F_1 hybrid. The same authors have not suggested, however, a mechanism by which somatic segregation is accomplished. Such segregation would occur if somatic nondisjunction took place early in the development of the F_1 plants, so that of the two daughter cells formed, one would receive both of the chromosomes derived from one member of the heterozygous chromosome pair present in the parent cell, and the other daughter cell would receive the two chromosomes derived from the second member of the heterozygous pair. As a result, both cells would retain the characteristic number of somatic chromosomes and at the same time be homozygous for either the type or rogue factors.

Besides assuming the phenomenon of somatic nondisjunction, the further assumption must be made that the daughter cells homozygous for the rogue factors increase more rapidly than cells containing the type factors, and consequently the F_1 plant becomes more roguelike as it matures. At sporogenesis only rogue tissue would take part in gamete formation; therefore type plants would not appear among the F_2 progeny.

Although somatic nondisjunction offers a hypothesis to account for somatic segregation it does not explain satisfactorily the failure of the type factors or "elements" to be present in the gametes of the F_1 hybrids. Certainly accepted instances of somatic nondisjunction are rare, the best examples being the gynandromorphs in *Drosophila* described by Morgan and Bridges (*9*) and attributed to the effect of the dropping out of the sex chromosome at an early division of the fertilized egg. The demonstration of the loss of a chromosome was possible from a knowledge of the factors linked with sex. As there are no factors known which are linked to the type allelomorphs of the rogue factors, the loss, by nondisjunction in the F_1 hybrid, of the chromosome carrying the type factor can not be detected. In addition, in the F_1 plants nondisjunctional

nuclear divisions would have to take place too regularly and too often to make the explanation seem reasonable.

As an alternative hypothesis, the idea of "mass" somatic mutation is advanced to account for the anomalous behavior of the rogues in heredity. Using the factor symbols here adopted the x factor of Gradus is assumed to mutate occasionally to the X factor of Gradus rogue. The factor pair xx when in a homozygous condition is relatively stable and mutation to X rarely takes place. However, the heterozygous Xx combination is very unstable and x mutates with great frequency to X, creating a homozygous and more stable condition of the germplasm in regard to the X factor. Mass somatic mutation of x to X occurs in the soma of the F_1 hybrid at an early stage in its development and would account for the prevailing absence of the x factor among the F_1 gametes. The same phenomenon would be true of Gradus plants in which the primary mutation of x to X took place. Such a mutation occurring early in the ontogeny of a Gradus type plant would produce an individual which would gradually become more roguelike as it matured. Or when the mutation takes place late in the development of the plant, the form of the individual would not necessarily be changed but the issue derived from the cell containing the X factor, would, if it entered into gamete formation, cause rogues to appear in the next generation.

The fact that occasionally an F_1 hybrid of Gradus $\times$ Gradus rogue is Graduslike at maturity rather than roguelike is interpreted as indicating that rare Gradus gametes are produced, as a consequence of mutation or otherwise, in which the X factor is replaced by an allelomorph comparable in stability to the x′ of Mummy.

It is probable that the rogues are an extreme manifestation of a series of mutations originating by changes in the x factor of Gradus. Thus the various sorts of intergrading intermediates described by Bateson and Pellew (1) may well represent various modifications of the factor x which may be designated as x″, x‴, etc. The xx″ or x″ x″ combinations produce intergrading intermediates in which mutation of either of x or x″ to X is more frequent than in Gradus type. The differences in the stability of the various modified x factors as exhibited in the rate of change of these factors to X, accounts for the existence of high and low rogue-producing strains of intermediates.

The difference in the proportion of rogues produced from the lower as compared with the upper nodes of intergrading intermediates, besides being due to a multiplication of the somatic cells in which a primary mutation of x to X has occurred, may conceivably be caused by an increase in the number of somatic mutations brought about by physiological changes in the protoplasm as the plant matures.

The mutation of x to X in Gradus, in the F_1 hybrid and in the intergrading intermediate is comparable to what Emerson (5) calls a recurring somatic mutation. The varieties in which rogues arise are of the sort earlier described by de Vries (*13*) as "ever-sporting," meaning that it is impossible to free the stock of the tendency to produce "sports" (mutations).

The results in the F_2 generation of crossing Gradus type with Mummy and Gradus rogue with Mummy have led to the assumption of a factor pair Yy in addition to the xx factors as partly responsible for stipule shape in Gradus and Gradus rogue. In Gradus there are at least two factor pairs xx and yy determining the ratio of width to length of stipule.

The y factor in Gradus is linked with the a factor for white flowers; its allelomorph Y is linked with the A factor for colored flowers in Mummy. The proof of the presence of two factors for stipule shape in Gradus and Gradus rogue lies in the fact that no linkage exists between the X factor (the mutated form of the x factor of Gradus) in Gradus rogue and the a factor for white flowers as determined from an analysis of the F_2 generation of the cross Gradus rogue with Mummy.

The allelomorph, in Mummy, of factor x is probably not identical with x but is very similar in its somatic expression. At least in combination with y of Gradus (the x′x′yy segregates from the F_2 generation of Rogue × Mummy), the x′x′yy plants have stipules very nearly like Gradus type. The factor allelomorphic to x and found in Mummy is called x′ to distinguish it from the x factor of Gradus. Additional evidence of a difference between x and x′ is the fact that x′ has never been known to mutate to X when x′ is present in the homozygous state. The factor x′ is apparently more stable than x.

In addition to the assumed inherent stability of the x′ factor found in Mummy it is probable that the Y factor also acts as a stabilizer of the germ plasm. The heterozygous F_1 plants of Gradus rogue × Mummy (YyXx′) have a germ plasm which is certainly less affected by the presence of the X factor than is the heterozygous Xxyy germ-plasm of Gradus rogue. With the Xx′ combination, recurring somatic mass mutation to the extent obtaining in the F_1 generation of Gradus type × Gradus rogue, does not occur. The various factors that entered the zygote take part in gamete formation at maturity and give rise to the expected F_2 combinations. Although Y behaves as a stabilizer of the F_1 germ plasm and retards mass mutation of x′ to X, the extent of the influence is difficult to determine. Nor is it known to what extent, if any, senility affects the relation of the X and x′ factors. It is possible that, with increasing age, the F_1 plants exhibit a change in the mutability of x′ to X, resulting in an increase of rogues among the F_2 segregates. Since other disturbing causes than somatic mutation in the F_1 hybrid may combine to upset the expected number or ratio of F_2 recombinations, the deviations of the observed number from the calculated number of variates in any one class can not be used, for the F_2 data at hand, to approximate the rate of change of x′ to X.

That changes in the X factor may occur in the F_1 hybrids here discussed is indicated by the exceptional F_1 plant (no. 9.727–6), which was similar in appearance and genetic behavior to the F_1 hybrids of Gradus type × Mummy. Additional evidence of mutation of X to some other form is shown in the behavior of two roguelike F_1 plants (No. 9.722–1 and No. 9.722–5). These plants apparently produced very few, or no, rogues in the next generation (families 0.1059 and 0.1093). The primary mutation or mutations of X in the F_1 parents was here delayed until late in development and, while not affecting the soma, radically changed the genetic character of the microspores and macrospores.

An analysis of the F_3 generation of the cross Gradus rogue with Mummy substantiates the hypothesis of Mendelian inheritance of the rogue factors complicated by recurring somatic mutation. The number of segregating to nonsegregating F_3 families is in close agreement with the theoretical number of 1 XX : 2 Xx′ F_2 rogue segregates expected from a monohybrid F_1 generation. The ratio of XX to Xx′ plants in the progenies of heterozygous F_2 rogues is disturbed by the phenomenon of recurring somatic mutation.

The discrepancy between observation and theory is great in respect to the ratio of rogues to nonrogues in the segregating F_3 families. However, the fact of segregation is considered more important than the ratios obtained. The instability of the heterozygous Xx′ combination, though not leading to a complete elimination of the x′ gametes, by mutation of x′ to X, is such as to produce an excess of X gametes over the number that would normally be formed if somatic mutation did not take place.

SUMMARY

(1) The Gradus variety of *Pisum sativum*, characterized by broad, wavy, emarginate stipules with a mean ratio of $\frac{\text{length of stipule}}{\text{width of stipule}}$ of 1.70, occasionally produces rogue mutations, characterized by narrow, flat, pointed stipules with a mean ratio of 2.35.

(2) Primary rogues are produced from Gradus types by a mutation of a single factor x to X. They are therefore heterozygous, of the formula xX. They give an F_2 of homozygous secondary rogues, XX, through recurrent (mass) somatic mutation of the x factor to X. Such recurrent mutation is believed to occur because of the instability of the combination of xX.

(3) The crosses Gradus × Gradus rogue, and reciprocal, likewise produce an unstable F_1 germ plasm in which mass, somatic mutation of x to X occurs.

(4) The effect of mass somatic mutation in the F_1 hybrid is to produce a germ plasm homozygous for the X factor in by far the greater number of the somatic cells. At gametogenesis only an occasional x gamete is produced. The F_2 generation consists almost entirely of rogues (XX), the Gradus type (xx) very rarely appearing.

(5) In addition to the x factor a second factor y for stipule shape is present in both Gradus and Gradus rogue. The factor y is linked with the a factor for white flowers.

(6) The English Mummy pea, a nonrogue producing variety, has a stipule ratio of 2.05 determined by a factor Y, allelomorphic to y of Gradus, and linked with the A factor for colored flowers.

(7) In crosses between Gradus and Mummy the number of cross-overs between AY and ay is approximately 20 per cent.

(8) Mummy, in addition to the Y factor, has a factor x′ which is allelomorphic to the x factor in Gradus type, therefore to the X factor of Gradus rogue. The x′ factor is practically identical with the x factor in its expression but differs in that it only rarely mutates to X, even in the combination x′X.

(9) The germ plasm of the F′ cross, Gradus rogue (yyXX) × Mummy (YYX′x′) is fairly stable. In the F′ hybrids, YyXx′, the factor x′ mutates to X but not to such an extent as to prevent at gametogenesis the formation of the usual gametes, XY, Xy, x′X, and x′y, as indicated by the appearance in the F_2 generation of rogues (having the X factor), Graduslike plants (having the x′ and y factors), and Mummylike segregates (having the x′ and Y factors).

(10) The expected 3 : 1 ratio of rogues to nonrogues in the F_2 and succeeding generations obtained from the heterozygous Xx′ plants, is disturbed by an excess of X gametes and consequently of XX and Xx′ zygotes.

(11) Inheritance of the factors for stipule shape X and y in Gradus rogue is Mendelian. Normal inheritance is obscured by somatic mutation of the factors x and x′ when in the Xx or Xz′ combination.

(12) In addition to determining a stipule ratio of 2.05 in Mummy, the factor Y acts as a stabilizer of the germ plasm. Somatic mutation of x′ to X in the F_1 cross between Mummy and Gradus rogue (Xx′Yy) does not take place to the extent in which mutation of x to X occurs in the F^1 crosses between Gradus type and rogue (XxYy) on account of the presence of the Y factor.

(13) Besides mutations of x and x′ to X, other mutations of x and x′ occur which affect stipule shape. The nature of the latter kind of mutations has not yet been investigated.

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PLATE 1

A.—Upper part of a mature type plant of Gradus. × ¼.
B.—Upper part of a mature rogue plant of Gradus. × ¼.


C


B


A

PLATE 2

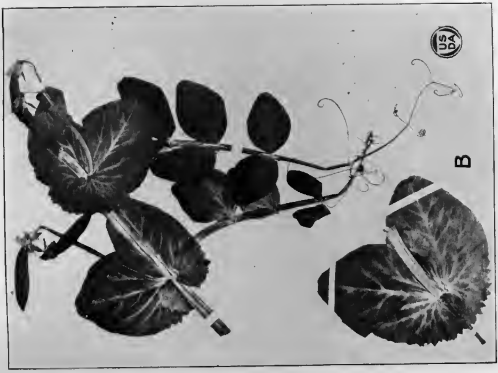
A mature plant of the Mummy variety (P16).
A.—The tip. $\times 1/5$.
B.—The part immediately below the portion shown in A. $\times 1/5$.
C.—Lower part of the stem. $\times 1/5$.

PLATE 3

A.—Upper part of a branch of a mature F_1 plant of Gradus type × Mummy. × ¼.
B.—Upper part of a branch of a mature F_1 plant of Gradus rogue × Mummy. × ¼.


B


A


B


A

PLATE 4

A.—Upper part of a mature broad (y) segregate from the F_2 generation of Gradus type × Mummy. Note the typelike emarginate stipules. × ¼.
B.—The part immediately below the portion shown in A. × ¼.

PLATE 5 [a]

A.—Upper part of an intermediate (Y) or Mummylike segregate from the F_2 generation of Gradus type × Mummy. Note the narrow pointed stipules. × ¼.
B.—The part immediately below the portion shown in A. × ¼.

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[a] Plates 4 and 5 are of plants from the same F_2 family.


B


A


B


A

PLATE 6

A.—Upper part of a mature broad segregate from the F_2 generation of Gradus rogue × Mummy. × ¼.
B.—The part immediately below the portion shown in A. × ¼.

PLATE 7

A.—Upper part of a mature intermediate segregate from the F_2 generation of Gradus rogue × Mummy. × ¼.
B.—The part immediately below the portion shown in A. × ¼.


B


A


B


A

PLATE 8 [b]

A.—Upper part of a mature rogue segregate from the F_2 generation of Gradus rogue × Mummy. × ¼.

B.—The part immediately below the portion shown in A. × ⅓.

[b] Plates 6, 7, and 8 are of plants from the same F_2 family.

A METHOD OF TREATING MAIZE SEED TO DESTROY ADHERENT SPORES OF DOWNY MILDEW[1]

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POSSIBLE INTRODUCTION OF ORIENTAL DOWNY MILDEWS

Introduction into the United States of the Sclerospora downy mildews, which attack maize and related crops in the Orient, would be especially disastrous. These diseases are by nature essentially destructive and cause tremendous losses in the Orient. According to all available information they would prove equally destructive in the southern portion of our own corn belt.

Despite the dangerous possibility of thus introducing such diseases, there is a fundamental need of importing maize, not in commercial quantities, but in small quantities for experimental purposes. As the extensive maize breeding of Mr. G. N. Collins, of the Office of Crop Acclimatization and Adaptation Investigations, has shown, there may be obtained from the peculiar types of maize growing in remote countries valuable characters which can be combined most advantageously with those of our own varieties.

Under the Federal Horticultural Board regulations (including Quarantines 21, 24, and amendments), which thus far have prevented the importation of these mildews, the procedure of introducing foreign varieties of maize and related Gramineae from infested countries is necessarily difficult. As is requisite, such seed is inspected, given such treatment as any insect pest or disease found on it may demand, and grown under constant observation in an isolated quarantine greenhouse. Seed from healthy plants thus grown may then be planted without restriction by the experimenters to whom the original shipment was consigned. This procedure, although reasonable and necessary, is time-consuming and laborious.

A method of treating maize seed which will eliminate with absolute certainty any possibility of introducing maize mildews on such seed would be highly desirable. It is the purpose of this paper to present a method which the writer, after considerable experiment, has found to meet these requirements.

The oriental downy mildews of maize and related Gramineae all belong to the genus Sclerospora of the phycomycetous order Peronosporales. Several species are involved, but in the main features of structure and reproduction they agree. Through the work of Raciborski (*9*),[2] Rutgers (*11*), and Palm (*8*) on the Javan maize mildew; of Butler (*2*) on the maize mildew of India; of Lyon (*5*, *6*), Miyake (*7*), and Lee (*4*) on the maize

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[2] Reference is made by numbers (italic) to "Literature cited," p. 859-860.

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and sugar-cane mildew of Queensland, Fiji, Formosa, and the Philippine Islands; and of Reinking (*10*) and Weston (*14*, *15*, *16*) on the maize mildews of the Philippines, our knowledge of these forms is fairly complete. We know that if they should be carried to the United States on maize seed, it would be by resistant mycelium within the seeds, or by conidia or oospores on them. That mycelium should be the means is most improbable. Palm's work on the Javan maize mildew and unpublished experiments of the writer on the Philippine mildew of maize show that in the case of these two Sclerosporas at least, mycelium, although frequently penetrating the seed of badly infected ears, apparently is unable to transmit infection to the seedling developing therefrom. In such vegetative propagative parts as cuttings of sugar cane, living mycelium of Sclerospora may be carried and transmitted; but, in the case of maize, which is propagated only by seed, this difficulty is avoided.

Conidia adherent to maize seed can not accomplish infection. In the Philippine maize mildews the writer has found that conidia do not survive drying even for as short a time as one hour. Butler (*2*) in India and Miyake (*7*) in Formosa find this to be true of the maize mildews of those countries also.

It is preeminently probable that oospores on or in the maize seed can transmit infection. In the case of the closely related downy mildew of millet (*Sclerospora graminicola* [Sacc.] Schroet.) there is every evidence that intercontinental spread has been accomplished in this way. Whether the same means of distribution operates in the maize Sclerosporas we do not know. It should be noted in this connection that, although none of the several oriental mildews of maize has ever been found to produce oospores on maize itself, nevertheless these resting spores may occur abundantly on related hosts from which they can reach adjacent maize plantings.

Since the conidia are so easily killed, it will be by the resistant oospores, if at all, that these downy mildews will be introduced on maize seed. Consequently seed treatment must be directed against the oospores. To be effective in the case of Sclerospora diseases, a method of seed treatment not only must destroy any of these resistant spores which may be present but also must meet certain unusual requirements. The efficacy of the usual types of seed treatment is customarily demonstrated by the failure of adherent spores to give growth of the disease-producing fungus when the treated seeds have been placed in suitable media. But this would prove no index in the case of Sclerospora, as germination of the oospores has never been seen, and growth of the fungus on artificial media has never been secured. To be successful for Sclerospora, then, a method must give conclusive visible evidence of the destruction of the oospores.

EXPERIMENTS IN SEED TREATMENT

With this end in view, experiments were made with the substances customarily used in seed treatment. Of these, concentrated sulphuric acid fulfills the requirements most successfully, since it gives visible evidence of oospore destruction and yet does not impair seed germination. This was determined as follows:

Oospores (both fresh and two years dried) of the Sclerospora species so common (*14*, *15*) on *Saccharum spontaneum* L. and *Miscanthus japonicus* (Thunb.) Anders. in the Philippines were mounted on a slide and carefully observed through the microscope while a drop of concentrated

sulphuric acid was drawn under the cover glass. Immediately the spores swelled and became distorted, either bursting at once or else less violently extruding oil drops and protoplasmic content while the wall softened and split open. Both content and wall rapidly darkened. Addition of India ink to the acid made more noticeable the extrusion of even small amounts of spore content. After five minutes all the spores in the field were obviously collapsed and exploded, or otherwise gave every evidence of being killed. The effect of first wetting the spores with alcohol was tried, a drop of alcohol being drawn under the cover of a dry mount, left about one minute, drawn off, and replaced by acid. In this case, the extrusion of the contents and the killing of the spores were even more rapid and complete. Water as a wetting agent was less effective than alcohol. There apparently was no difference between the wetting action of 80 to 95 per cent ethyl, methyl, or even denatured alcohol, or between the destructive effects of chemically pure or commercial concentrated sulphuric acid.

Experiments were next made to determine what effect this spore-destroying treatment would have on maize seed. Dry seeds of typical dent, flint, sweet, pop, and waxy-endosperm varieties of maize were put in separate glass jars, covered with concentrated sulphuric acid, and stirred occasionally with a glass rod during the 5 to 20 minutes of treatment. The acid was then drained off, and the seed washed for one hour in running water. Half of each lot of seed was planted, its germination recorded, and the character of the resulting plants observed. The other half was carefully dried, and its keeping quality tested by planting experimental lots from time to time. Although no quantitative results were obtained, it was found that even after the most severe treatment the viability was retained by a considerable percentage of the seed for as long a period as three months, even under the unfavorable rainy season conditions of the Philippines.

To prevent the persistence of minute air bubbles that might protect occasional spores from the acid, similar lots of seed were first given a preliminary wetting with alcohol for about a minute, the alcohol drained off, and the acid added. No difference either in immediate wetting effect, in subsequent action of acid on the seed, or in ultimate germination of the seed was apparent when 80 to 95 per cent ethyl, methyl, or denatured alcohol was used. Also there was no apparent difference between the effect of chemically pure or commercial concentrated sulphuric acid. As a result of the action of the acid, especially after treatments of 15 to 20 minutes, the surface of the seed became somewhat blackened, but the germination percentage was not decreased beyond practicable limits by even the longest treatments. Fresh acid was used for each lot of seed for fear that the black gelatinous material remaining in the acid might dilute it sufficiently to decrease its efficiency.

Some of these experiments in which representative kinds of maize were used are summarized in Table I. Calamba yellow flint is a typical example of the flinty types grown by the Christian Filipinos quite generally throughout the Philippines, Boone County White represents the American dent corn that has been introduced and successfully grown on large haciendas in several parts of the islands, while Manobo waxy is typical of the small, rapidly maturing varieties of maize cultivated by some of the non-Christian tribes in the mountains.

TABLE I.—*Effect of treatments with sulphuric acid on germination and growth of three types of maize*

Lot.	Number of kernels.	Treatment.[a]			Growth after 4 days.					Growth after 10 days.					Growth after 16 days.					Yield after 3½ months.			
					Number of plants.			Average height.	Number of leaves.	Number of plants.			Average height.	Number of leaves.	Number of plants			Average height.	Number of leaves.				
		Alcohol.	H_2SO_4	Planted, 1919.	Total.	Normal.	Abnormal.[b]			Total.	Normal.	Abnormal.[b]			Total.	Normal.	Abnormal.[b]			Plants.	Ears.	Nubbins.	Barren.
CALAMBA YELLOW FLINT																							
		Minutes. Ethyl, 85–95 per cent.	*Minutes.* Commercial concentrated.					*Inches.*					*Inches.*					*Inches.*					
1a	40	0	5	May 24..	28	26	2	2	2–3	32	26	6	6	3–5	32	30	2	9	5–6	18	6	12	0
1b	40	½	5	...do....	34	28	6	2	2–3	34	34	0	6	4–5	34	34	0	9	5–6	20	4	5	11
2a	40	0	10	...do....	2	2	0	1½	2	4	2	2	5	3–4	4	4	0	6	4–5	2	1	0	1
2b	40	½	10	...do....	28	16	12	1½	2–3	28	28	0	5	4	28	10	0	6½	5–6	22	10	12	0
3a	40	0	15	...do....	32	26	6	1½	2–3	32	32	0	5½	4–5	32	32	0	9	5–6	15	3	7	5
3b	40	½	15	...do....	20	14	6	1½	2–3	22	18	4	5	3–4	22	18	4	8	5–6	6	1	0	5
4a	40	0	20	...do....	4	.....	.....	(c)	.....	14	2	12	2½	3	14	2	12	5	4–5	7	1	3	3
4b	40	½	20	...do....	1	.....	.....	(c)	.....	4	4	0	4	3–4	4	4	0	5	4–5	1	0	1	0
A	20	.....	.....	...do....	20	20	0	1½	2	20	20	0	6½	5	20	20	0	12	7	12	8	4	0
B	20	½	.....	...do....	20	19	1	2	2–3	20	20	0	6½	4–5	20	20	0	12	6–7	10	7	2	1
C	20	1	.....	...do....	20	17	3	1½	2–3	20	20	0	6	4–5	20	20	0	11	6–7	5	3	1	1
D	20	5	.....	...do....	19	19	0	2	2–3	19	19	0	6	4–5	19	19	0	12	6–7	9	5	2	2
			Chemically pure concentrated.																				
4c	40	0	20	May 28..	12	8	4	1	2	16	10	6	4	3–4	.....	.....	.....	.....	.....	15	12	2	1
4d	40	½	20	...do....	22	12	10	1	1–2	22	18	4	3½	3–4	.....	.....	.....	.....	.....	18	16	0	2
		Denatured, full strength.	Commercial concentrated.																				
1c	10	0	5	Nov. 23.	8	8	0	1	2	8	8	0	3½	3	.....	.....	.....	.....	.....	6	3	0	3
1d	10	½	5	...do....	7	4	3	1	2	6	3	[d] 3	4	3	.....	.....	.....	.....	.....	4	2	2	0
2c	10	0	10	...do....	6	6	0	1	2	6	5	1	3	3	.....	.....	.....	.....	.....	4	2	1	1
2d	10	½	10	...do....	8	8	0	1	2–3	8	8	0	3	3	.....	.....	.....	.....	.....	6	1	1	4
BB	10	½	0	...do....	.....	.....	.....	.....	.....	8	8	0	3	3	.....	.....	.....	.....	.....	7	2	3	2

BOONE COUNTY WHITE DENT

		Ethyl, 85-95 per cent.																				
1a	40	0	5	May 24..	24	20	4	1	2	26	24	2	4½	3-4	26	24	2	6	4-5	11	8	3	0
1b	40	½	5	...do.....	16	8	8	1	2	18	16	2	4	3-4	18	16	2	6	4-5	8	4	4	0
2a	40	0	10	...do.....	8	.....	.....	(c)	.....	12	10	2	3½	2-3	12	10	2	4½	4	4	2	2	0
2b	40	½	10	...do.....	4	4	0	1	1	6	6	0	3½	2-3	6	6	0	4	4	2	2	0	0
3a	40	0	15	...do.....	10	4	6	1	1	14	12	2	4	2-3	14	12	2	6	4-5	6	3	2	1
3b	40	½	15	...do.....	4	4	0	1	1	8	8	0	4	3	8	8	0	6½	4	5	0	3	2
4a	40	0	20	...do.....	24	12	12	1	1-2	26	24	2	4½	3-4	26	24	2	6	4-5	12	7	3	2
4b	40	½	20	...do.....	30	14	16	1	1-2	28	26	2	4	3-4	28	28	0	6	4-5	15	6	6	3
A	20	.....	.....	...do.....	18	17	1	1½	1-2	20	20	0	6½	3-4	20	20	0	12	5-6	12	7	1	4
B	20	½	.....	...do.....	20	20	0	1	1	20	20	0	6½	4	20	20	0	12	5-6	13	4	7	7
C	20	1	.....	...do.....	19	19	0	1	1-2	19	18	1	6	4	19	18	1	12	5-6	8	3	4	1
D	20	5	.....	...do.....	19	19	0	1	1-2	19	19	0	6	4	19	19	0	12	5-6	7	2	1	4
			C. P. concentrated.																				
4c	40	0	20	May 28..	20	16	4	1	1-2	22	20	2	3	3-4	.....	.....	.....	.....	.....	14	8	1	5
4d	40	½	20	...do.....	16	8	8	1	1-2	16	8	8	3	3-4	.....	.....	.....	.....	.....	10	2	2	6

MANOBO WAXY

		Denatured, full strength.	Commercial concentrated.																			
3b	40	½	15	...do.....	12	6	6	1	1-2	6	6	[e] 0	2	2-3	.....	.....	.....	.....	.....	4	1	3	0
4b	40	½	20	...do.....	6	2	4	1	1	4	4	[f] 0	2	2	.....	.....	.....	.....	.....	3	2	1	0
B	20	½	0	...do.....	19	19	0	1½	2	19	19	0	5	4-5	.....	.....	.....	.....	.....	13	11	2	0

[a] After treatment all lots were washed in running water for one hour and were then planted immediately.

[b] Under "abnormal" plants are included those showing distortion, discoloration, or other evidences of atypical growth. It should be noted, however, that such growth results not only from chemical injury, as from acid, but also from mechanical injury, from flooding, or from attack by ants or other insects.

[c] Plants just emerging at end of 4-day period.

[d] One abnormal plant died.

[e] Three abnormal plants died.

[f] Two abnormal plants died.

Table I is significant only to the extent of showing that the method gives practical yields even after treatment more than rigorous enough to insure destruction of adherent spores. Too many extraneous factors are involved to allow significant comparison of such points as percentage of germination after different treatments or for different varieties. For example, the dryness and age of the seed were not in all cases the same, the location of the plots did not insure equal pollination, and the season of May to September, 1919, when most of the trials were made, terminated in six weeks of successive and violent typhoons that beat down and destroyed many plants.

From a large number of such experiments the following routine for the treatment of maize seed was formulated.

METHOD OF TREATING MAIZE SEED TO DESTROY ADHERENT SCLEROSPORA SPORES

(1) Wet the seed with alcohol for one half to one minute; drain, and, while the seed surface is still damp—

(2) Cover with concentrated sulphuric acid, allow to remain 5 to 10 minutes, stirring from time to time, then drain off the acid.

(3) Wash for one hour in running water, stirring the seed occasionally to insure complete removal of the acid.

(4) Plant at once, or dry thoroughly and save for future planting.

After carefully trying all phases of this method of treatment the writer is convinced that it meets successfully the requirements of the situation earlier outlined in this paper. By it oospores of Sclerospora that may be adhering to the maize seed may be destroyed, while at the same time the vitality of the seed is but little impaired. If planted at once, the percentage of germination is only slightly reduced, and most of the resulting plants are normal in growth and seed production. If dried and preserved, the percentage of germination declines gradually, but even after three months a sufficient number of normal plants will result to meet all practical purposes.

Preliminary experiments indicate that this method of treatment is equally effective against spores of the Physoderma disease of maize, which in the Orient involves two causal species (*3, p. 114; 12, p. 245–247*), one of them already introduced and destructive in the southern United States (*13*). Preliminary experiments have shown also that this treatment can be used successfully in the case of teosinte (*Euchlaena luxurians* Schrad.), coix (*Coix lachryma-jobi* L.), and some varieties of sorghum (*Andropogon sorghum* [L.] Brot.).

In its essential feature, i. e., the use of concentrated sulphuric acid, this method is by no means new. This reagent has been employed by several investigators for sterilizing the surface of many different varieties of seed. Brigham (*1*), for example, has used sulphuric acid to free popcorn seeds from mold spores customarily adherent to them; and the same reagent is used by the Federal Horticultural Board inspectors to destroy anthracnose spores on cottonseed. As modified to meet the exacting requirements of maize seed suspected of carrying Sclerospora, however, the method, it is hoped, will fill a peculiar need, enabling experimental quantities of seed of desirable varieties to be imported from quarantined regions afflicted with Sclerospora mildews, and insuring that, after treatment, the seed may be planted immediately without restriction and without danger.

SUMMARY

In various parts of the Orient there are several downy mildews (Sclerospora spp.) that cause severely destructive diseases of maize. Yet it is desirable that seed of varieties with useful characters be imported even from such regions for experiment and breeding. This involves the danger of introducing these dreaded diseases into our own country by oospores adhering to the maize seed. Therefore such importations are grown in quarantine greenhouses to produce seed under constant observation, a costly and time-consuming procedure. There is need of some method of seed treatment which with absolute certainty will destroy any oospores which may be adherent, and make possible immediate and unrestricted planting of the seed. Such a method not only must give conclusive visible evidence of destroying the resistant Sclerospora oospores which might be on the seed, but also must leave the viability of the seed unimpaired. The writer finds that treatment with sulphuric acid after preliminary wetting with alcohol successfully fulfills these requirements. Details of the method are given in this paper.

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INFLUENCE OF THE SUBSTRATE AND ITS HYDROGEN-ION CONCENTRATION ON PECTINASE PRODUCTION [1]

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INTRODUCTION

Former investigations by the authors (*9*)[2] have shown that *Rhizopus tritici* Saito produces a substance of the nature of an enzym which has the power to dissolve the middle lamellae of raw sweet-potato (*Ipomoea batatas*) disks. This macerating principle was found to be thermolabile and to be produced abundantly in sweet-potato decoction in cultures one or two days old. It was found that while the mycelium itself retained some of the enzym which is called pectinase in these investigations, a portion of it was excreted into the culture solution on which it grew. Investigations have also proved that pectinase is produced by a number of other species of Rhizopus, some of which are able to cause a typical softrot of sweet potatoes. The amount of enzym produced was not equal in all cases. The most parasitic species did not necessarily produce the largest amount of enzym under cultural conditions. For example, *Rhizopus nigricans* Ehrb., which is the common softrot-producing organism in sweet-potato storage houses, produced a small amount of enzym in culture.

In order to study the action of the enzym on raw sweet-potato disks, the organism was grown for two or three days in a solution of sweet-potato decoction. The mycelium was then removed and treated according to a method described elsewhere (*9*). The ability of the enzym, contained both in the mycelium and in the solution, to macerate raw disks was tested. Measured portions of the solution were pipetted into small flasks, some of which were steamed to inactivate the enzym. Raw sweet-potato disks 1 cm. in diameter and 1 mm. in thickness were dropped into the steamed and unsteamed solutions which were then held at 45° C. Maceration was usually completed in from 2 to 4 hours in the solution containing the active enzym. The disks in the steamed controls had not been acted upon in that length of time. If, however, the disks in the control solutions were examined at the end of 24 hours, they were frequently found partially or completely macerated, the cells separating along the line of the middle lamellae in a manner typical of those in active enzym solution. In searching for an answer to this curious phenomenon two possible explanations presented themselves; first, that the enzym was not actually completely inactivated, although the solutions were steamed in an Arnold sterilizer for 10 minutes; second, that some other substance was produced which acted in a manner identical with the enzym itself.

When a modified Czapek's nutrient solution was used, a solution employed quite generally in these investigations, the results were even

[1] Accepted for publication Jan. 22, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 877–878.

more striking. It was found that in the case of this solution steaming caused no change, the steamed solutions macerating as readily as the unsteamed. These results indicated that the enzym was not secreted, but that some other substance was produced which had the power to dissolve the middle lamellae.

These results indicated two possibilities; first, that the enzym was not secreted when the fungus was grown on certain substrates or only in a small amount; and, second, that although the enzym was not secreted the organisms made the solution sufficiently acid to cause maceration of raw sweet-potato disks. In view of these facts, the authors undertook to make a study of the influence of the substrate on the production of pectinase, and of the changes in acidity produced by the fungus in different media as measured in terms of hydrogen-ion concentration. It will be shown in the discussion later that investigations have enabled the authors to explain some of the curious and puzzling results with respect to the parasitism of some of the species of Rhizopus causing soft-rot of the sweet potato.

Since *Rhizopus tritici* has been employed in so many of the investigations by the writers in this particular field, it was decided to use it in these studies. *Rhizopus tritici* grows readily in culture. It is parasitic on the sweet potato and produces an abundance of pectinase. Although not as common in storage as *Rhizopus nigricans*, it lends itself to physiological studies herein outlined more readily than does the latter species.

INFLUENCE OF THE SUBSTRATE ON PECTINASE PRODUCTION

METHODS OF EXPERIMENTATION

In order to determine what influence the substrate had on the production of pectinase, 10 different media were employed as follows: Bean, prune, Irish-potato, carrot, turnip, and sweet-potato decoctions, beef bouillon and a modified Czapek's, Pfeffer's and Richard's solutions. The vegetable and fruit decoctions were prepared by using 500 gm. of the fruit or vegetable in 1 liter of water, cooking for one hour and then filtering. Measured portions of these decoctions were put in small flasks, which were then plugged and autoclaved for about 20 minutes at 15 pounds pressure. The beef bouillon was prepared according to the usual method for preparing this medium. Some modifications were made in Czapek's, Pfeffer's, and Richard's solutions. For example, the $NaNO_3$ in Czapek's solution was replaced by an equal amount of NH_4NO_3. Glucose, 50 gm. per liter, was used as a source of carbon. The substitution of NH_4NO_3 for $NaNO_3$ was made because previous experiments showed that *Rhizopus tritici* thrived better when the nitrogen was derived from ammonium nitrate. Investigations have also shown that the fungus can not utilize cane sugar, hence glucose was substituted for it.

Pfeffer's and Richard's solutions were made according to the following formulae:

	Pfeffer's.	Richard's.
KH_2PO_4	5.0 gm.	0.5 gm.
KNO_3		4.0 gm.
$MgSO_4$	2.5 gm.	0.5 gm
NH_4NO_3	10.0 gm.	10.0 gm.
$FeSO_4$	Trace.	Trace
Glucose	100 gm.	60.0 gm.
Water	1,000 cc.	1 000 cc.

The original Pfeffer's and Richard's solutions call for cane sugar, but, in view of the fact that the fungus used in these investigations does not utilize it, glucose was employed instead. All the different media were prepared in large quantities and held in flasks stoppered with cotton and covered with oiled paper until ready for use. Just previous to using, 30 cc. of the solutions were pipetted into each of fifteen 100 cc. Erlenmeyer flasks which were then autoclaved for 20 minutes at 15 pounds pressure. Ten flasks of each solution (set) were inoculated after which they were incubated at 35° C. The remaining 5 flasks of each set were held as controls.

At the end of the growth period, usually three to four days, the mycelium from all the flasks of a single series was collected into one compound sample and prepared for macerating experiments, according to methods previously described. The solutions on which the fungus grew in each set were made into one compound sample and equal portions (about 30 cc.) pipetted into small flasks to which raw sweet-potato and carrot disks were added. A portion of each of the solutions was steamed for 10 minutes to inactivate the enzym which, together with flasks of the original solutions which had not supported a fungous growth, were used as controls. An accurately weighed amount of mycelium (0.25 gm.) ground in pure quartz sand was used in determining the macerating action of the hyphae. The mycelium was not extracted in water prior to the addition of the raw disks, since previous experiments showed (9) that the rate of maceration was not influenced thereby. The ground mycelium was included in the system.

The hydrogen-ion concentrations of the solutions and of the controls (not inoculated) were determined by the electrometric method as rapidly as possible after the hyphae were removed, in all cases during the same day.

EXPERIMENTAL DATA

In this series of experiments 10 different solutions were employed, 6 being of vegetable origin, 3 synthetic solutions, and 1 of beef bouillon. In one or two series of experiments in which a modified Czapek's nutrient solution was employed as a substrate it was noted that no pectinase seemed to be produced, although it was abundantly secreted in sweet-potato decoction with which it was being compared. It was also noted in these cases that even in the absence of pectinase a certain amount of maceration of sweet-potato disks occurred. In view of these facts, experiments were undertaken to study the regulating action of certain substrates on the production of the enzym. The substrates here employed were selected as being representative of the vegetable decoctions and synthetic solutions in common use. It was also believed that if the substrate exercised any influence on the production of pectinase that fact would be brought out by these media.

MYCELIAL GROWTH

In these series of experiments the dry weight of the mycelium was not determined. The mycelium was all used in maceration experiments. In general, the growth and fruiting of the fungus on the media of vegetable origin were good, being much better than they were when the fungus was grown on the synthetic solutions or on beef bouillon. Bean, carrot, and turnip decoction yielded the best growth and fruiting. A fair growth took place in prune decoction, but little or no fruiting. The

growth and fruiting on Irish-potato decoction were good but somewhat inferior to what they were on bean, carrot or turnip. The growth was poorer on sweet-potato decoction than on any of the vegetable preparations. The growth on the three synthetic media and on beef bouillon was not as good as on the vegetable decoctions. A better growth was made on beef bouillon than on Czapek's, Pfeffer's, or Richard's solutions. The poor growth on Czapek's solution was rather surprising in view of the fact that Rhizopus usually grows well and fruits abundantly on it. The time for terminating the experiment was gaged by the stage of fruiting of the fungus. It was shown by Brown (*2*) and by Harter and Weimer (*9*) that the maximum amount of the macerating principle was contained in the mycelium just at or just preceding fruiting. Fruiting began a little earlier on the vegetable media than on the synthetic ones, consequently the experiments in which the vegetable media were used were terminated usually one day sooner than the others.

HYDROGEN-ION CONCENTRATION

The hydrogen-ion concentrations of the uninoculated solutions and the solutions on which the fungus grew are shown by Table I.

TABLE I.—*Hydrogen-ion concentration of inoculated solutions and controls (uninoculated at the end of the experiments in terms of* P_H)

Media.	Experiment 1.		Experiment 2.	
	Control.	Inoculated.	Control.	Inoculated.
String-bean decoction	4.88	7.82	4.80	7.99
Prune decoction	3.95	3.58	3.91	3.53
Irish-potato decoction	5.68	7.48	5.60	7.80
Carrot decoction	5.18	4.36	4.98	4.47
Turnip decoction	5.05	4.03	4.83	4.26
Sweet-potato decoction	5.22	3.24	5.05	3.31
Czapek's solution	4.84	2.34	4.14	2.47
Pfeffer's solution	3.53	2.56	3.48	2.61
Richard's solution	3.44	2.51	3.36	2.46
Beef bouillon	8.29	8.18	8.04	8.52

A survey of Table I shows that there is about as close an agreement in P_H values between the two experiments as could be expected from two experiments carried out at different times and under somewhat different conditions. The greatest variation noted is in the controls in connection with Czapek's nutrient solution. The solutions for the two experiments were prepared at one time so that those used for the second experiment stood for some time longer. A certain amount of evaporation would probably take place and chemical reactions are not unlikely.

A further examination of the table reveals other curious facts with respect to the hydrogen-ion concentration. The growth of the fungus caused some change in the hydrogen-ion concentration of all the solutions although in the case of beef bouillon and prune decoction it was not large. On the other hand, the change produced by the fungus when growing on string bean and Irish potato decoction and on Czapek's solution was quite marked. In experiment 1 in string-bean decoction the hydrogen-ion concentration was decreased from P_H 4.88 to 7.82 and

on Irish-potato decoction from P_H 5.68 to 7.48. The hydrogen-ion concentration was increased from P_H 4.84 to 2.34 on Czapek's solution.

If a comparison is made of the hydrogen-ion change produced in the six vegetable decoctions, it will be seen that in some cases it is increased and in others decreased. This is contrary to what might be expected on theoretical grounds. Before these experiments were conducted it had been many times demonstrated that *Rhizopus tritici* made sweet-potato decoction more acid. For this reason it was predicted that it would produce similar changes when growing on other vegetable decoctions prepared in a similar manner. However, it was found that some of the decoctions (carrot, turnip, sweet potato) became more acid while others (string bean, Irish potato) became less so.

The synthetic media were all more acid at the close than at the beginning of the experiment, Czapek's nutrient solution showing a greater increase in hydrogen-ion concentration than either Pfeffer's or Richard's solutions. Beef bouillon, a solution not well suited to the growth of *Rhizopus tritici*, was very little changed in acidity, becoming less alkaline and more alkaline in experiments 1 and 2 respectively.

The outstanding conclusion to be drawn from these experiments alone is that it is not safe to class any one fungus as an acid or an alkaline-producing organism. These experiments too clearly demonstrate that an organism may be an acid producer on one medium and the reverse on another. The class to which an organism belongs would clearly seem to depend upon the media used and should be determined in each individual case.

ENZYM PRODUCTION

The amount of enzym produced was measured by the time required to macerate raw sweet-potato and carrot disks 1 mm. thick suspended in the solution on which the fungus grew and in a water suspension of the mycelium. The time required to effect loss of cohesion of the cells of disks by the enzym secreted into the solution of the different media and retained by the mycelium is shown by Table II. It must be remembered that these results are only approximate, since it is not always easy to tell just when loss of coherence is complete. It is believed, however, that they are accurate within the limits of ±5 per cent.

TABLE II.—*The time (in hours) required to macerate raw sweet-potato and carrot disks by the enzym in the solution and in a water suspension of the mycelium*

Media.	Experiment 1.			Experiment 2.		
	Solution (sweet potato).	Hyphae.		Solution.		Hyphae (sweet potato).
		Sweet potato.	Carrot.	Sweet potato.	Carrot.	
String-bean decoction	6.00	7.0	7 to 24	4.5	7 to 24	7 to 24.
Prune decoction	None in 24.	7 to 24		None in 24	None in 24.	7 to 24.
Irish-potato decoction	3.75	5.0	7 to 24	3.0	6.5	7 to 24.
Carrot decoction	2.75	4.5	7 to 24	3.0	3.0	5.0
Turnip decoction	2.25	4.0	5	2.0	2.0	3.0
Sweet-potato decoction	2.75	5.5	7 to 24	3.0	5.5	6.75.
Czapek's solution	None in 24.	Considerable in 30.	None in 30.	None in 24.	None in 24.	None in 24.
Pfeffer's solution	do.	do.	do.	do.	do.	Do.
Richard's solution	do.	do.	do.	do.	do.	Do.
Beef bouillon	do.	do.	do.	do.	do.	Do.

It was demonstrated that a macerating enzym was produced by *Rhizopus tritici* when grown on all the vegetable decoctions, with the possible exception of the prune, and that this enzym was quite active in some cases (carrot, sweet potato, and turnip). An examination of experiment 2, Table II, shows that loss of coherence of both sweet-potato and carrot disks was complete in the turnip decoction in two hours and in the carrot decoction in three hours, and in the suspension of hyphae grown on these decoctions in three and five hours respectively. The rate of maceration by the enzym in the sweet-potato decoction and by that in the hyphae grown on this solution was about the same as that obtained in many previous tests. Although there was a fair growth of mycelium on prune decoction, the writers were unable to demonstrate the presence to any extent of pectinase either in the solution or in the hyphae. With this exception, the vegetable media all produced a demonstrable amount of pectinase. There was considerable variation in the amount produced in the different media, the complete loss of coherence being shown by some solutions in less than one-half the time required by others.

The production of pectinase could not be demonstrated in any of the synthetic solutions or in beef bouillon or the mycelium grown on them. From these results it seems safe to conclude that there is something in the composition of most of the vegetable decoctions which stimulates the production of pectinase, which is not present in the synthetic media or in beef bouillon.

A probable explanation of the difference between the synthetic media and the vegetable decoctions with respect to the production of pectinase by the fungus growing on them may be sought in the regulatory action of the substrate. A number of investigators have shown a quantitative regulation of the production of enzyms, while Knudson (*15*) demonstrated a qualitative regulation of tannase with *Aspergillus niger* and *Penicillium* sp. He found that these fungi produced gallic acid by the fermentation of tannic acid when the latter was added to a modified Czapek's nutrient solution, but if supplemented with glucose no tannase was formed. A number of other substances when used as a source of carbon failed to stimulate the secretion of tannase. Katz (*14*) studied the regulatory action of certain chemical substances in the substrate on the secretion of amylase by *Penicillium glaucum*, *Aspergillus niger*, and *Bacillus megatherium* and found that while the amylase secretion was not prohibited by the presence of substances chemically allied to starch, their effect was to greatly inhibit it. He found that the different fungi did not act identically and cites as proof the results obtained with *A. niger* and *P. glaucum*, in which case sugar had a much less inhibiting effect on the production of the enzym with the former than with the latter. Similar conclusions were reached by Duclaux (*6*) with *P. glaucum* and *A. glaucus*, though he considered only the enzym excreted into the culture medium. A number of other investigators, among them Kylin (*16*), have made similar studies. He worked with *P. glaucum*, *P. biforme*, and *A. niger*, and found no qualitative regulation of the enzyms (diastase, invertase, and maltase), though a quantitative regulation was conclusively proved. In the case of *P. glaucum* the regulatory secretion of diastase was greater than in that of *A. niger*. In this same connection the results obtained by Young (*26*) with *A. niger* may be cited. He showed that inulase was produced in greatest amount in the mycelium when inulin was used as the source

of carbon, but was likewise produced when other carbohydrates were employed. The substances most closely allied to inulin were most efficient in the production of the enzym. Investigations along similar lines have been made by Went (*22*), Wortmann (*24*), Dox (*5*), Pfeffer (*19*), Brunton and MacFayden (3), Harter (*8*), and others. Went, for instance, showed that *Monilia sitophila* secreted a number of enzyms, some of which were produced only when the particular substances on which they act were present in the culture solution. Brunton and MacFayden found that a bacterium formed diastase when cultivated on starch paste but not when grown on meat broth. Dox, on the other hand, demonstrated that the enzyms were secreted by *Penicillium camemberti*, regardless of the chemical nature of the substrate. He found that by cultivating the fungus on any particular substratum the quantity of the corresponding enzym could be increased, but that no enzym not normally produced by the organism could be developed by any special method of nutrition. Harter likewise showed that when different carbohydrates were used alone or in combination in the culture medium, although amylase was produced when sugars alone were employed, it was secreted in greatest abundance when starch was the only source of energy.

The preceding review of some of the literature shows that the quantitative regulation of enzyms is a rather common phenomenon. The qualitative regulation, however, has been demonstrated in only a few cases. Failure to demonstrate the presence of an enzym does not necessarily constitute positive proof that it is not secreted. It is a well known fact that some enzyms act only under certain conditions—that is, in the presence of certain acids or alkalies or electrolytes or other substances, the so-called co-enzyms. For example, it has been shown that the presence of either the chlorin or bromin ion is absolutely essential to the activity of pancreatic amylase (*1*). It is a fact, however, that the writers have been unable to demonstrate the production of pectinase by *R. tritici* on Czapek's, Pfeffer's, and Richard's solutions and on beef bouillon. On the other hand, it was freely produced in all vegetable decoctions with the exception of prune decoction, where its secretion was doubtful.

With these facts in mind the writers suspected that there might be some substance or substances in the vegetable decoctions which were stimulating the production of pectinase that were absent in the synthetic media and in beef bouillon and that this substance was probably one or more of the pectic compounds. Experiments were therefore initiated in which pectin obtained in as pure a state as it was possible to make it was introduced alone and in combination with glucose into Czapek's modified nutrient solution as the available sources of carbon.

The pectin was obtained from the carrot, the method described by Hunt (*10*) being followed for the most part in its preparation. A number of flasks were prepared, using Czapek's modified nutrient solution as the substrate. In some cases dextrose (20 per cent), in others pectin (1 per cent), and in still others dextrose (20 per cent), and pectin (1 per cent) in combination were supplied as the source of carbon. Thirty cubic centimeters of these solutions were placed in each flask and after inoculation with a spore suspension of *Rhizopus tritici* the cultures were incubated at 35° C. At the end of 4 days' growth the mycelial felts from all of the flasks of a single series were combined into one compound sample and prepared according to the usual method with acetone and ether for macerating experiments. Likewise, the solutions on which the

fungus grew from a single experiment were made into one sample and immediately used for the maceration of raw sweet-potato disks. A portion of this solution which was first steamed to inactivate the enzym together with the uninoculated solution, served as controls. Hydrogen-ion determinations were made of the inoculated solutions and of the uninoculated controls at the close of the experiment.

A good growth was obtained in each of the different series. The best growth and fruiting, however, were obtained when dextrose and pectin were combined in the same solution.

Some interesting results with respect to the hydrogen-ion concentrations were likewise obtained. In one experiment Czapek's solution plus 20 per cent dextrose with an original hydrogen-ion concentration of P_H 5.24 had a P_H of 1.93 after the fungus had grown on it for 4 days. When 1 per cent pectin alone was substituted for the dextrose the solution had an original P_H of 4.38 and (after the fungus had grown upon it) a final hydrogen-ion concentration of P_H 3.76.

In another experiment the following results were obtained: Czapek's solution plus 20 per cent dextrose with an initial P_H of 4.94 had a final P_H of 1.85. When 20 per cent dextrose and 1 per cent pectin were used the initial P_H was 4.09 and the final 1.70. If, on the other hand, pectin alone was used the original hydrogen-ion concentration was P_H 4.43 and the final P_H 3.90. The results show clearly that the pectin itself while supporting a good growth of mycelium is not so efficacious in the production of acid as dextrose.

Equally striking results were obtained with respect to the production of a middle lamellae dissolving enzym. It has already been pointed out that pectinase was not produced on Czapek's nutrient solution with dextrose as a source of carbon. Comparative experiments as detailed above showed that only a feeble macerating principle could be demonstrated either in the mycelium or in the solution on which the fungus grew when Czapek's solution plus 20 per cent dextrose was used or when the same solution plus 20 per cent dextrose plus 1 per cent pectin were employed as the source of energy. On the other hand, when pectin alone was used as a source of carbon a vigorous cell wall dissolving enzym was secreted. In one series of experiments coherence of the cells of sweet-potato disks immersed in the solution was entirely lost in 3¼ hours when pectin alone was used. When dextrose was employed 24 hours were required. There was no maceration in 24 hours in the steamed control when pectin was used. When glucose was used maceration was completed in 24 hours, which was probably caused by the acid (P_H 1.93) in the solution.

A second series of experiments gave results similar to those discussed above. When pectin alone was used as the source of carbon maceration in the solution and by means of the mycelium was completed in two and three hours, respectively. On the other hand, when glucose alone or in combination with pectin was employed 22 hours were required for the complete loss of coherence of the cells in the solution and in a water suspension of the mycelium. It is interesting to note in this connection that maceration was completed in the steamed controls in 22 hours, which may have been due to the acid (P_H 1.85 in 20 per cent dextrose and P_H 1.70 in 20 per cent dextrose + 1 per cent pectin) formed.

The results of the experiments as detailed above seem to indicate clearly a rather marked quantitative if not qualitative regulation of

pectinase. Although the qualitative regulation of pectinase has been demonstrated here for the first time, it is a well-known phenomenon for other enzyms. A brief review of some of the literature bearing on other enzyms showed that some of them, diastase for instance, are produced more abundantly when starch forms the only available source of carbohydrates in the solution.

INFLUENCE OF THE HYDROGEN-ION CONCENTRATION OF THE SUBSTRATE ON PECTINASE PRODUCTION

METHODS OF EXPERIMENTATION

Two different solutions, sweet-potato decoction and Czapek's modified solution, were used in the investigations of the influence of the hydrogen-ion concentration of the substrate on pectinase production. These two solutions were employed for two reasons: First, it has proved that *Rhizopus tritici* makes a good growth on both of them; second, they are easily prepared and thus available for experimental work at all times. The original solutions were prepared in sufficient quantity for a number of experiments. In the case of Czapek's nutrient solution the chemicals were put into a large enamel-ware vessel and the water added. The sugar was then added and the mixture heated for about one-half hour. A precipitate which formed during the process of heating was removed by filtering. The sweet-potato decoction was prepared according to the method described on page 862, except that 800 gm. of potato were used for each liter of water. From this point on the methods employed were the same for both solutions. Both solutions were made up to a strength such that when 30 cc. were diluted to 50 cc. they were of the desired concentration. The general method for adjusting a solution to a definite hydrogen-ion concentration described by Karrer and Webb (13) was employed. These authors, using Czapek's solution and beet decoction, worked out a method whereby the addition of a definite amount of acid, alkali, or water to 30 cc. of the stock preparation would give a definite hydrogen-ion concentration. They showed that it was possible to obtain a range of P_H 1.2 to 10 + and 1.2 to 9.8 in beet decoction and Czapek's solution respectively by varying the amounts of N/5 HCl and N/5 KOH. The writers, selecting out of the series given by Karrer and Webb such P_H values as they wished to use, employed their method and made up Czapek's nutrient solution and sweet-potato decoction to these hydrogen-ion concentrations. Although the hydrogen-ion concentrations obtained were not identical with those obtained by Karrer and Webb, they gave a range sufficient to meet all the needs of these experiments. The final amount of the solution in each flask was 50 cc. Exactly 30 cc. of the stock solution (Czapek's solution or sweet-potato decoction) were added to each flask by means of a self-filling burette. The desired amount of water was added and the solutions were then autoclaved for 20 minutes at 15 pounds pressure. The required amounts of acid and alkali were then added to each flask under aseptic conditions. The burette was first sterilized with 95 per cent alcohol and then washed with sterile distilled water. Care was taken at every step to prevent contamination. The quantity of acid, alkali, or water to be added to make the solutions up to 50 cc. depended upon the hydrogen-ion concentration desired.

The acid and alkali with which the dilutions were made were held in two liter flasks. The flasks were plugged with rubber stoppers through

which two glass tubes passed, one of which was long enough to extend nearly to the bottom of the flask. The liquid was drawn out through the long tube. The other tube, through which the air entered the flask, was stoppered with cotton to prevent the entrance of contaminating organisms with the inflowing air. The flasks so prepared were autoclaved for 20 minutes at 15 pounds pressure.

The culture flasks, after being finally prepared, were allowed to stand for two days before inoculation in order to determine which if any of them were contaminated. In spite of all the possible precautions, some of the flasks in every experiment became contaminated. These were discarded and a portion of the remaining flasks were inoculated with a spore suspension of *Rhizopus tritici*. Some of the flasks were held as controls. The cultures were incubated in the dark at a constant temperature of 35° C.

The duration of the experiment varied from 5 to 8 days. At the close of the experiment a portion of the mycelium and of the solution on which the fungus grew was prepared according to methods already described and used for macerating tests. Another portion of the mycelium was used in obtaining the dry weight of the fungous material produced. A portion of the solution was used to determine the hydrogen-ion concentration and the amount of dextrose present in Czapek's solution only. The sugar was determined by means of a Fric saccharimeter. The hydrogen-ion concentration and sugar content of the controls and inoculated flasks were determined on the day the experiment was terminated.

Fifteen flasks of each hydrogen-ion concentration were prepared, 10 of which were inoculated. The remaining 5 were held as controls.

EXPERIMENTAL DATA

It has already been shown that pectinase was not produced in Czapek's nutrient solution when dextrose was used as a source of carbon. It was found, however, that when pectin was employed in the substrate as the only source of carbon in Czapek's solution a powerful middle lamellae dissolving enzym was secreted. When pectin was combined with dextrose as a source of energy pectinase was produced, but its action was much slower, being presumably secreted in a much smaller amount. It was likewise pointed out that *Rhizopus tritici* produced a macerating substance on several vegetable decoctions (turnip, sweet potato, Irish potato, carrot, and bean) but not on synthetic media (Pfeffer's, Richard's, and Czapek's solution and beef bouillon). Furthermore, it has been demonstrated that some substance which causes maceration of raw sweet-potato disks is produced when *Rhizopus tritici* is grown on Czapek's nutrient solution. That this substance was an acid seems likely as some of the experiments to be detailed below will show. In view of the fact that Czapek's nutrient solution became rapidly acid when *R. tritici* was grown on it, it was suspected that the production of the acid interfered with the secretion of pectinase. It is also interesting to note in the way of comparison that when sweet-potato decoction in which pectinase was abundantly produced was used as a substrate the final acidity as measured in P_H, although increased, was never as high as in Czapek's solution.

A series of experiments were therefore outlined to determine what effect the original hydrogen-ion concentration of the substrate (Czapek's

solution, sweet-potato decoction) would have on the secretion of pectinase. The methods used in these experiments have been outlined already. The results of these experiments are shown in Tables III to VI.

Table III gives the results of experiment 1, in which the P_H values of the original control solutions (column 1) varied from 1.04 to 8.53. In this experiment the fungus grew for 5 days.

TABLE III.—*(Experiment 1) showing hydrogen-ion concentration and dextrose content of the control and inoculated Czapek's solution, the time required by them to macerate sweet-potato disks, and the dry weight of the mycelium produced*

P_H of control.	P_H of inoculated.	Dextrose in control.	Dextrose in inoculated.	Time required to cause maceration in control.	Time required to cause maceration in inoculated.	Dry weight of mycelium.
		Per cent.	*Per cent.*			*Gm.*
1.04	1.01	14.7	15.25	Complete in 5 hours	Complete in 5 hours	None.
1.71	1.76	14.95	15.25	Complete in 24 hours	Complete in 24 hours	Do.
2.62	2.27	14.95	14.55	Some in 48 hours	Some in 48 hours	0.0283
3.58	2.18	14.95	13.75	Nearly complete in 48 hours	Complete in 48 hours	.0728
5.82	2.17	14.95	13.40	....do	....do	.0972
6.01	2.18	14.95	13.30	Complete in 48 hours	....do	.1005
6.57	2.15	14.95	13.00	....do	....do	.1147
7.28	2.20	14.95	12.55	....do	....do	.1357
7.61	2.32	14.90	12.20	Some in 48 hours	....do	.1553
7.91	2.39	14.85	12.00	Very little in 48 hours	Nearly complete in 48 hours	.1620
8.12	2.59	14.70	12.40	Doubtful if any in 48 hours	Very little in 48 hours	.1748
8.53	2.66	14.55	12.40	None	Practically none in 48 hours	.1780

In experiment 2 (Table IV) the fungus was allowed to grow for seven days. Carrot disks were macerated in about the same time as the sweet-potato disks. If there was any measurable difference the carrot disks were macerated in a little less time than the sweet-potato disks.

TABLE IV.—*(Experiment 2) Showing the hydrogen-ion concentration and dextrose content of the control and inoculated solutions, the time required by them to macerate sweet-potato and carrot disks, and the dry weight of the mycelium produced*

P_H of control.	P_H of inoculated.	Dextrose in control.	Dextrose in inoculated.	Time required to cause maceration in control.	Time required to cause maceration in inoculated.	Dry weight of mycelium.
		Per cent.	*Per cent.*			*M.*
1.67	1.63	15.9	15.9	Complete in 22 hours	Complete in 22 hours	None.
2.67	2.22	15.9	14.9	Slight maceration in 30 hours.	Slight maceration in 30 hours.	0.0340
3.22	2.09	15.9	14.0	A little maceration in 30 hours.	A little maceration in 30 hours.	.0766
4.39	2.18	15.9	14.0	....do	....do	.0812
4.81	2.18	15.9	14.0	....do	....do	.0814
5.69	2.12	15.9	13.5	....do	....do	.1040
6.46	2.18	15.9	12.8	....do	....do	.1283
6.78	2.46	15.8	12.2	No maceration in 30 hours	No maceration in 30 hours	.1539
6.97	2.47	15.4	11.7	....do	....do	.1679
7.23	2.59	15.4	11.1	....do	....do	.1718
7.80	2.58	15.4	9.2	....do	....do	.1734

TABLE V.—*(Experiment 3) Showing the hydrogen-ion concentration of the control solution (sweet-potato decoction) at the beginning and end of the experiment, also of the inoculated solution, and the time required by the solution and mycelium to macerate sweet-potato disks*

P_H of control at end of experiment.	P_H of inoculated solution at end of experiment.	P_H of control at beginning of experiment.	Time required to complete maceration.			
			In inoculated unsteamed solution.	In inoculated steamed solution.	In uninoculated solution.	By hyphae ¼ gm. in 25 cc. H_2O.
			Hours.	*Hours.*	*Hours.*	*Hours.*
1.27	1.27	1.28	2	2.25	42	.....
2.05	2.10	2.10	7–24	48	48	.....
2.67	2.55	2.71	2.25	24	Slight in 48	5.0
3.80	3.12	3.83	2.00	4.5	None in 48	4.0
4.66	3.20	4.69	2.00	24.0	do	4.0
5.06	3.36	5.20	2.25	24	do	5.0
6.14	3.48	6.17	2.25	48	do	5.0
7.12	3.57	7.19	2.25	None in 48	do	.....
7.58	3.60	7.67	2.50	do	do	.....
8.28	3.69	8.33	2.50	.....	.....	5.5

TABLE VI.—*(Experiment 4) Showing the hydrogen-ion concentration of the uninoculated sweet-potato decoction at the beginning and end of the experiment, also of the inoculated solution, dry weight of mycelium produced, and time required to macerate sweet-potato disks*

P_H of control at beginning of experiment.	P_H of control at end of experiment.	P_H of inoculated solution at end of experiment.	Dry weight of mycelium.	Time required to complete maceration.			
				In inoculated unsteamed solution.	In inoculated steamed solution.	In uninoculated solution.	By hyphae ¼ gm. in 25 cc. H_2O.
			Gm.	*Hours.*	*Hours.*	*Hours.*	*Hours.*
1.24	1.24	1.27	.....	3.00	6	18	.....
2.01	2.01	2.02	.....	24.00	24	18	.....
2.69	2.63	2.54	0.0965	3.25	48	None in 48	4.75
3.63	3.57	3.17	.0115	2.50	24	do	3.50
4.33	4.31	3.29	.1268	3.00	24	do	3.75
5.13	5.05	3.51	.1214	3.00	24	do	4.00
6.05	6.02	3.51	.1186	3.00	24	do	4.50
7.29	6.32	3.58	.1388	3.25	48	do	4.50
7.99	7.53	3.72	.1280	3.25	None in 48	do	4.75
8.30	8.21	3.86	.1297	3.50	do	do	4.75
9.12	8.86	8.14	.0693	None in 48	do	do	5.50

In experiment 4, the fungus felts from five flasks were collected and the dry weight determined. The average weight is given in Table VI. The mycelium from the other flasks was used in determining the rate of maceration.

An examination of Tables III to VI shows some interesting facts with respect, first, to the changes in hydrogen-ion concentration of the solution produced by the fungus; second, to the influence these changes have on the production of pectinase; third, to the consumption of sugar (in Czapek's solution); and fouth, to the amount of mycelium produced. It will be seen that growth was entirely inhibited at a P_H of 1.7. The maximum limit of alkalinity for growth was not obtained, since the alkali in the solutions prepared to be about P_H 11.0 appeared to react with the sugars, thereby reducing the alkalinity. It was found in general, however, that the growth was best in the most alkaline solutions so long

as it was not alkaline enough to inhibit growth. Growth was better in sweet-potato decoction than in Czapek's nutrient solution at approximately the same hydrogen-ion concentration.

The fungus tends in all cases to make the solution more acid. The degree of acidity reached was greater in Czapek's solution than in sweet-potato decoction. In experiment 1 (Table III) no growth took place at a P_H of 1.04 and 1.71 and the hydrogen-ion concentration was therefore not much changed. As the hydrogen-ion concentrations of the control solutions decrease, however, the change in the P_H value of the inoculated solutions becomes greater, as, for example, P_H 8.53 (Table III) was changed to 2.66. It will be seen from Table III and the other tables also that the P_H of the inoculated solutions gradually increase with the decrease in the hydrogen-ion concentration of the control solutions.

That many fungi and bacteria make the substrate acid or alkaline is well known. Currie (*4*) showed that citric acid was produced by *Aspergillus niger* in a nutrient solution and Wehmer (*20*) gave the generic name Citromyces to a group of fungi which he believed was characterized by its ability to produce the same acid. Lafar (*17*), and Lind (*18*) found that oxalic acid was produced by certain species of Penicillium, Botrytis, and Citromyces. Weimer and Harter (*21*) showed that *Rhizopus tritici, Diplodia tubericola, Mucor racemosus, Penicillium sp.* and *Botrytis cinerea* increased the acidity of Czapek's nutrient solution when glucose was used as the source of carbon. Young and Bennett (*25*) found that *Fusarium oxysporum* when grown on Richard's solution with a P_H 5 first made the solution acid and then alkaline so that in 40 days a P_H of 7.4 was reached. Bacteria appear to produce acid in the solution, though not to so marked a degree. For example, Jones (*11*) found that a strain of Pneumococcus when inoculated into a medium with an initial reaction of P_H 7.0 grew poorly and developed a P_H of 6.2. If, on the other hand, the initial reaction was 7.6 a good growth and a final P_H of 5.4 resulted. *Streptococcus viridans* was found by Grace and Highberger (*7*) to change a broth with an initial hydrogen-ion concentration of between P_H 7.1 and 7.3 to a P_H 6.6 in 6 days. Similar changes in the hydrogen-ion concentrations were noted by Wolf and Harris (*23*) and by Karrer (*12*) with certain bacteria and fungi, respectively. A comparison of the results of other investigators with those obtained by the authors seems to indicate that *Rhizopus tritici* renders the substrate more acid than most fungi or bacteria. As a matter of fact, it will be seen that the degree of acidity produced (P_H 2.09, Table IV) while not prohibitive of growth, which is practically stopped in a P_H 1.7, closely approximates it.

From Tables III and IV it is seen that pectinase is not produced in Czapek's nutrient solution when adjusted to any of the hydrogen-ion concentrations tested. This conclusion is drawn from the fact that, although a certain amount of maceration took place in certain solutions, especially in those with the highest hydrogen-ion concentrations, there was just as much in the steamed as in the unsteamed solutions. In the solution with an initial P_H of 1.01 (Table III) no growth of the fungus took place and yet maceration was complete in 5 hours. When the initial P_H was 1.76, 24 hours were required to complete maceration. At this concentration no growth occurred. At all other concentrations there was a normal amount of mycelium produced and the final hydrogen-ion concentration of the inoculated solutions varied from P_H 2.15 to 2.66

(Table III). At a concentration of P_H 2.66 no maceration occurred. At the other concentrations about 48 hours were required for the cells to completely lose their coherence. There is therefore a degree of acidity such that the acid has no action on the middle lamellae in 48 hours (P_H 2.66, Table III). At a P_H of 2.59 little maceration took place in 48 hours. In the remaining solutions (Table III) the acidity is about sufficient to dissolve the middle lamellae in 48 hours. At P_H 1.76 maceration is complete in 24 hours and at 1.01 in 5 hours. Similar data are shown in Table IV.

The data presented in Tables V and VI were obtained by growing *R. tritici* on sweet-potato decoction adjusted to the different P_H values. These show that the decoction does not become as acid as Czapek's solution. No growth occurred in P_H 1.27 and 2.03 (Table VI), but at all other concentrations about the same amount of mycelium was produced. The hydrogen-ion concentration of the solutions when there was growth was considerably increased; however, in no case was the increase as great as that found in Czapek's solution. At the two higher concentrations the maceration produced was doubtless due to the action of the acid, since in one case (P_H 1.27, Table V), although there was no mycelium produced, coherence of the cells was lost in 2 hours, and in another case (P_H 2.10, Table V) in 7 to 24 hours. In all the other concentrations the middle lamellae were completely destroyed in from 2 to 2½ hours in the unsteamed solutions. In the steamed solution there was practically no maceration in 48 hours.

The conclusions to be drawn from these data are, first, that pectinase is not produced in Czapek's nutrient solution at any hydrogen-ion concentration tried and, second, that its production in sweet-potato decoction is not interfered with when the hydrogen-ion concentration is not so high as to prevent growth. In other words, pectinase is produced by *R. tritici* when growing in sweet-potato decoction at any hydrogen-ion concentration that will permit its growth. Pectinase was also present in the mycelium.

The amount of sugar present in the control (not inoculated) and the inoculated solutions was determined for Czapek's solution only. At the higher hydrogen-ion concentrations no fungous growth took place and no sugar was used. As a matter of fact, there appears to be a slight increase in the amount of sugar present in the controls over that in the inoculated solutions, probably due to some concentration of the sugars as a result of evaporation. A measurable amount of sugar was consumed by the fungus at all other hydrogen-ion concentrations, the larger amount being at the higher P_H values. It will be noted that the amounts of sugar consumed at the lower hydrogen-ion concentrations are proportionately greater than in the less alkaline solutions. This is also true in the controls. No doubt this variation may be accounted for, at least in part, by the reaction between the sugar and alkali. The largest amount of mycelium was produced at the lower hydrogen-ion concentrations.

An examination of Tables III and IV shows that in Czapek's nutrient solution the amount of mycelium produced increases with the decrease in hydrogen-ion concentration, varying from 0.0283 gm. to 0.1780 gm. (Table III) and from 0.0340 to 0.1734 gm. (Table IV). No mycelium was produced at the highest hydrogen-ion concentration. In experiment 3, the dry weight of the mycelium was not determined, but in experiment 4 (Table VI) there was a more or less gradual increase in dry

material as the hydrogen-ion concentration decreased, with one exception. In the solution with an original P_H of 8.86, which is apparently too alkaline for normal growth, the dry weight was somewhat less than it was in the solution with a P_H of 8.21 (Table VI).

It has been intimated that the maceration which takes place in solutions after they have been steamed is due to the action of some one or more acids produced in the solution by the fungus. A number of acids have been shown by different investigators to be formed by fungi in nutrient solutions, among them being oxalic, acetic, and formic. These three organic acids, together with hydrochloric acid and sodium hydroxid, were made up to different hydrogen-ion concentrations and their ability to dissolve the middle lamellae determined. The results showed that maceration could be brought about by the acids in high hydrogen-ion concentrations and by the alkali at a P_H of about 12 or above. Oxalic acid at a P_H of 2.13 macerated raw sweet-potato disks in from 24 to 48 hours at a P_H of 2.44 in 72 hours. No maceration occurred in 72 hours by this acid at a P_H of 2.76. Acetic acid appeared to require more time to complete maceration than oxalic acid at the same concentration. Acetic acid of P_H 2.13 macerated the disks in 72 hours, while at P_H 2.40 the amount of maceration was very slight in that length of time and was entirely absent at a P_H of 2.44. In a P_H 1.99 of formic acid maceration was nearly complete in 6 hours, at P_H 2.16 in 24 hours, and at P_H 2.23 in 54 hours. At hydrogen-ion concentrations of P_H 2.40 and 2.57, there was slight and no maceration, respectively, in 54 hours by this acid. In full strength formic acid maceration was complete in 3 hours. Hydrochloric acid of a P_H 2 will induce slow maceration. A comparison of these results with those obtained when Czapek's solution was used shows that the loss of coherence of the cells of sweet potato disks occurs in pure solutions of these acids at about the same hydrogen-ion concentration as in Czapek's solution and in about the same length of time. Oxalic acid macerates a little more rapidly than acetic acid at the same concentration, and formic acid a little more rapidly than either oxalic or acetic.

The results of these investigations seem to indicate that no pectinase is produced in Czapek's nutrient solution when glucose is used as a source of carbon. However, an acid is produced in sufficient hydrogen-ion concentration to cause a dissolution of the middle lamellae identical with that caused by pectinase. It was found also that the original P_H value of the solution had no influence on the secretion of pectinase. In all concentrations of Czapek's solution in which the fungus grew the hydrogen-ion concentration was increased, the greatest increase being in the solutions with the highest P_H values.

A middle lamellae splitting enzym was produced when pectin alone was used as a source of carbon in Czapek's nutrient solution and to a less extent when combined with glucose. It had been suspected that the failure to obtain maceration in Czapek's solution was not due to the absence of the enzym, but to its inactivation by the acid formed. Brown (*2*) showed that the addition of some of the organic and mineral acids to extracts of Botrytis mycelium retarded the action of the enzym, the time required to macerate potato disks being increased with the increase in the concentration of the acid. A point was finally reached when inactivation was complete. The retarding action of all the acids, was found to be about the same up to a certain concentration beyond

which the retarding action of the mineral acids increased much more rapidly. A concentration was finally reached where maceration was caused by the acid. Brown in his studies used dilutions of normal acids, so his results can not be compared directly with those of the writers, who studied the action of the acids in terms of hydrogen-ion concentrations. That the acid formed in Czapek's solution did not inactivate the enzym may be inferred from the following facts. When Czapek's solution, which was made acid (P_H 1.8) by the growth of the fungus, was changed to a P_H of 7.8 and raw sweet-potato disks added no maceration took place. It had been previously shown that the enzym was not inactivated at P_H of 10. The solution was not alkaline enough to inactivate the enzym. Some of the data obtained with sweet-potato decoction may be cited as further evidence. It has been shown (Table V) that maceration in sweet-potato decoction is complete in 2¼ hours at a P_H of 2.55. On the other hand, in Czapek's solution with a P_H of 2.66 (Table III) which is even less acid no maceration occurred in 48 hours. Although there is a difference in the composition of these two media, it is reasonable to assume that since the acid was not sufficiently strong in the sweet-potato decoction (P_H 2.55) to inhibit the action of the enzym it would not be able to do so in the less acid Czapek's solution (P_H 2.66).

The investigations of the authors showed that the presence of pectinase in the mycelium grown on Czapek's nutrient solution could not be demonstrated, while it occurred regularly in the hyphae produced on sweet-potato decoction. If the enzym was produced, its occurrence in the hyphae on Czapek's solution as well as on sweet-potato decoction would be expected. Furthermore, the enzym in the mycelium, if it occurred there, would not be inactivated by the acid in the substrate, since the mycelium is always suspended in distilled water, which is practically neutral.

SUMMARY

(1) *Rhizopus tritici*, a fungus capable of causing the softrot of sweet potatoes was used in all these experiments. On suitable media it produces a powerful enzym, called pectinase, which dissolved the middle lamellae of raw sweet-potato and carrot disks.

(2) A comparison of the production of the enzym was made on the following media: String bean, prune, Irish potato, carrot, turnip, and sweet-potato decoction, and on Czapek's, Peffer's, and Richard's synthetic media and on beef bouillon.

(3) It was found that the cell wall dissolving enzym was produced on all the vegetable media except prune decoction, but not on the synthetic media with glucose as a source of carbon or on beef bouillon.

(4) If pectin was used alone as a source of carbon in Czapek's nutrient solution, an active enzym was produced. When pectin was combined with glucose the action on raw sweet-potato disks was feeble.

(5) When *Rhizopus tritici* was grown in Czapek's nutrient solution with glucose as a source of carbon the substrate became sufficiently acid to cause dissolution of the middle lamellae so that coherence of the cells was completely lost in from 6 to 24 hours.

(6) The production of the macerating principle by the fungus when growing on Czapek's nutrient solution and sweet-potato decoction was not influenced by adjusting these solutions to different P_H values. The enzym was not produced in Czapek's solution at any hydrogen-ion

concentration tested. On the other hand, it was secreted in sweet-potato decoction at all concentrations tried.

(7) The best growth as measured by the dry weight of the mycelium took place at the lowest hydrogen-ion concentration.

(8) The amount of glucose consumed in Czapek's solution is correlated with the amount of mycelium produced.

(9) The substrate influences the production of pectinase; the hydrogen-ion concentration does not. The results indicate a well-marked qualitative action of the substrate on the production of the macerating enzym, in that when vegetable decoctions which probably contain pectin are used and when nutrient solutions to which pectin is supplied as a source of carbon is employed pectinase is produced.

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THE MICROSCOPIC ESTIMATION OF COLLOIDS IN SOIL SEPARATES[1]

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In a recent work upon soil colloids,[2] in which a description of the soil materials classified as colloids was given, it became desirable to compare the values obtained by an adsorption method for determining the colloidal content of soils with values obtained by some method independent of adsorption phenomena. The most satisfactory method for this purpose would be a mechanical separation of colloidal from noncolloidal soil materials. But it has been shown in previous publications[2,3] that a mechanical analysis probably does not effect a complete separation. However, it seemed possible that colloidal matter not separated from the soil by mechanical analysis might be estimated by microscopical means. Then the sum of the colloid extracted and that estimated microscopically would give a value for the total colloid in the soil by methods quite independent of adsorption.

For the purpose of separating the colloids from the coarser particles of the soils the samples were repeatedly treated by agitation with distilled water containing a trace of ammonia, rubbed with a rubber pestle, and the supernatant liquid decanted and centrifuged. From 40 to 60 such treatments yielded practically all of the colloid extractable by this method. For convenience of manipulation the soils were divided, during the washing and rubbing process, into the colloidal fraction made up of particles less than 0.001 mm. in diameter, a fine fraction made up of particles ranging approximately from 0.001 to 0.050 mm. in diameter, and a coarse fraction consisting of particles larger than about 0.050 mm.[2]

The adsorptive capacities of these fine and coarse residues indicated that they contained colloidal material.[2] Observations of the residues with the ultramicroscope showed that the mineral particles had been fairly well cleaned of adhering colloidal matter by the washing and rubbing, and that the colloidal material remaining in these fractions was in the form of lumps or aggregates made up, at least superficially, of very large numbers of particles less than 0.001 mm. diameter. But since the light coming to the eye from the ultramicroscope is reflected from the surfaces of the particles under examination it was possible that the colloidal aggregates thus observed were simply mineral grains completely coated with colloids or that, at least, the colloidal aggregates

[1] Accepted for publication Apr. 7, 1923.

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inclosed minute mineral particles. The clean appearance of the obviously mineral particles was, of course, a strong indication that such was not the case, but the element of doubt remained nevertheless.

Under the petrographic microscope in transmitted light these colloidal aggregates were found to be almost universally transparent and, in the rare cases where this was not so, to be highly translucent. Therefore any mineral grain included within the colloidal aggregate would be readily visible provided that either its color or index of refraction was different from the color or index of the colloid. Observation of birefringence between crossed nicol prisms would, of course, readily differentiate between minerals and colloids regardless of similarity of color or refractive index. Such observation, however, showed that practically all of the colloidal aggregates were free from mineral inclusions. Presumably, aggregates containing such had been broken up by the repeated rubbing and washing process.

Therefore, since the residues consist of easily determinable minerals and colloidal aggregates, one readily distinguishable from the other, a microscopic estimation of the relative quantity of each in a given sample was easily obtainable. Owing to the extreme variation of size of particles a straight count would have been subject to large errors. Therefore, the estimation was made by means of a checker-work eyepiece micrometer, relative space occupied by the minerals and colloids being the basis of the calculation. It is believed, on the basis of results obtained with synthetic samples, that errors due to the varying thickness of the particles, both minerals and aggregated colloids, are fairly well balanced and do not appreciably affect the results. Quartz is the predominant mineral in the fractions, and since the specific gravity of this mineral (2.66) and that of the colloids (2.53–2.68) extracted from the soils are very near together, it was believed that differences in specific gravity would not give rise to any serious error.

In order to test this method of microscopic estimation, four synthetic samples were made up, each containing a known amount of colloidal material. Air dried Marshall soil colloid, which had been extracted mechanically and graded by means of the supercentrifuge, was mixed with quartz in the proportion of 1.2 gm. colloid to 0.8 gm. quartz and 0.8 gm. colloid to 1.2 gm. quartz. The first of these mixtures was ground dry to pass a 200-mesh sieve. In order to facilitate any tendency of the colloid to form coatings on the mineral particles, the second sample was ground wet, dried and subsequently rubbed lightly with a pestle to pass a 200-mesh sieve.

Since quartz is practically without cleavage, the particles formed by grinding have a tendency to assume shapes in which the dimensions vary little in different directions within the same particle, although the dimensions of different particles may vary widely. This statement also holds good for the colloidal aggregates. Such a similarity of fracture facilitates a microscopic estimation of the relative quantities of the different constituents present, and therefore does not afford a very difficult test of the method. But, since quartz is the predominant mineral constituent of soils, the results obtained with quartz are probably very near those obtained on the soil separates themselves.

For the purpose of testing the method under more unfavorable conditions, Orangeburg subsoil colloid, extracted mechanically and graded by the supercentrifuge, was mixed with hornblende which has a tendency

to cleave into oblong particles with the greater dimensions several times the magnitude of the smaller, thus presenting a widely different shape from the colloidal aggregates with which it was to be compared. Two samples were prepared consisting of 0.3 gm. colloid to 1.7 gm. hornblende and 0.4 gm. colloid to 1.6 gm. hornblende. The first of these was mixed dry and the second wet, as with the Marshall colloid-quartz samples.

The results of the microscopic examination of these samples are given in Table I.

TABLE I.—*Microscopic estimation of colloids in synthetic samples*

Sample.	Colloid present.	Colloid observed.
	Per cent.	*Per cent.*
Marshall colloid and quartz, mixed dry	60	57
Marshall colloid and quartz, mixed wet	40	45
Orangeburg colloid and hornblende, mixed dry	15	12
Orangeburg colloid and hornblende, mixed wet	20	23

These results showed that the colloid in the fine and coarse residues of the soils could be estimated with fair accuracy by this microscopic method.

Microscopic examinations were therefore made of the fine and coarse residues of eight soils which had been subjected to the repeated washing and rubbing process. The results are given in Table II. The quantity of the unextracted colloid in the fine and coarse residues, as determined by this microscopic method, are given in column 3. The additional data in Table II are given for the purpose of showing the relative amounts of "extractable" and "unextractable" colloids in the soils.

From Table II it will be seen that from 9.4 to 42.4 per cent of the soil was made up of colloids extractable by the methods employed. The microscopic examination of the residues shows that from 25 to 97 per cent of the fine residues and 2 to 25 per cent of the coarse residues were colloidal aggregates not extractable by the methods employed.

These results throw considerable doubt upon the results of several investigators who have determined the colloidal contents of soils and clays by purely mechanical methods. Hissink[4] and Sven Odén,[5] for example, have dispersed colloids in soils by very elaborate washing and rubbing processes. It is of course possible that they may have effected complete dispersion by their methods, but apparently no investigations were undertaken to ascertain whether, or to what extent, aggregates of undispersed colloids remained in their soil suspensions. Williams,[6] in the course of a very elaborate and painstaking separation of colloids from soils by washing, rubbing and boiling, did examine his residues microscopically. Details of his microscopic method are not given, but it is apparent that he used an ordinary chemical microscope and mounted his residues either in air or water. Owing to the wide differences in refractive indices between these mounting media and the ordinary soil minerals, scarcely more than surface phenomena could be

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observed under these conditions; and consequently the distinction between lumps of aggregated colloids and soil minerals would reduce itself purely to a question of personal judgment based on appearance. Colloidal aggregates might bear very close resemblances to corroded mineral particles and ferruginous or rutilated quartz, for example. Even with a completely equipped petrographic microscope careful cognizance must be taken of the pseudo-similarities of the optics as well as the resemblances of some crystalline and amorphous materials, such as the similarity between colloids subjected to strain and the matted structure of such minerals as chlorite.

TABLE II.—*Microscopic estimation of unextracted colloids in fine and coarse residues of soils*

Sample.	Quantity of fractions separated mechanically, as part of whole soil.	Quantity of unextracted colloid in residues determined microscopically, as part of residue.	Unextracted colloid in combined fine and coarse residues, as part of whole soil.	Unextracted colloid, as part of total colloidal material in soil.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Cecil clay loam, soil:				
Colloid extracted	9.4	.....	.....	.....
Residue finer particles	7.6	74	.....	.....
Residue coarser particles	83.8	4	9.0	48.9
Cecil clay loam, soil:				
Colloid extracted	9.4	.....	.....	.....
Residue finer particles	10.4	55	.....	.....
Residue coarse particles	78.3	4	8.9	48.6
Huntington loam, soil:				
Colloid extracted	10.3	.....	.....	.....
Residue finer particles	21.9	48	.....	.....
Residue coarse particles	64.0	8	15.6	60.2
Huntington loam, subsoil:				
Colloid extracted	13.3	.....	.....	.....
Residue finer particles	19.4	25	.....	.....
Residue coarse particles	63.3	10	11.2	45.7
Sassafras silt loam, subsoil:				
Colloid extracted	14.4	.....	.....	.....
Residue finer particles	20.6	38	.....	.....
Residue coarse particles	61.9	2	9.1	38.7
Sharkey clay, soil:				
Colloid extracted	42.4	.....	.....	.....
Residue fine and coarse particles combined	53.1	42	22.3	34.5
Sharkey clay, soil:				
Colloid extracted	31.5	.....	.....	.....
Residue finer particles	38.9	52	.....	.....
Residue coarse particles	31.8	25	28.2	47.2
Vega Baja clay loam, soil:				
Colloid extracted	30.5	.....	.....	.....
Residue fine particles	38.6	97	.....	.....
Residue coarse particles	31.8	14	41.9	57.9

The calculations given in column 4 of Table II show that from 8.9 to 41.9 per cent of the whole soil was composed of colloidal aggregates not extractable by the methods used. It should be mentioned, however, that the Vega Baja soil which showed a total colloidal content of 72.4

per cent is an exceptional soil. Usually the quantity of colloid present is much lower. Basing the calculations on the colloidal matter alone, it is seen from the table that 34.5 to 60.2 per cent of the total colloids in the soils were not extracted by the methods used. Although this method of repeated rubbing and washing for the extraction of colloids from soils was carried out as carefully and thoroughly as possible, and although we are confident that no more than traces of colloidal material could have been extracted by further washing and rubbing, it by no means follows that other more efficient methods might not be devised.

As practiced in this bureau, microscopic estimation of the amount of colloidal aggregates in the fine and coarse residue is, at best, a long and tedious process. In order to insure fair sampling of the sample under study several mounts must be examined, and several areal counts should be made on each mount. Experience showed that a total of 10 to 12 counts on about four slides gave averages very comparable with those obtained by triple that number of counts on as many more mounts. Nevertheless when only 12 counts are made, the method is too long for routine application.

Unfortunately, the preliminary washing and rubbing are essential in order to remove coatings of the adhering extractable colloid. These coatings are rarely thick enough to interfere with the determination of the mineral; but the microscopic estimation of the quantity of colloid in the coatings would be extremely difficult and inexact. This necessity for washing the soils adds tremendously to the time consumed. Nevertheless, the method is of value in special studies.

SUMMARY

(1) A microscopic method for estimating the quantity of colloidal soil aggregates in soil separates is given.

(2) This method was applied to the residues left after extracting, by repeated rubbing and washing, all colloid possible from eight samples of soil. The results showed that from 34.5 to 60.2 per cent of the total colloidal material in the soil was in the form of colloidal aggregates not extractable by washing and rubbing.

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MORPHOLOGY AND HOST RELATIONS OF PUCCINIASTRUM AMERICANUM[1]

By B. O. DODGE

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The orange rusts have always been considered as practically the only rusts of economic importance on species of Rubus in America. *Kuehneola uredinis*, it is true, is known to cause considerable damage to fruiting canes of certain cultivated varieties of blackberries. *Pucciniastrum americanum* (Farl.) Arth. is not rare on wild raspberries but its effects on the host have been found heretofore to be altogether negligible. Brief notes dealing mostly with its relationships to the European form *P. arcticum* have appeared from time to time but no reference has been made to damage to cultivated raspberries. An outbreak of this rust, therefore, of such severity as to cause defoliation and spotting of canes of certain varieties, becomes a subject of interest.

One of the native hosts of this rust is the wild *Rubus strigosus*, and like the orange-rusts and the stemrust, Kuehneola, the Pucciniastrum will no doubt be found wherever the natural hosts may grow. The rust has been reported rarely on *R. occidentalis*, but "*R. neglectus*," a hybrid between the red and the black raspberries, seems to be rather susceptible according to Farlow (*9, p. 13*).[2] At Bell, Md., where an outbreak occurred in 1922, Mr. G. M. Darrow has gathered together from all parts of the world a number of species and horticultural varieties of Rubus and has originated many new forms in connection with his breeding work. During July it was found that the leaves of several different hybrids were dropping off prematurely, leaving the canes entirely bare below. The lower surface of the leaves was evenly covered with uredospores, giving them nearly the same appearance as blackberry leaves heavily infected with the Kuehneola. The leaves at the growing tips were sometimes only slightly affected and the attacks seemed to increase in severity from the tips downward, showing that the rust had been present for some time.

Conical peridia (Pl. 1, C), each surmounted by a corona, were easily found on leaves just being attacked, and as the spores were identical with those found on the fallen leaves there can be no question that this defoliation was being caused by what has heretofore been considered a harmless rust. From July 28 to August 4 a survey was made to ascertain which species, varieties, and hybrids were being attacked. Mr. Darrow cooperated with the author in going over these records.

[1] Accepted for publication Jan. 22, 1923.

[2] Reference is made by number (italic) to "Literature cited," pp. 893-894.

Certain varieties were represented by as many as 75 plants, others by only 1 or 2, and conditions for infection were perhaps more favorable on one side of the field than on the other, so that a full report on these observations would not fully represent the actual degrees of susceptibility. Among others, the following seedlings were found to be quite badly infected: Ranere × Cuthbert,[3] Cuthbert × *R. lasiostylus*, La France × Ranere, Van Fleet × Ranere, Laxtonberry × Ranere, Cuthbert, Gregg × Cuthbert, Brighton × La France, Brighton × *R. lasiostylus*, *R. coreanus* × Brighton, Shaffer seedlings, Shiebley × King, *R. leucodermis* × King, Newman 23 × *R. coreanus*, seedlings of Hailsham open pollinated, Syracuse open pollinated, Brighton selfed. The Ranere (St. Regis) was infected, but not as heavily as were the Cuthberts.

Certain hybrid seedlings were so severely infected as to result in almost complete defoliation, petioles and canes also being attacked. These very susceptible kinds include such hybrids as June × Cuthbert, Latham × Brighton, Latham × Ranere, Erskine × Ranere, *R. coreanus* × Brighton, etc.

A number of varieties in this plot were only slightly infected, but, as previously noted, the number of plants in many cases was too small to prove that they were resistant. About 50 plants each of the following forms were carefully examined at this time, but no rust was found: *Rubus Idaeus*, open pollinated seedlings of the Mahdi (which is a cross between *R. Idaeus* and a European blackberry), *R. innominatus* × Antwerp, *R. innominatus* × King, *R. innominatus* (open pollinated seedlings). A few self-pollinated Cuthbert seedlings in this field showed no rust, but many plants of this variety in a garden near by were heavily infected.

Separated from these plots by about 40 rods was a third plot, in which several plants of wild *Rubus occidentalis*, *R. strigosus*, *R. leucodermis*, King, Cuthbert, Ranere, etc., were growing. A few pustules were found on one unnamed variety. A month later the rust was very abundant on the King, June, Newman 23, Cuthbert, and some was found on *R. strigosus*, but *R. occidentalis*, *R. Idaeus* (Hailsham variety), and 5 Asiatic raspberries were not affected.

Mention has been made of finding the rust on leaf stalks and canes. The cankers on the latter are sometimes 2 or 3 inches long. A species of Gloeosporium (not anthracnose) was also common in large cankers on the varieties whose canes were most heavily attacked by the rust. It is possible that this fungus became established in the lesions first formed by the rust; at any rate the combined effect of the two fungi will be to kill the canes. The calyx and carpels of late-fruiting or everbearing varieties were sometimes badly rusted. It is certainly curious to see the golden-yellow pustules deeply embedded in the juicy red carpels. There was more rust on the fruit of a late-flowering form of *R. strigosus* than on the leaves.

A press of work prevented a satisfactory examination of species of wild and cultivated raspberries in other localities to learn how common the rust was in the vicinity. It is clear, however, that the rust had found in these hybrids very susceptible hosts.

[3] The Black Pearl, Kansas, Cumberland, Gregg, and Plum Farmer, are American horticultural varieties of black raspberry which were originated as seedlings or by selection from *Rubus occidentalis*, of which *R. leucodermis* is a western variety; the Shaffer, Columbian, and Royal Purple are of American origin, probably derived from the form *Rubus neglectus*, which is a hybrid between *R. strigosus* and *R. occidentalis;* the Cuthbert, King, Victor, Erskine, June, Latham, Ranere, and Brighton, are American horticultural varieties of red raspberries, and related to *R. strigosus; R. Idaeus*, the Antwerp, La France, Laxtonberry, and the Hailsham, are European red raspberries; *R. coreanus* and *R. innomiatus* are native of China.

An inspection of the berries grown at the Government farm at Arlington, Va., was not made until the latter part of September. From 6 to 50 hills each of Cuthbert, King, Ranere, Antwerp, wild *Rubus occidentalis*, Kansas, Black Pearl, Cumberland, Plum Farmer, Shaffer, Columbian, and Royal Purple are grown. The Cuthbert red raspberry was the most severely attacked; much defoliation had resulted, and the remaining lower leaves were well covered with uredinia. Considerable rust was found on the Columbian, Royal Purple, and the Victor. The Ranere (St. Regis) and King varieties bore some rust, but not enough to cause any damage. No rust was found on any of the black raspberries grown here, and as *R. occidentalis* (at Bell, Md.) showed no rust it may be assumed that our black raspberries are very resistant. It is not strange to find that crosses between the black and the red varieties in many cases prove to be very susceptible. Whether the strain of the rust which made its appearance in the vicinity of Washington, D. C., is more virulent than that heretofore found on *Rubus "neglectus"* and *R. strigosus* is not known, but it is evident that many of the hybrids at Bell are more susceptible than either of the parents.

The host is occasionally erroneously identified by collectors, especially in the case of the red raspberry, which is often confused with the black raspberry. For example, in the Path. Coll., B. P. I., is found No. 2280, N. A. Uredinales, one gathering at Madison, Wis., October 6, 1906, and another at Mackinac, Mich., September 6, 1894, in which the host is given as *R. occidentalis* when it is a red raspberry. Arthur (*2, p. 190*) states that *R. occidentalis* was erroneously given as a host for *Pucciniastrum americanum* in Bull. Torrey Club (*1, p. 468*). In the same article a specimen from Algoma, Wis., was referred to *P. arcticum*, and the host is given as *R. occidentalis*. The rust is clearly of the *P. americanum* type, and the host is *R. strigosus*. Davis (*6*) has shown that *R. occidentalis* can be infected by *P. americanum* from *R. strigosus*. We have not found pustules of this rust on the wild black raspberry in the vicinity of Washington, D. C., but more careful search would very likely result successfully. The rust is undoubtedly more widely distributed than is commonly supposed. Specimens on cultivated raspberries have been referred to other species by mistake. In the Path. Coll., B. P. I., we find one packet of the rust on a red raspberry originally labeled *Lecythea Ruborum* Lev.; No. 32, Economic Fungi, Seymour and Earle, *Phragmidium Rubi-Idaei* (P) Wint. is *Pucciniastrum americanum*. One gathering of Pucciniastrum on *R. strigosus* was sent to the author as orange-rust in response to a request for red raspberry plants infected with the Gymnoconia.

THE MORPHOLOGY OF THE UREDINIUM

Farlow (*9*) recognized that the rust which he had found on a hybrid raspberry differed somewhat with respect to the shape of the peridium from *Pucciniastrum arcticum* of Europe. The peridium was more sharply conical than that of *P. arcticum*, which is said to be low and broad. He called attention to a good illustration of the peridium of the American form by Dietel in Hedwigia (*7, p. 331*) under the name *Phragmidium gracile*. Farlow proposed the variety name *americanum*, which Arthur (*1, p. 468*) later raised to specific rank. Davis (*5*) has shown that *Pucciniastrum arcticum* is very common in Wisconsin on *Rubus triflorus* and questioned at that time whether the differences in the morphology noted by Farlow may not after all be due to host differences. He is now convinced (*6*),

however, that the two forms are distinct and reports the work of one of his students who failed to infect *R. strigosus* with uredospores from *R. triflorus*, whereas *R. strigosus* and *R. occidentalis* were infected with spores from *R. strigosus*. The reader is not informed just what was done in the way of attempting to infect *R. triflorus* with spores from either host. The leaves of *R. arcticus* and *R. triflorus*, practically devoid of tomentum on the lower surface, are certainly quite different in texture from those of our common raspberries. No one would question that there may be, as we have shown, certain hosts that are more susceptible than others, and it is possible that differences in texture of tissues attacked by the rust could very well account for the differences or modifications in the form and size of sori which originally led Farlow to suggest that the form on *R. strigosus*, etc., be called *P. arcticum* var. *americanum*. The presence of *P. americanum* at Bell, Md., where pustules occurred so abundantly on leaf blades, midribs, petioles, canes, floral envelopes, and even on the fruit, offered a rare opportunity to study the morphology of the sorus.

Our sections show that there is a great variation in the form of the uredinia developing on the same plant. Some are sharply conical with coronate peridia; others are low and broad. Pustules were found to vary in size from 50 μ to 1 mm. in diameter. The peridia of uredinia developing on the under side of the leaf (Pl. 1, C) conform to the type described by Farlow. On the larger veins and especially on the petioles they tend to grow much larger and round out, sometimes becoming nearly globular (Pl. 1, B). The corona is not conspicuous in such peridia, especially where they are rather deep-seated, originating beneath three or four layers of cells.

Sori on canes are much flattened, elliptical or lenticular (Pl. 1, D, E, F, G, and 3, A). In such cases the heavily cutinized epidermis effectively prevents the protrusion of the peridium (Pl. 1, G), so that the peristomal cells and most of the peridium often disappear through disorganization. Spores are discharged through the passageway opened by the crushing and thrusting aside of the peridial and host cells above (Pl. 1, A).

Deep-seated sori are apt to occur in the calyx and fruit where the apex of the peridium seldom appears through the epidermis. Adjacent sori merge by the dissolution of side walls, large saclike cavities which are packed with spores resulting. Sori that do not lie much over two or three cells deep in the tissues beneath the epidermis are erect (Pl. 1, F), broadly elliptical, or lenticular. While individual sori on canes are large, they increase in length by coalescence (Pl. 1, G). Very frequently the vegetative hyphae mass together, crushing aside the host cells of the cortex. If these primordia lie near the forming cork layers, or lie deep down in the cortex, the uredinia will be inverted, the "peridium" being formed on the inner side (toward the center of the cane) (Pl. 1, D, E).

Two sori may originate from the same primordium, one having a peridium on the side toward the epidermis, the other on the side next to the cork cambium, which lies quite deeply embedded in the cortex. The sori shown in Plate 1, F, originate from separate primordia; the one above is erect, the other inverted.

ORIGIN OF THE SORUS AND ITS PERIDIUM.

The origin of the sorus is most easily studied from sections of the rust as it occurs on leaf stalks. The vegetative hyphae are not well provided with a granular or stainable cytoplasm, but the cells giving rise to the

primordium increase in size and their contents become granular and stainable and the nuclei show very distinctly (Pl. 2, A). Cells become arranged side by side in a compact plectenchyma. The terminal cells which eventually constitute the peridium elongate rapidly, lose their granular cytoplasm and nuclei by disorganization, and act as buffer cells to push aside and crush the host cells above. In Plate 2, B, two or three terminal cells at each end of the section are more heavily shaded in the drawing to indicate that they were more deeply stained than the others; the final divisions by which peridial cells arise will soon take place. In this way the sorus continues to increase in breadth for some time. In the formation of the teleutospores of species of Gymnosporangium (*8*) terminal cells of a sorus primordium function purely as buffer cells and entirely disappear as the sorus matures. It is the subterminal cells from which the teleutospore buds are formed. This is not the case in *Pucciniastrum americanum* on petioles, where the buffer peridium persists more or less and is usually recognizable in an old sorus, while the subterminal cells disappear.

Later the author will consider spore formation as found in *P. agrimoniae*, the species studied by Ludwig and Rees, but if Plate 2, A and B, of this paper is compared with their figure 1 in Plate VIII it will be seen that the only essential difference is in the layer of somewhat shorter or more flattened subterminal cells which the author found in various stages of disorganization, indicated by the way these cells take the orange G. In their figure the subterminal cells in the four central rows are not as thick as those below them. Sections presenting such features as are shown in Plate 5, A, of this paper certainly can be interpreted to mean that the layer of cells, b, which extends across the sorus, is composed of active basal cells, since these cells take the gentian violet stain rather deeply, while the cells of the "hyphal plate" and vegetative cells below take scarcely any stain. The cells s take the orange and safranin and certainly appear to be degenerating. The cells u will develop into spores. Toward the center of this sorus space has been formed and the intercalary cell below the spore is elongating into a stock as it disorganizes. If this method of spore formation were continued, the uredospores would be borne in chains, and essentially there is very little difference between the two methods. In very compact deep-seated primordia, conditions may be such as to necessitate the formation of the first spores in this way. A greater upward thrust against the overlying tissue would result if the basal cell elongates as a whole or at its upper end in preparation for division. In Plate 5, B, are shown the first spores formed in a sorus where the pressure was soon relieved at the center by a break in the overlying tissues. Large wedge-shaped uredospores with perfectly definite stalks can be seen. Nearer the margin of this sorus there was still considerable resistance at the left and above, so that the spore initial buds developing from the basal cells, b, are pushing out to the right where the tissue was less compact. The relationship of peridial cells, p, intercalary cells, i, and basal cells, b, is very evident in sections of this sorus. Further evidence that pedicellate spores occur in more mature sori is scarcely necessary. At the right in Plate 5, C, is shown a perfectly typical pedicellate spore. At the left three adjacent basal cells are budding; nuclear division is occurring in the cell at the middle.

If the tangled mass of hyphae constituting the sorus primordium is exceptionally large the development of a clear-cut peridium is more or

less interfered with, and uredospores often appear to be formed from almost any cell of the vertically oriented hyphae. The dark area in the sorus shown in Plate 1, G, indicates where disorganization of abnormal spores or of a portion of the primordium tissue is occurring, so that space will be provided and the host tissue above ruptured. More detailed studies of the process involved in spore production in such atypical sori should be made in order to determine to what extent these apparent variations from true form are really the results of abnormal conditions.

The "hyphal plate" layer mentioned by Ludwig and Rees is commonly present along the base of the sorus in *P. americanum* and seems to be almost continuous with the peridium, as shown in Plate 1, B. The origin of this tissue is perfectly clear. The columns or chains of cells constituting the plectenchymatous primordium are the much enlarged, vertically oriented branches of the ordinary hyphae which originally formed a small tangled mass at the commencement of the uredinium. These hyphae can not be distinguished from the purely vegetative hyphae by the size, form, or staining properties of their cells. Now, the sorus increases in breadth simply by the lateral extension of branches from this undifferentiated tissue. Certain branches grow out vertically and parallel the other columns of cells and form peridial, intercalary, and basal cells in regular order, so that there are bound to be platelike layers of cells, especially along the base at the margins, which connect up with the peridium.

In order to secure further proof of the homology of intercalary cells and uredospore stalks, the author gathered and fixed material showing various stages in the development of uredinia of *P. agrimoniae* and *P. hydrangeae* on November 2 at Occoquan, Va. A study of sections of *P. agrimoniae* showed that Ludwig and Rees have given a good figure of a young sorus. There is ample evidence, on the other hand, proving that intercalary cells which degenerate are cut off below the peridium (Pl. 5, D, E) and that such cells are also formed with at least the first uredospores (Pl. 5, F). Many cases were observed where there could be no doubt that the basal cell forms one or more buds, spore initials (Pl. 5, G), each of which in turn divides to form a spore supported by a stalk. The stalk may disappear without much or any elongation. A few other stages in spore formation are figured here showing that *P. agrimoniae* does not differ materially in the organization of its uredinium from *P. americanum*.

Much of the material of *P. hydrangeae* was probably gathered too late in the season, as most of the sori sectioned had aborted or undergone degeneration after having developed the chains of cells constituting the primordium. Such stages are, in this condition (Pl. 5, J), apt to be misleading. In a few cases the uredinia appeared to be healthy and normal. Some evidence was found showing that the intercalary cells are cut off as the peridium is formed (Pl. 5, K). Certain irregularities in the structure of this tissue need further study. One could find stages where the first spores were being formed, showing that the uredospores originate as a result of the cutting off of a spore initial from the basal cell as a whole and not as a bud. In this species the cell supporting the spore must be considered the homolog of a true stalk cell or pedicel. Young spores supported by intercalary cells are shown in Plate 5, H.

Plate 3, A, B, shows more clearly that the peridium is composed of the terminal cells of the sorus primordium. The subterminals take the

orange stain more deeply than the others, therefore this layer is easily recognized in sections. Disorganization, followed by collapse, occurs, so that these interstitial cells are represented finally by more lines of degeneration products.

METHODS OF SPORE FORMATION

The formation of uredospores in Puccinia from a definite layer of basal cells which send out buds (spore initials) is well understood. Nuclear division is followed by cell division, cutting off from the spore initial the stalk cell below and the spore above. Such spores are thus not borne in chains, even though the basal cells may continue to bud and give rise to a number of spores.

Liro (*10, p. 490, 492, 493*) states that the uredospores of *Melampsorella cerastii* and *M. feurichii* are borne in caeomalike sori, that is, in chains. Magnus (*12*), while confirming Liro regarding the first species, disagrees with him as to the second, figuring stalked spores in a sorus of *M. feurichii*, and further holds that it would be impossible for a spore to be formed out of such stalk cells. He creates the new genus Milesina, especially characterized by stalked uredospores, leaving in Melampsorella *M. cerastii*, which he and Liro agree has catenulate spores.

Ludwig and Rees (*11*), who made a study of the uredinium in *P. agrimoniae*, find that the apical cells of the primordium elongate and their contents stain less deeply. The terminal cells become the first peridial cells, and the other cells of the chains are the uredospores, no intercalary cells being formed. These authors further state that the spore chains arise from a layer of basal cells just above a "hyphal plate layer," which is a tissue extending across the base of the sorus and connecting at the margins with the peridium. Stages in spore formation are not shown, although they follow Magnus in their explanation of the probable method. Since intercalary cells were not found, they suggest that the peridium is formed in a way analogous to that in the ordinary aecidium. They also suggest that the genera now included in the Pucciniastratae may be separated into two subgroups, the fern rusts having pedicellate spores in one group, and Pucciniastrum, Melampsorella, Melampsoridium, characterized by spores borne in chains with each chain maturing but one spore at a time, in the other.

The writer has not made an extensive study of the order of cell division in spore formation in *P. americanum*, but there can be no question that the uredospores are frequently borne singly on pedicels. On the other hand, if the very important fact that intercalary cells are present is disregarded, there is considerable evidence showing that the first spores, especially in deep-seated sori on petioles and canes, are borne somewhat as Magnus describes for Melampsorella. In other words, in *P. americanum* the first spore initials arise as the result of tangential divisions of basal cells and not by budding. Any claim that the upper daughter cell may sometimes become a spore at once, the sister cell remaining as a basal cell, would be difficult to disprove.

FORMATION OF THE OSTIOLE

The formation of the ostiole and the peristomal cells in *P. americanum* can be followed very readily by studying sections of sori which are not very deeply seated. About the time the first spores appear in the cavity beneath the central part of the buffer peridium, it will be seen that a few

cells at the center or apex are beginning to disorganize and collapse at their lower ends (Pl. 1, A, and 5, B). As the peridium is lifted these central cells sustain considerable pressure from the sides so that they eventually become distorted, compressed below, enlarged above, and are finally thrust somewhat out of place as they break through the epidermis. The spinelike thickenings which are being formed during the rupturing processes may in some way facilitate the final dislodgment of the peristomal cells in older sori. The cell ps in Plate 4, E, is slightly more than half the length of that of the adjacent peridial cell, p. Originally these two cells were the same length; therefore, the difference seen later is due, not to the thickening of the wall of the lower end, as thought by Farlow, but to disorganization which is not confined to the peristomal cells alone. It is interesting to see that while the lower end of such cells is becoming disorganized and distorted the cytoplasm and nuclei in the upper part are still active, holding the gentian violet stain to the last or until the spines are formed.

It is now a well-recognized fact that peridial cells of an aecium are the homologs of aeciospores, being merely the specialized terminal cells in the chain of spores. The figures in this paper suggest that these homologies can be carried over to the uredinium of Pucciniastrum, where the peridial cell and intercalary cell below are comparable to the uredospore and its stalk, which supports the theory first advanced by Sappin-Trouffy (*13, p. 86*) and further defended by Christman (*4*).

Stalked spores arising from budded basal cells were not found in the limited study of *P. hydrangeae* made by the author. Such types as are shown in Plate 5, I, suggest that in older sori the intercalary cell may elongate to take the form of a stalk and that the basal cell may bud the same as we find in the other species.

It is a certainty that spores are sometimes formed atypically in *P. americanum*, so the author has hesitated to interpret the figures in Plate 3, which show (at the right) spores without stalks or supporting intercalary cells. The author has relied on the positive evidence offered by finding intercalary cells and pedicellate spores in just such sori, rather than on such negative evidence as these figures might be interpreted to afford. In the orderly process of spore formation in uredinia there is a hymenial layer of basal cells from which spore initials regularly arise, but all sorts of abnormalities are liable to occur here as in other fungi, so that a spore might be formed from almost any cell, peridial, intercalary, or basal. Every well-nourished cell in the primordium is potentially sporogenous, but usually one of the daughter cells of the spore initial is in effect sacrificed, thus providing additional food for the one that is to become the spore; the other, although incidentally becoming a disjuncting intercalary cell or an elongated stalk, finally degenerates.[4]

Magnus' figure 7, Plate XIV (*12*), in support of his view is not convincing. He may have overlooked the stalk cells or their homologs, intercalary cells, as he did the peridial cells in Hyalopsora which Bartholomew (*3*) shows very plainly to be present in this genus. Ludwig

[4] L. Kursanov, in a recent paper which has just come to hand (Recherches morphologiques et cytologiques sur les Urédinées. Bul. Soc. Nat. Moscow, v. 31, p. 1–129, pl. 1–4, 25 fig. Oct., 1922), traces the development of terminal sterile cells in the uredo sori of *Puccinia allii, Triphragmium ulmariae, Uredo (Pucciniastrum) pirolae*, and *Hyalopsora polypodii-dryopterdis*. He figures intercalary cells directly beneath the sterile terminal cells in the last two species and states that they arise by the division of the subterminal cells. This may very well be their method of origin in the species of Pucciniastrum which the writer has under discussion, but it would not affect their homology. Instances of the cutting off of the intercalary cell from the upper part of the aecidiospore initial are well known. Kursanov has not followed fully the development of the uredo spores in *Pucciniastrum pirolae*, so that the reader is left in doubt as to whether they are provided with true stalk cells or not.

and Rees, as noted, state that the uredospores of *Pucciniastrum agrimoniae* are borne in chains and that the terminal cells of the chains elongate to become the peridial cells, the other cells developing one by one into uredospores as described by Magnus for Melampsorella. These authors did not find intercalary cells either in the spore chains or below the peridial cells. If this were true, the peridium would be formed in a manner analogous to that of the aecidium, as they pointed out.

Some of the strongest hints as to the trend in evolution are suggested by what it is thought are atypical processes; but if in addition to the method of spore formation which the author has observed in Pucciniastrum it should be rarely found that every cell below the peridium in the cell chains develops into a uredospore, as claimed by Ludwig and Rees, the doctrine of homologies would be weakened. The buffer cell in the telium of Gymnosporangium may be the homolog of the peridial cell in Puccinastrum; this depends on just how the first intercalary cell in the latter is cut off. It is not the homolog of the peridial cell of the ordinary aecidium, but rather it is comparable to the hymenial basal cell or its sister peridial initial. If the uredospores of *Melampsorella cerastii* are always borne in chains, and those of Hyalopsora are borne singly on pedicels, then the species of Pucciniastrum form a good series of connecting links, *P. hydrangeae* being more nearly like Melampsorella, especially if we should find intercalary cells in the latter.

The complete life history of *P. americanum* is unknown. Three other species of the genus have their aecial stage on Tsuga and two on Abies. The occurrence of our rust on canes of the current season suggests that the fungus may live through the winter, thus obviating the necessity for the alternate aecial stage in certain localities. No explanation has been offered to account for the presence of the deep-seated, inverted sori on canes, which are very common. It is possible that the inversion may facilitate spore discharge the following spring as the cortical parenchyma peels off, exposing the cork tissue below. Such inverted sori were not found on leaves or petioles.

It has been shown that the form and size of sori vary greatly, depending upon what tissue they are found, indicating that *P. arcticum* and *P. americanum* are not distinct species. In the event that it can be shown that *Rubus triflorus* and *R. arcticus* can not be infected with spores from *R. strigosus* and related forms, distinct names for the strains or biologic forms might still be desirable.

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PLATE 1 [a]

Pucciniastrum americanum (Farl.) Arth. on June × Cuthbert Red Raspberry

A.—Section through the center of a young uredinium on leaf stalk. Four layers of host cells lie above the sorus. Cells next to the sorus have been crushed by the expansion of the buffer tissue. Disorganization of cells at the center. Uredospores formed irregularly, intercalary cell replaces stalk.

B.—Vertical section through a mature sorus on a leaf stalk. Only two layers of host cells above. The buffer tissue has been flattened by the pressure from within and will persist more or less as a peridium, although the cells at the center are fully collapsed and will soon disappear. No corona would have been formed. Uredospores stalked.

C.—Vertical section of sorus on lower side of leaf. One cell of the corona showing.

D.—Inverted broadly elliptical sorus on young cane. "Peridium" or buffer tissue facing the cork cambium, which is deep-seated; e, epidermis; ck, cork layers.

E.—Inverted sorus still more deeply seated. Buffer tissue directly against the cork.

F.—Two lenticular sori on a cane. The upper one lies just beneath the epidermis and is erect; the lower is inverted.

G.—Erect double sorus on large cane. Peridium of the part at the left has entirely disappeared, cavity filled with spores. At the right peridium still evident although disorganizing. Degeneration of tissue at the center of the sorus (dark area), normal spore production below. Note the position of the epidermis above the sorus and of the cork cambium below it; e, epidermis, cp, cortical parenchyma, ck, cork.

B to F, photographed with Leitz 8 mm. lens and No. 10 oc.; A, 4 mm., 6 oc.; G, 16 mm., 10 oc.

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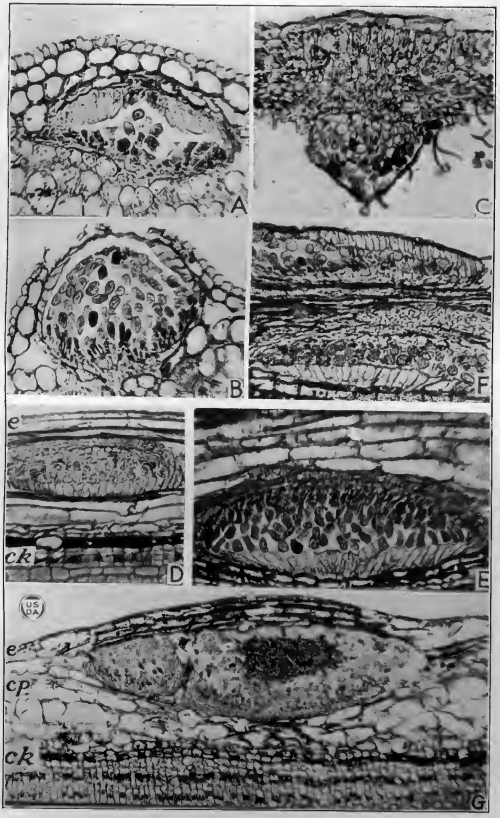
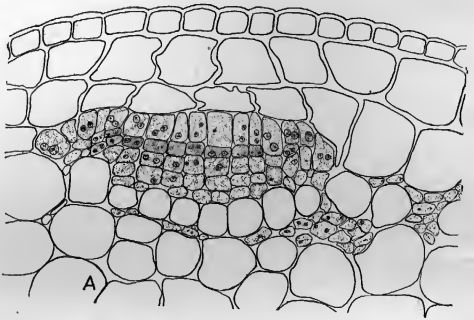
[a] The photographs shown in Pl. 1, and all the sections which were studied in the preparation of this paper were made by Miss Ruth Colvin.

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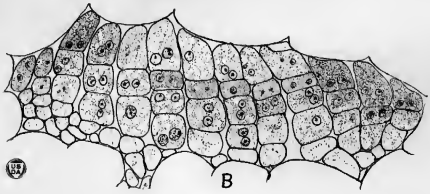

B

PLATE 2

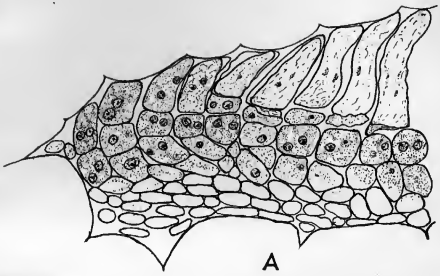
A.—Primordium of sorus on leaf stalk of raspberry, showing its position in the host tissues. Peridial or buffer cells, each accompanied by a disorganizing intercalary cell, at the center of the sorus. Cell division by which intercalary cells are formed has not occurred at the margins.

B.—Primordium of sorus on petiole of raspberry. Terminal cells elongating, intercalary cells disorganizing. Cells at the margin just before division.

PLATE 3

A.—Part of section of a young sorus on leaf stalk. Peridial cells fully elongated at the right, contents disappearing, intercalary cells collapsing.

B.—Part of section of a small and rather flat sorus on a cane. Buffer tissue of erect cells; intercalary cells in all stages of disorganization. Five uredospores at right center.



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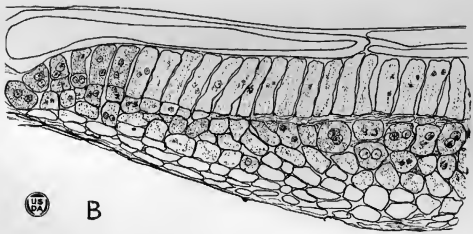

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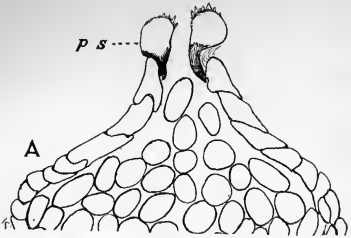
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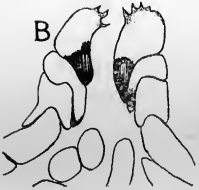
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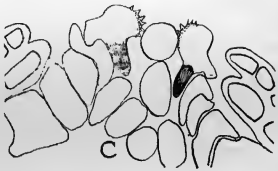
C


D


PS
P
E

PLATE 4

The origin of peristomal cells in *P. americanum*. Deep shading of the peristomal cells, PS, indicates disorganization; P, peridial cell. (See text for further explanation.)

PLATE 5

P. americanum

A.—Columns of cells in a young, compact sorus. Uredospores arise by division of spore initial cells: p, peridial cell; i, intercalary cell; u, uredospore; s, stalk; b, basal cell.

B.—Formation of stalked spores in a sorus in which space was provided by the early rupture of overlying tissues. Spore initial buds are arising from basal cells (at the left) so that the first spores will be borne on stalks.

C.—Uredospores formed from budding basal cells. Nuclear division occurring in the bud from the second basal cell.

P. agrimoniae

D.—Row of cells at margin of sorus, wedge-shaped intercalary cell below the peridial cell p.

E.—Fully elongated peridial cells supported by intercalary cells beneath which are normal basal cells, not spores.

F.—Typical spore initial bud si, from basal cell (right); cell chain consisting of young uredospore, intercalary cell, basal cell, and "hyphal plate" cell; stalked spores.

G.—Short stalk cells. Whether we find two spores or only one connected with the basal cell may depend on the way the cell is sectioned.

P. hydrangeae

H.—Four young uredospores, u, each supported by an intercalary cell.

I.—Intercalary cell elongating somewhat.

J.—Chains of cells in an aborted sorus. Intercalary cells beneath the peridium.

K.—Cell chains at the margin of a normal young sorus; wedge-shaped intercalary cells degenerating.

A
B
C
D
E
F
G
H
I
J
K
p
ps
u
s
b
i
si

WATERY-ROT OF TOMATO FRUITS[1]

A PHYSIOLOGICAL FORM OF OOSPORA LACTIS; EFFECT ON THE HOST; PENETRATION OF THE CELL WALLS BY ENZYMIC ACTION

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INTRODUCTION

A new rot of tomato fruits closely resembling the rot caused by *Bacillus carotovorus* has frequently been found in shipments of southern grown tomatoes and sent to the Office of Cotton, Truck, and Forage Crop Disease Investigations by the Bureau of Markets' inspectors since the spring of 1921. It is also prevalent in the vicinity of Arlington, Va., and Washington, D. C.

This rot is characterized by the extremely watery appearance and condition of the affected tissues, by the absence of any other discoloration, and by the occasional oozing of water from the surface—features by which it can readily be distinguished from other fungous softrots. The rotted areas usually develop in the form of sectors extending from the stem scar toward the blossom end. In very humid air they are partly covered with a white velvety to granular fungous growth, but under average atmospheric conditions this is absent. The illustration shown in Plate 4, A, is fairly typical of this rot except for the low point of origin and the presence of a surface growth.

That this rot has frequently been mistaken for the rot caused by *Bacillus carotovorus* is quite likely, as the two are similar in macroscopic appearance. However, it differs from the bacterial rot in rapidity of development; in fact, fruits kept in the laboratory 10 days after this rot had made considerable progress were not completely softened, while those infected by *B. carotovorus* usually collapsed in about 3 days.

As an examination of the affected fruits obtained from the Bureau of Markets always disclosed the presence of an Oospora, experiments were made to determine the relation of this fungus to the disease.

INVESTIGATION

MATERIAL AND METHODS

The Oospora used in the inoculations was grown chiefly on carrot agar, as it grew better on it than on most other kinds of media. It grew well also on turgid raw carrots kept in a moist atmosphere, producing a distinct rot (Pl. 4, C), but this material was used only for morphological comparisons.

In the inoculation work, green, ripe, and partly ripe tomato fruits free from blemishes were submerged for 30 minutes in a 1:1000 aqueous solution of bichlorid of mercury, washed in distilled water, and inoculated with a pure culture of the Oospora obtained from the rotted fruits.

[1] Accepted for publication Jan. 22, 1923.

This treatment caused some discoloration of the fruits but no softening. It had no visible effect on susceptibility to infection by this fungus, for fruits treated with weak solutions of bichlorid of mercury or formaldehyde as well as fruits not treated were quite as readily infected.

Controls were liberally used in all the experiments. Both the controls and the inoculated fruits were usually kept in closed glass chambers to prevent contamination from the air, but inoculated fruits kept in open dishes were quite as readily infected.

The decomposition of the cell constituents was observed on roots of carrots and on green tomato fruits. Sections of carrot roots, chiefly from the heart, and of green tomato fruits, 250 and 500 μ thick, respectively, and free-hand sections of varying thickness were used as fresh material. Pieces of green tomato fruits from spots 2 days old were treated with Flemming's medium killing and fixing solution, embedded in paraffin, and sectioned and stained for the study of fixed material.

The cultures were made as described by Brown (*3*)[2] on thin layers of media (carrot decoction, and beef bouillon + 2½ per cent glucose) in small flasks or Petri dishes, inoculated with an optimal quantity of spores, and kept at a temperature of 22° to 26° C.

The action of the enzyms on the host cells was determined by means of the live organism, the ground-dried organism, the extract from the ground-dried organism, the filtrate from cultures, and the alcoholic precipitate from the filtrate.

The organism was separated from the culture medium by passing the liquid through a double layer of Whatman's No. 50 filter paper. A few small cells passed through the filter but showed no signs of germination during the experiment.

The enzymic material was precipitated by adding 4 volumes of 95 per cent alcohol to each volume of the filtrate. The liquid was then filtered and the precipitate washed in 95 per cent alcohol, dried in a warm air current, dissolved in a very small quantity of distilled water, and used immediately.

The ground-dried organism was used in aqueous suspension. The extract of the same material was prepared by soaking the powdered fungus in water for 24 hours.

The decomposition experiments were run for 24 hours at temperatures ranging from 24° to 45° C. Growth of microorganisms was prevented by adding chloroform or toluene to the liquid in the proportion of 10 to 25 per cent, but duplicate experiments in which no antiseptic was used were also made. Chloroform and toluene inhibited growth without apparently hindering the action of the enzym.

RESULTS OF INOCULATION

The results obtained with both pricked and unpricked fruits are summarized in Table I.

Of the 277 pricked fruits inoculated, 208, or 75 per cent, became infected. The ripe fruits seemed to be somewhat more susceptible than the green fruits, but both were easily infected through punctures. The infections of unpricked fruits took place only through the stem scar. This was frequently observed in preliminary experiments not recorded. Moreover, it appears to be the cause of the position of the rotted areas of this type on most shipped fruits.

[2] Reference is made by number (italic) to "Literature cited," p. 905.

TABLE I.—*Results of inoculating tomato fruits with the Oospora associated with watery-rot*

	Green.	Ripening.	Ripe.	Total.	Fruits inoculated.				Fruits infected.			
					Green.		Ripening.		Ripe.		Total.	
					Number.	Per cent.	Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Pricked.....	160	90	27	277	118	74	64	71	26	96	208	75
Unpricked.....	16	7	5	28	0	0	0	0	[a]2	0	0	0

[a] Infected through the stem scar.

Although these infections were obtained with an apparently pure culture of Oospora, the possibility of contamination by *Bacillus carotovorus* was not overlooked. The cultures were repeatedly plated and examined microscopically for the presence of bacteria but no evidence of bacterial contamination was found in either the plates or microscopic mounts. Moreover, the fungus pricked into halved potato tubers caused only a slight superficial growth, even when allowed to stand for a considerable period of time, while *B. carotovorus* produced a very decided rot (Pl. 4, B). The infections of the inoculated tomato fruits were therefore unquestionably caused by the fungus.

The stems and leaves of 15 vigorous tomato seedlings about 4 inches tall were thoroughly sprayed with the Oospora spores and kept in a moist chamber 60 to 72 hours but no infections developed. Ten similar tomato seedlings were thoroughly pricked in stems and leaves and smeared with the spores of Oospora, but the fungus was unable to invade the tissues. It therefore seems to have little if any parasitic action on tomato plants.

MORPHOLOGY OF THE FUNGUS

The Oospora causing watery rot of tomato fruits consists of a hyaline, septate mycelium with granular contents and numerous short branches arising near the septa at an angle of about 45° from the main filament (Pl. 3, A) and other longer branches of a two-or-three-forked type (Pl. 3, B–D).

The hyphae vary from 2.5 to 7.5 μ in diameter. The younger branches are narrower than the main filament but the taper is very gradual.

Reproduction is accomplished by the breaking up of the hyphae into their cells which serve as spores. The branches shown in Plate 3, A, divide into numerous short cells which round at the ends (Pl. 3, E, F, H) and separate. The rounding appears to begin in the apical cells (Pl. 3, G), but occurs in all cells of a branch almost simultaneously. At a certain stage of their development these cells appear to be chains of spores arising from the main part of the mycelium (Pl. 3, E, F), but by the time they lose their coherence, or shortly afterward, the main filament itself breaks up by cell division and separation into numerous cells of different lengths, which round at the ends, and when short are often indistinguishable from those formed from the lateral branches. They are hyaline and granular and are capable of germinating immediately.

The transverse diameter of these sporelike cells (oidia), which for convenience will be referred to hereafter as spores, varies from 2.5 to 7.5 μ; the length from 3.2 to 40 μ and in some cases even to more than 60 μ.

Germination of the spores may start from the end (Pl. 3, I, L), from the side (Pl. 3, J, K), or from a corner (Pl. 3, I, M). Development from a corner of the cell is quite similar in point of origin to the development of the branches shown in Plate 3, A.

COMPARISON WITH OOSPORA LACTIS

The Oospora obtained from rotted tomato fruits shipped from the Gulf States was compared morphologically with the *Oospora lactis* that commonly grows on the surface of tissues in the cracks of ripe tomatoes; with two cultures of *O. lactis* received from Dr. Charles Thom, one isolated by him from pickle scum, the other sent to him from Germany; and with an Oospora isolated from green tomato fruits affected by watery-rot at the Government Experimental Farm, Arlington, Va. The two Oosporas causing watery rot and the one from Germany did not always break up into their cells so readily and completely as those from the cracks of ripe tomatoes and from pickle scum, but this habit varied with the age of the culture and with the kind of culture medium used. Aside from this difference, which was not constant, and slight differences in quantity of mycelial growth, there were no peculiarities, except in parasitism, by which one form could be distinguished from the others. The Oospora received from the Gulf States and the one obtained from green tomato fruits at the Government Experimental Farm reproduced the watery-rot in a large percentage of the fruits inoculated, but the others appeared to have no such parasitic action on tomato fruits. It would seem, therefore, that the Oospora causing watery-rot of tomato fruits, both in the Gulf States and at the Government Experimental Farm, is a physiological form of *O. lactis*. Consequently we have given it the trinomial *O. lactis parasitica*, form phys., to distinguish it from the parent species. As it is indistinguishable morphologically from *O. lactis*, it needs no further description.

It would be interesting to know how this form compares with the 9 varieties of *Oospora lactis* that Schnell (*8*) grew on sliced potato tubers, but as he made no inoculations on tomato fruits an accurate comparison of their parasitism is impossible. Six of the 9 varieties grown on sliced potatoes, 4 of which grew also on sliced cucumbers and 2 on plums, produced a discoloration of the potato tissues which would distinguish them from the form isolated from green tomatoes. Two of the others produced slimy colonies on sliced potatoes—a character not obtained with the watery-rot fungus. The remaining strain, viz, Oid. l. 557, made a feeble growth on potato tubers, agreeing in this respect with the one isolated from tomato fruits affected by watery-rot, but this has no significance with reference to its parasitism on tomato fruits.

TEMPERATURE RELATIONS

The effect of temperature on growth and infection by *Oospora lactis parasitica* is shown in Table II.

The minimum temperature obtained for germination of the spores, for growth of the mycelium, and for infection of pricked tomato fruits was approximately 2° C., the optimum temperature 30°, and the maximum 38.5°, except for infection of fruits, which was 37.5°. As there was a difference of 1° to 2° between the temperatures of adjoining chambers and some fluctuation within each chamber, these temperatures are only approximately correct.

TABLE II.—*Relation of temperature to growth and infection by Oospora lactis parasitica*

Temperature.	Growth on culture media.[a]	Infection of pricked tomato fruits.	Germination of spores.
	°C.	°C.	°C.
Maximum	38.5	37.5	38.5
Optimum	30.0	30.0	30.0
Minimum [b]	2.0	2.0	2.0

[a] Carrot agar and glucose agar.
[b] The experiments on the minimum temperature were run about a month.

EFFECT ON THE HOST

ACTION ON THE CUTICLE

It was shown in the inoculation experiments described above that this fungus is unable to infect uninjured tomato fruits except through the stem scar or other similar areas not covered by the cuticle. This is also illustrated in Plate 1, A. The fungus lay in masses on the surface of the fruit but was unable to penetrate it. However, when once inside the fruit it invaded the epidermal cells quite readily.

ACTION ON THE PROTOPLASM

Invasion of the host cells is soon followed by a gradual consumption of their protoplasmic contents. Some of the steps in this process are illustrated in Plate 1, in which B and C show an early stage, D a medium early stage, and E–J late stages. This action of the fungus on the protoplasm of the invaded cells is quite evident soon after the penetration of the wall, as it causes dissolution of the protoplasm in advance of the growing tip (Pl. 3, R). These transparent areas, or digestion vacuoles immediately surrounding the filaments, are shown more fully in Plate 1, B–D, and Plate 3, U. It would seem from these figures that the proteolytic enzym secreted by the fungus acts chiefly in the region of the growing tip. There was apparently no preference for the nucleus, as it often remained intact after most of the other cell contents had disappeared.

Quite different results were obtained with the ground dried organism, the filtrate, and the alcoholic precipitate from the filtrate. Repeated experiments with these substances produced no visible effects on the protoplasm. The failure to obtain a proteolytic action with any of these substances may be due to a rapid deterioration of the enzym, to inhibitors, or to lack of suitable technic.

ACTION ON THE MIDDLE LAMELLA

The growing fungus, as shown in Plate 1, dissolves the middle lamella slowly, causing the loss of cell coherence. This loss of coherence and the breaking up of the fungus filaments into their individual cells produce the extremely watery consistency which distinguishes this rot from other softrots in which the fungus filaments remain intact and hold the host cells together. A similar action was produced on the middle lamella by the ground dried organism, the extract from the ground dried organism, the filtrate, and the alcoholic precipitate from the filtrate of cultures 2 to 3 days old, but this activity diminished as the age of the culture

increased. In fact, the alcoholic precipitate from cultures 6 days old and the filtrate from cultures 21 days old produced no visible effect on the middle lamella. This deterioration or inhibition of the action of the pectinase from age is quite different from the rapid action obtained with pectinase from cultures of *Bacillus carotovorus* 21 days old.

ACTION ON THE CELLULOSE

The passage of this fungus through the cell walls is shown by the drawings in Plate 3, N–T, and by the intracellular filaments in the photomicrographs of Plate 1. The drawings in Plate 3, N–T, were made from partly destroyed cells of a disintegrating area of the fruit. Only the tips of growing hyphae are shown passing through the somewhat wavy and more or less separated walls. The penetration of the cell walls of normal cells by young germ tubes was also observed by means of the microscope. The more important details of this process will be described later.

No visible effect was made on the walls by the filtrate, by the alcoholic precipitate from the filtrate, nor by the mycelium in the presence of sufficient chloroform or toluene to inhibit its growth. Moreover, bits of filter paper placed in fresh cultures of the fungus and allowed to remain there for 10 days to 2 weeks did not disintegrate. The fungus filaments passed between the fibers, causing the paper to tear apart somewhat more readily after the breaking up of the hyphae than similar bits of paper kept in distilled water, but microscopic examination of the fibers failed to reveal any corroding effects. There was apparently no chemical action on the filter paper.

It might seem from the foregoing facts that pressure rather than enzymic action enables the fungus to penetrate the cell walls as described for *Pythium debaryanum* by Hawkins and Harvey (7), but further observations do not substantiate this means of penetration for Oospora lactis.

Before a fungous filament can penetrate a cell wall by means of pressure, it must attach itself to the wall, or, if in a cell, to the protoplasm in order to prevent pushing itself away from the wall as it elongates. Spores of this fungus germinated either in water or in culture solution do not attach themselves to the slide or the cover slip. Moreover, when germinated in cells of tomato fruit tissue they do not adhere to the wall or the protoplasm. When the tip of such a sporeling comes into contact with the wall, its more or less continuous growth in length usually pushes it aside, which causes it to slide along the wall. Not infrequently the position of the whole filament is thus changed, as well as the position of other sporelings lying in contact with it. In fact, such a filament may even shift its position in such a way as to remove its tip some little distance from the wall. Such short filaments go through the walls more easily at the corners of the cell because there is less chance to slide along the wall. When a filament has passed through a wall it pierces other walls more rapidly because the anchorage thus obtained holds the growing tip against a single point better than does the free spore end of a germ tube that has no anchorage.

The phenomena accompanying the penetration of a cell wall by a germ tube of this fungus throw some light on the means by which it is accomplished. By placing spores of the fungus on the top of thin sections of tomato fruit tissue mounted and covered on a glass slide and furnished with a constant supply of water, the growth activities of the

spores that settle in the cells as well as the penetration of the wall and the effects resulting from it are easily observed by means of the microscope. Some short germ tubes lying near a wall and approaching it perpendicularly go directly through it without the use of any support or anchorage to increase their pressure. The opening made in the first half of the wall is a hole, not a basin or general depression such as would be produced by pressures, although the second half of the wall, i. e., the wall of the cell undergoing invasion, is sometimes pushed back. Whenever an invading filament completely fills the hole in the first half of the wall it attains a certain amount of anchorage which no doubt enables it to make some use of its growth pressure. This causes the second half of the wall to bend back before the filament has passed through it (Pl. 3, P, T), but it is not essential to the penetration. Moreover, this bending is usually absent, because the hole made in the first half of the wall, as shown by focusing sharply with the microscope, is usually a little larger than the filament (Pl. 3, V). It is also destitute of radiating cracks or fragments such as would be likely to accompany the bursting of the wall by pressure. By pressing the cover slip with a needle so as to produce vertical and lateral movements the angle between a germ tube and the wall through which it has passed may be varied more than 90°. This is caused by the pressure of the liquid against the filament, which changes its position in the wall without bending it at the edge of the hole. The two ends of such a filament usually move in opposite directions, especially when the part extending through the wall is three or four times as long as the part in the original cell. If the filament were rigidly fixed in the wall, the angle between it and the wall would not change unless the filament were bent at the edge of the hole. Moreover, an occasional filament can be made to slide in the hole. These phenomena are possible only when the hole is larger than the filament. In view of these facts, it would seem that this fungus invades the cells by means of a cellulose-dissolving enzym (cellulase) secreted by the growing tips while in contact with the wall.

COMPARISON WITH BACILLUS CAROTOVORUS

As this Oospora and *Bacillus carotovorus* [3] cause similar rots of tomato fruits, a comparison of their effects on the host is interesting. Oospora invades the cells and destroys the protoplasm before it causes much separation of the walls (Pl. 1, B–F). Although it is also found in the intercellular spaces, it apparently makes little use of them except as passages. *Bacillus carotovorus*, on the other hand, remains in the intercellular spaces until it destroys the middle lamellae of the adjoining cell walls (Pl. 2, A–D) and enters the cells usually after it has destroyed their coherence. That an earlier entrance is sometimes effected, however, is evident from Plate 2, E. In later stages (Pl. 2, F–H) it not infrequently fills the cells.

ENZYM VERSUS PRESSURE

If we assume that the penetration of the cuticle by all fungous parasites is by "sheer mechanical presssure," as concluded for *Botrytis cinerea* by Brown (*4*, *5*) and by Blackman and Welsford (*1*) and for *Sclerotinia*

[3] An excellent history of the work done on the softrots of vegetables (also tomato) caused by the *Bacillus carotovorus* group of organisms is published in "An Introduction to Bacterial Diseases of Plants," by Erwin F. Smith (9).

Libertiana by Boyle (*2*), we may be greatly misled. This would apply also to the conclusions of Hawkins and Harvey (7) regarding the penetration of cellulose walls. Fungi produce a variety of enzyms by means of which they decompose substances and obtain food. Moreover, it is quite likely that they produce many more enzyms than have been isolated. Failure to isolate an enzym therefore does not disprove its existence, especially as the physiological factors involved in the production of enzyms and the activators and inhibitors controlling their activities are little understood. Moreover, the observation by Hasselbring (*6*) that the cavities made in the waxy covering of *Berberis Thunbergii* berries by the anthracnose fungi are much larger than the germ tubes and the conclusions by Ward (*10*, *11*) from an exhaustive study of the brownrust of bromes that there is absolutely no relation between thickness of walls, number and size of stomates, hairs, and other mechanical structures and immunity to rust, present a striking contrast to the pressure theory of parasitic invasion. That growth pressure often accompanies enzymic action is obvious. It may also speed up the process of penetration and in certain cases serve as the chief, if not the sole, means of invasion, but it plays only a minor and nonessential part in the invasion of the tomato fruit cells by *Oospora lactis* and no doubt acts only in a secondary capacity in most cases. This is fortunate, for if the development of disease-resistant fruits depended upon thickness of walls, quality and resistance would often be diametrically opposed.

Since Oospora passes through the cellulose walls of two adjoining fruit cells without attaching itself to the wall or protoplasm and not infrequently makes an opening larger than the filament without causing a depression in the wall of the occupied cell, nor lateral cracks, or ruptures, in the wall of the invaded cell, its means of penetration can not be ascribed to pressure. The only other known means by which a fungus can make an opening of this description is by the use of an enzym, such as cellulase, which has been isolated from fungi.

There are features in the penetration of cell walls by some fungi, especially species of the genus Pythium, which cast considerable doubt on the penetration of cell walls by pressure. In his study of *Pythium gracile*, Ward observed that an oospore which had germinated at some little distance from a cress seedling, produced as it grew several bends in its germ tube and passed around a small algal cell at right angles before reaching a cress cell. On coming into contact with this cress cell its "apex became closely pressed against the cuticle, apparently lifting the whole hypha slightly in the process," evidently a result of pressure, but thereafter made no further movement nor change in its position as it produced a small hole in the cuticle and cellulose wall, passed through, and enlarged to normal size within the cell. As this fungus filament consisted of a single cell, the pressure within it was equally distributed. If the pressure had been sufficient to penetrate the host cell wall, which was many times as thick as the fungus wall, it would have straightened the fungus filament. As a matter of fact, however, it did not straighten a single bend nor change its position in the least, although such filaments bend easily, even by the motion of delicate water currents. Moreover, if pressure had caused the penetration of the wall, the fungus would have made a hole as large as its filament instead of a small hole, for the pressure on every unit area of its wall surface was equal. A more probable cause of this type of penetration is that cellulase is formed solely at a single point on the tip of the filament.

In view of the foregoing evidence, it would seem that the pressure theory of cell wall penetration by fungi is not so well supported as the enzym theory.

DISTRIBUTION OF THE FUNGUS

As this physiological form of *Oospora lactis* has been isolated frequently from tomato fruits shipped from the Gulf States, it is probably common in at least several of the Southern States. It is difficult from the reports of the Bureau of Markets' inspectors to estimate how often it really occurs in shipped tomatoes, as they report all such rots as "soft rot." It was found to be common at the Government Experimental Farm, Arlington, Va., and in gardens in the vicinity of Washington, D. C., where no doubt it has carried on its parasitic activities for some time, but has been overlooked because of the similarity of the rots produced by it and *Bacillus carotovorus* and the not infrequent association of the two organisms in the same fruits.

POSSIBLE MEANS OF CONTROL

As this fungus infects tomato fruits quite readily between 9° and 32° C. and can infect them at temperatures ranging from 2° to 37.5°, the practicability of controlling it by means of low temperatures seems doubtful.

Some experiments were made to determine the effects of antiseptics on the control of this organism. The fungus was immersed for 30 minutes in an aqueous solution of the antiseptic and transferred to sterile carrot decoction, in which, if it were still viable, it grew readily. The results are summarized in Table III.

TABLE III.—*Effect of antiseptics on the viability of Oospora lactis parasitica*

Antiseptic.	Concentration of solution.		Subsequent growth in carrot decoction.
	Antiseptic.	Water.	
Chlorid of lime	1	40	None.
Potash alum	(a)	(a)	Considerable.
Potassium permanganate	1	400	None.
Copper sulphate	(a)	(a)	Good.
Mercuric chlorid	1	5,000	None.
Formaldehyde, 37 per cent	1	300	Do.
Water	.....	.....	Abundant.

[a] Saturated.

Immersing the fungus for 30 minutes in an aqueous solution of chlorid of lime 1:40, potassium permanganate 1:400, formaldehyde (37 per cent) 1:300, or mercuric chlorid 1:5000, prevented its subsequent growth, but a similar treatment with a saturated solution of potash alum or copper sulphate was ineffective. The resistance of this fungus to copper sulphate—a fungicide of wide use—is surprising. Although the experiments with this treatment were repeated several times, only negative action was obtained.

Some experiments were also made on the use of the antiseptics as washes. Tomato fruits varying in maturity from green to ripe were lightly pricked in several places and submerged for 5 minutes in an aqueous suspension of Oospora spores, then drained, washed in an antiseptic solution for 30 minutes, and placed in moist chambers. From 20

to 40 pricked fruits, 8 unpricked fruits, and a number of controls equal to the number of treated fruits were used in each treatment. The results are summarized in Table IV.

TABLE IV.—*Effect of washing green to ripe tomato fruits for 30 minutes in an antiseptic to control watery-rot*

Fungicide.	Strength of solution; parts antiseptic to water.	Pricked fruit infected 6 days after treatment.	Unpricked fruit infected 12 days after treatment.
		Per cent.	*Per cent.*
Potash alum	1:50	31	25
Do	1:40	30	.....
Chlorid of lime	1:50	20	.....
Do	1:40	14	12½
Formaldehyde (37 per cent)	1:300	30	0
Do	1:240	22	0
Control	.....	57	37½

The percentage of infected fruits is higher in the pricked than in the unpricked series. Moreover, it is higher than in shipped fruits. As shipped fruits usually become infected through the stem scar instead of through the epidermis, they are probably comparatively free from punctures and therefore compare more nearly with the unpricked series. Formaldehyde and chlorid of lime (calcium hypochlorite) caused considerable reduction in the percentage of infected fruits. It is quite likely that sodium hypochlorite, which is a cheap convenient solution, would give more effective results than chlorid of lime. As the latter is partly insoluble it should, if used on a commercial scale, be dissolved in a separate tank so the clear liquid can be drawn off for use, or dissolved in a washing tank having a removable perforated sheet a few inches above the bottom to prevent the solid particles from adhering to the fruits.

The lower effectiveness of these antiseptics in the presence of fruits as compared with their effect on free spores (Table III) is probably due to chemical reaction of the germicides with substances on the surface of the fruits, which reduces their strength.

The economical use of a fungicide for washing tomato fruits should not be measured by its effect on a single rot, for if it controls one, it will give at least partial control of several others that cause heavy loss during shipment. The selection of a solution for this purpose should therefore be based on its control of this whole group of rots as well as on its cheapness and ease of handling.

SUMMARY

(1) A new rot of tomato fruits, closely resembling the rot caused by *Bacillus carotovorus*, has been common since the spring of 1921 in tomatoes shipped from the Gulf States. It is also prevalent in the vicinity of Arlington, Va., and Washington, D. C.

(2) The rot is characterized by dark-colored water-soaked areas which start in the stem scar and spread slowly toward the blossom end of the fruit without the production of a pronounced odor.

(3) The causal organism is a physiological form of *Oospora lactis*, which, though incapable of penetrating the epidermis, usually enters the fruits through the stem scar, but may enter through any place not cov-

ered by the cuticle, such as wounds, cracks, and punctures. It shows a little preference for ripe fruits, but infects green fruits quite readily. It invades the cells, destroys their protoplasmic contents, and causes loss of cell coherence through a slow dissolution of the middle lamellae of the cell walls.

(4) The minimum temperature for the germination of its spores, growth of its mycelium, and its infection of tomato fruits is approximately 2° C., the optimum 30°, and the maximum between 37.5° and 38.5°.

Immersing the fungus spores (cells) in an aqueous solution of an antiseptic for 30 minutes and transferring them to sterile carrot decoction had the following effects on their viability: Chlorid of lime 1:40, potassium permanganate 1:400, formaldehyde (37 per cent) 1:300, and mercuric chlorid 1:5,000, no growth; potash alum or copper sulphate in saturated solution, considerable growth. The percentage of tomato fruits infected after inoculation with this fungus was considerably reduced by washing them for 30 minutes in an aqueous solution of chlorid of lime 1:40 or formaldehyde (37 per cent) 1:240. It would seem from these results that an effective wash could be developed for the control of a large number of these rots during shipment.

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PLATE 1

Sections of a 2-day-old spot of a green tomato fruit affected by watery-rot (*Oospora lactis parasitica*.

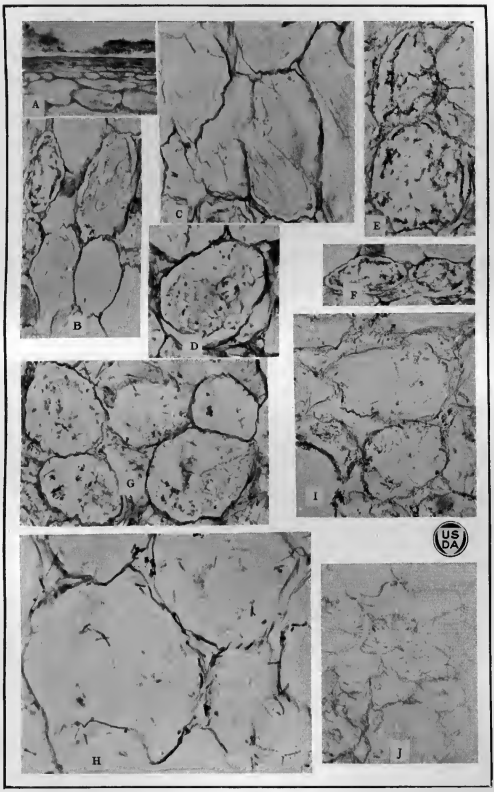
A.—Masses of the fungus lying on the surface unable to penetrate the cuticle.

B–G.—Consumption of the protoplasm by the fungus; B–C, early stages; D, medium early stage; E–G, late stages.

H.—Cells beginning to lose their coherence after destruction of their protoplasm.

I.—A more complete stage of cell separation than shown in H.

J.—Very late stage. Protoplasmic contents completely destroyed; cells free; walls thin, irregular, and inconspicuous.



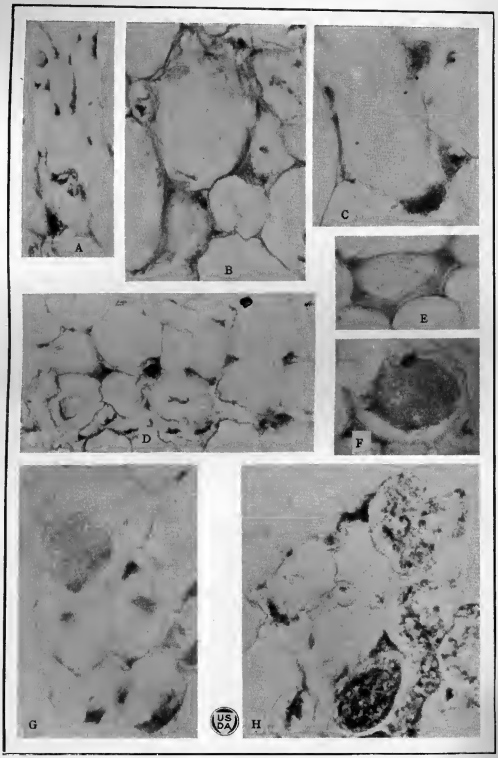

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PLATE 2

Sections of a tomato fruit infected by *Bacillus carotovorus*.

A.—Early stage of invasion; bacteria confined to the intercellular spaces.

B–D.—Medium early stages. B–C.—Intercellular spaces enlarging through dissolution of the middle lamellae of the adjoining cell walls; bacteria beginning to invade the cells.

D.—Cells losing their turgidity and coherence; middle lamellae dissolved.

E.—Bacterial invasion of a firmly attached cell.

F–H.—Late stages. Cells occupied by the bacteria.

PLATE 3

Oospora lactis parasitica

A–D.—Types of branches. A.—Short branches which arise near the septa at an angle of about 45° with the filament and break up into more or less rounded, irregularly shaped cells which function as spores. B–D.—Long branches which bear lateral branches of type A, but also break up into numerous cells capable of immediate germination.

E–F.—Segmented branches of type A; also early stages in the segmentation of the central filament.

G.—Order of segmentation in branch of type A.

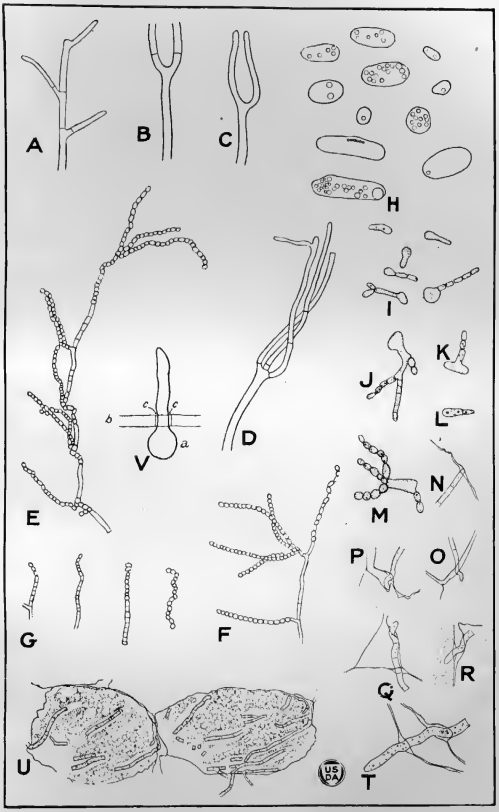
H.—Highly magnified detached cells of lateral branches and central filament.

I–M.—Germinating detached cells.

N–T.—Penetration of irregular, wavy, somewhat separated walls of partly destroyed cells by tips of growing hyphae.

U.—Cells of tomato fruit tissue containing hyphae surrounded by digestion vacuoles.

V.—Penetration of the cell wall of a tomato fruit cell by a germinating spore. (*a*) Germinating spore. (*b*) Cell wall. (*c*) Hole made in the wall by the germ tube. Somewhat diagrammatic.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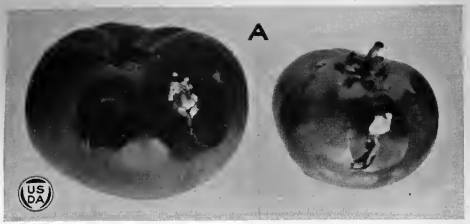
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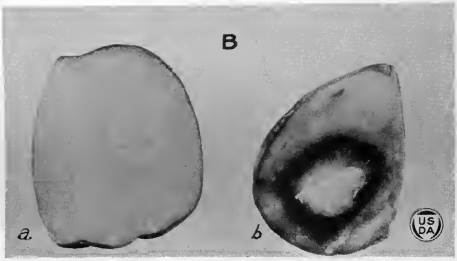
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b

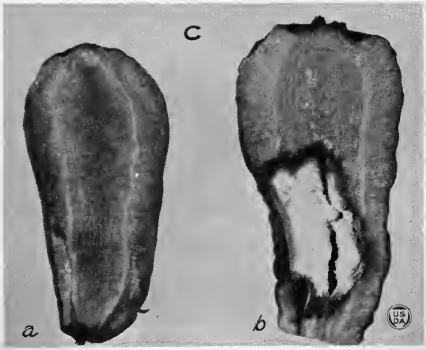
C
a
b

PLATE 4

A.—Watery-rot of tomato fruits produced by *Oospora lactis parasitica*. The fruits were inoculated in needle punctures below the stem scar and kept in a moist atmosphere.

B.—Halved potato tubers. (*a*) Inoculated with *Oospora lactis parasitica;* (*b*) inoculated with *Bacillus carotovorus*.

C.—Halved carrot roots. (*a*) Pricked with a sterile needle; (*b*) inoculated with *Oospora lactis parasitica* isolated from watery-rot lesions of tomato fruit.

INFLUENCE OF THE ABSOLUTE REACTION OF A SOIL UPON ITS AZOTOBACTER FLORA AND NITROGEN FIXING ABILITY[1]

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INTRODUCTION

In a preliminary report the writer (*2*)[2] called attention to the apparent close correlation existing between the absolute reaction of a soil extract and the presence of Azotobacter in the soil. The data presented in this report showed that when the hydrogen-ion concentration of the soil extract exceeded 1×10^{-6} the soil, with very few exceptions, failed to initiate the growth of Azotobacter when introduced into a suitable mannite culture solution. On the other hand, when the hydrogen-ion concentration of the soil extract was less than 1×10^{-6} similar cultures almost always developed typical Azotobacter films. The data, though meager, indicated that the maximum hydrogen-ion concentration endured by Azotobacter in soils was near that represented by a P_H of 6.

In the preliminary report only 90 soils were examined and the hydrogen-ion concentration of the soil extract was determined by the Clark and Lubs colorimetric method as modified for soils by Gillespie (*7*). Some investigators place little credence in the colorimetric method for determining hydrogen-ion concentrations, particularly in a medium as complex as a soil extract. Gillespie, however, found only slight differences in the hydrogen-ion concentration of soil extracts determined by this method and in suspensions of the same soils determined electrometrically. Since the publication of the preliminary report 418 soils from widely varying localities and conditions have been subjected to similar examinations, the resulting data being the basis of this paper. The hydrogen-ion concentration of these soils has been determined colorimetrically upon an extract and electrometrically upon a suspension of the soil.

The soils of Series I were collected within a few miles of the station either by the writer or one of his assistants. These soils represent practically all types and conditions of soil found in this immediate vicinity. Those of Series II were also collected near the station and in many instances at or near the point where soils of Series I were taken. These collections were made 2 years later to serve as a control on the first examinations. Where soils are duplicates of former samples, it has been indicated in Table VII by recording the former numbers. The soils of Series III were collected, as indicated in Table IX, from different counties in the State of Kansas by the county agents. Sterile containers were sent out and requests were made to collect soils representative of conditions in the area covered. The directions called for removing the inch or two of surface soil and collecting from at least four points within a short distance of each other in order to obtain as far as possible a representative sample of the immediate area. In Series IV the soils

[1] Accepted for publication Jan. 22, 1923. Contribution No. 51, Department of Bacteriology, Kansas Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 937–938.

were obtained from a number of different States. These were secured, in most instances, through the various experiment stations. The collector was requested to send samples, where possible, from alkaline or limed, and acid or unlimed, adjacent soils.

METHODS

Soon after the soils reached the laboratory they were well mixed and four 300 cc. Erlenmeyer flasks containing 50 cc. of mannite cultural solution were inoculated from each soil. Ten cubic centimeters of a suspension prepared by shaking 1 part of soil with 2 parts of sterile water was used as an inoculum. The suspension was allowed to stand a few minutes to let the heavier soil particles settle out. Two of the cultures were immediately sterilized in the autoclave to act as controls on total nitrogen determinations.

The culture medium employed had the following composition:

Magnesium sulphate	0.2 gm.
Di-basic potassium phosphate	0.2 gm.
Sodium chlorid	0.5 gm.
Ferric chlorid	Trace.
Calcium chlorid	Trace.
Mannite	20.0 gm.
Distilled water	1,000.0 cc.

This medium was rendered slightly alkaline to phenolphthalein with sodium hydroxid. In all experiments, except those reported in Table V, a small quantity of sterile calcium carbonate was added to each culture flask before inoculating. In all cases the cultures were incubated at room temperature for 3 weeks, after which total nitrogen determinations were made according to the modification of the Kjeldahl method suggested by Latshaw (*10*). The quantities of nitrogen reported represent the average of duplicate cultures after deducting the average of duplicate controls.

During the incubation period frequent examinations were made both macroscopically and microscopically, to ascertain the character of the growth. When "no film" is reported, no growth resembling Azotobacter took place during the first two weeks of incubation. After approximately 2 weeks of incubation a heavy fungus growth usually appeared, especially where no Azotobacter growth, or a nontypical Azotobacter film developed. After the development of a fungus film the growth became so complex that it was difficult to detect Azotobacter either macroscopically or microscopically. Such was the appearance of those cultures in which Azotobacter is reported as questionable. It is believed that the results would have been more consistent and striking if incubation had been reduced to 2 weeks. This would have avoided, to a large extent, the complications arising from the growth of fungi.

The microscopic examinations were made by placing on a slide a loop of that part of the surface growth which appeared most characteristic of Azotobacter, covering with cover glass, and examining with the 1/6 objective. If typical Azotobacter were present in appreciable numbers, the picture was so striking as to be almost unmistakable. If Azotobacter are not present in a soil in sufficient numbers and vigor to develop a visible film or to produce sufficient growth to be observed microscopically by the methods employed, it is questionable whether they are of any significance in the nitrogen economy of a soil.

In this examination of soils for Azotobacter it will be noted that three methods of detecting their presence were employed—the formation of a film, the microscopic examination, and the quantity of nitrogen fixed. Under the heading "Azotobacter" in Tables V, VII, IX, and XI a + (plus) sign has been placed where, in the opinion of the writer, the major evidence indicated the existence of a vigorous Azotobacter flora in the original sample of soil and a − (minus) sign where the evidence did not indicate the presence of a vigorous Azotobacter flora.

The colorimetric hydrogen-ion determinations were made by the Clark and Lubs method as modified for soils by Gillespie. The soils were ground to pass a 40-mesh sieve and a weighed quantity mixed with five times its weight of water, shaken well and centrifugalized until the supernatant liquid was practically clear. The water used in the preparation of the soil extract and suspension was freshly distilled from a mixture of sulphuric acid and potassium dichromate into a flask containing barium hydroxid. From this it was distilled into a third empty flask and again distilled. The P_H of water thus obtained was from 5.7 to 6.0 and was affected by the minutest trace of acid or alkali. All glassware coming in contact with the soil extract was washed in this water. Buffer solutions were prepared according to Clark and Lubs (*1*) and were checked, and adjusted if necessary, at frequent intervals on a Leeds and Northrup type K potentiometer.

Electrometric hydrogen-ion concentrations, or differences in potential between the soil solution and the hydrogen electrode, were made by using a Leeds and Northrup type K potentiometer in connection with saturated KCl—calomel and hydrogen electrodes similar to the one described by Hildebrand (*9*). The ratio of soil to water used was the same as employed in colorimetric determinations; i. e., 1 to 5. Six hydrogen electrodes were connected by switches to the potentiometer so that six samples could be run at the same time. Hydrogen was bubbled through the cells continuously at a rather rapid rate, the cells being constantly shaken. A maximum difference in potential was usually recorded in 10 to 30 minutes, after which the difference decreased very slowly. The length of time required to reach the maximum reading apparently depended, other things being equal, upon the rate of flow of hydrogen. The influence of the rate of flow of hydrogen upon the length of time necessary to obtain maximum difference of potential is illustrated in the data presented in Tables I and II. Neutral or alkaline soils usually required a longer time to reach the maximum difference in potential, and the agreement between duplicates was not, as a rule, as close as it was with acid soils.

TABLE I.—*Time required for electrodes to record maximum difference in potential; hydrogen passed over electrodes slowly (readings recorded as millivolts)*

Soil No.	Time (in minutes).													
	15	20	30	35	40	45	50	55	60	70	80	90	95	100
376	613	....	625	....	....	....	655	....	673	683	687	690	....	[a]693
376	618	....	654	....	....	....	678	....	685	690	694	695	....	[a]696
380	503	....	515	....	....	554	563	565	[a]566	....	....	....	....	....
380	517	....	540	....	....	563	[a]566	566	566	....	....	....	....	....
381	....	520	....	523	....	[a]524	....	524	....	....	....	....	....	....
381	....	500	....	524	....	[a]526	....	526	....	....	....	....	....	....
410	....	633	....	....	642	....	....	670	....	695	....	....	[a]703	703
410	....	654	....	....	691	....	....	697	....	[a]702	....	....	702	700

[a] Maximum reading.

TABLE II.—*Time required for electrodes to record maximum difference in potential; hydrogen passed over electrodes rapidly (readings recorded as millivolts)*

Soil No.	Time (in minutes).						
	5	10	15	20	25	30	35
74	678	688	[a] 691	690	690	.....	.....
74	668	681	680	[a] 682	682	.....	.....
75	674	683	[a] 688	688	688	.....	.....
75	670	685	688	690	[a] 692	.....	.....
76	583	[a] 587	586	585	584	.....	.....
76	590	[a] 592	591	590	588	.....	.....
24	648	.....	[a] 675	.....	674	.....	.....
24	630	.....	[a] 667	.....	664	.....	.....
26	662	.....	[a] 670	.....	666	.....	.....
26	665	.....	[a] 670	.....	670	.....	.....
28	[a] 690	.....	690	.....	686	.....	.....
28	682	.....	680	.....	[a] 688	.....	.....
209	[a] 576	574	573	.....	.....	.....	.....
209	535	570	[a] 577	.....	.....	.....	.....
210	540	[a] 546	546	.....	.....	.....	.....
210	536	546	[a] 550	.....	.....	.....	.....
191	.....	.....	[a] 562	.....	562	.....	560
191	.....	.....	564	.....	[a] 566	.....	564
192	.....	.....	[a] 562	.....	560	.....	558
192	.....	.....	560	.....	[a] 564	.....	564
265	.....	.....	[a] 515	.....	515	514	.....
265	.....	.....	[a] 515	.....	515	514	.....
166	575	.....	.....	[a] 588	.....	586	.....
166	565	.....	.....	[a] 586	.....	584	.....
38	556	.....	.....	[a] 563	.....	562	562
38	525	.....	.....	557	.....	[a] 561	560
8	548	.....	.....	582	.....	[a] 585	585
8	548	.....	.....	577	.....	584	[a] 585

[a] Maximum reading.

The platinum electrodes used were coated with platinum black and tested on a standard acetate solution before using. Several determinations could usually be run with one coating of platinum black. Duplicate samples of soil were always run and as a rule the results agreed within 10 millivolts. If the disagreement were much greater than this, the sample was again run. Sharp and Hoagland (*13*) state that "Duplicate determinations on soil suspension usually agreed within 0.01 to 0.02 volt." Plummer (*11*) says "Duplicate readings on the same sample of soil could easily be read to 0.02 volt," while "It was almost impossible to get such closely agreeing results as 0.02 volt with different samples of the same soil." The data presented in Tables I, II, III, and IV illustrate the millivolt readings of duplicate samples run on different electrodes. In Table IV are shown the millivolt readings of samples of the same soil run on different dates and also the slight effect upon the reading of varying the ratio of soil to water.

TABLE III.—*Effect on electrode readings of varying the method of saturating the electrode and suspension with hydrogen*

Soil No.	Hydrogen run over electrode continuously.					
	Electrode number—					
	1	2	3	4	5	Average.
401	615	608	595	601	619	608
362	557	550	552	553	554	553
378	542	544	541	541	538	541
377	545	542	549	541	551	546

Soil No.	1,000 cc. hydrogen run over electrode.					
	Electrode number—					
	1	2	3	4	5	Average.
401	[a] 597	[a] 611	[a] 611	[a] 615	611	609
362	550	550	550	550	.....	550
378	543	542	530	540	538	539
377	550	[a] 530	[a] 537	[a] 538	554	542

Soil No.	1,000 cc. hydrogen run over electrode, then run continuously.					
	Electrode number—					
	1	2	3	4	5	Average.
401	629	620	622	624	614	622
362	553	553	557	555	.....	554
378	543	543	533	543	530	538
377	555	554	550	552	552	553

[a] These samples apparently did not have sufficient hydrogen passed through to saturate the electrode and suspension, as is evidenced by the increased reading when further passage of hydrogen took place.

TABLE IV.—*Difference in potential of same soil determined on different dates, effect of varying ratio of soil to water, and variations in difference in potential as determined with five different electrodes*

Soil No.	Ratio of soil to water	Electrode number—					
		1	2	3	4	5	Average
390 [a]	1 to 5	534	544	541	539	532	537
390 [b]	1 to 5	538	540	539	532	536	537
369	1 to 5	678	679	677	678	676	678
395	1 to 5	530	528	528	528	529	529
395	1 to 10	536	533	536	536	539	536
387	1 to 5	493	494	493	493	493	493
387	1 to 10	495	494	494	495	494	494
384	1 to 5	650	653	650	652	652	651
397	1 to 5	563	566	567	566	566	566

[a] April 5. [b] May 31.

In converting volt readings into P_H use has been made of the tables prepared by Schmidt and Hoagland (*12*) adding 91 millivolts to the readings to convert them into N/10 KCl–calomel electrode readings. Some investigators regard the difference in potential between saturated and N/10 KCl–calomel electrodes to be of a value other than 91 millivolts. To convert the P_H values here recorded into those of any other difference in potential between saturated and N/10 KCl–calomel electrodes, it is only necessary to add or subtract, as the case may be, 0.017 from the figure here recorded for each millivolt above or below 91. All determinations were made at room temperature, and Schmidt and Hoagland temperature correction factors were used to convert room temperature readings into 25° C. readings.

Hydrogen was purchased in cylinders and washed through a saturated solution of mercuric chlorid, alkaline potassium permanganate solution, alkaline pyrogallic acid solution, and distilled water before entering the hydrogen electrode cell. The connection between the calomel and hydrogen electrodes was made through a glass stopcock. The end of this immersed in the soil suspension was drawn out to a capillary opening and the cock was kept closed during the determination. Between successive determinations, however, the connection was refilled with fresh saturated potassium chlorid.

RESULTS

Data relative to the type of growth and the quantity of nitrogen fixed in culture media inoculated from soils, together with the absolute reaction of the soil extract determined colorimetrically and the soil suspension determined electrometrically of 418 soils, are recorded in Tables V, VII, IX, and XI. In Tables VI, VIII, X, and XII some of the data have been rearranged to show the possible association, or correlation, existing between the hydrogen-ion concentration of the soil solution and the presence or absence of Azotobacter in the soils. In these various tables under the heading "Azotobacter" a + (plus) sign has been recorded to indicate the presence of Azotobacter and a − (minus) sign to indicate the absence of Azotobacter in the soils. These data are not absolute. Sometimes it was very difficult to differentiate between the presence or absence of Azotobacter, There are a few instances where an Azotobacter film developed in only one of duplicate samples, or where either the film or Azotobacter cells were questionable, accompanied by poor fixation of nitrogen, that Azotobacter have been recorded as absent. On the other hand, there are a few instances where similar conditions were accompanied by good fixation of nitrogen that have been recorded as containing Azotobacter. An effort has been made to weigh the evidence in questionable cases as carefully as possible and record Azotobacter as present if it were believed that the data indicated the presence of an active Azotobacter flora, and as absent if the available data did not indicate the presence of such a flora.

It should be borne in mind that the chance for contamination of Azotobacter free soils with Azotobacter was not entirely eliminated. Especially was this true in Series III and IV, for nearly all of the soils in these two series were collected by men inexperienced in bacteriological technic and were shipped long distances. Sometimes one or more of the individual containers were broken open upon arrival at the laboratory.

Even in the laboratory the possibility of contamination could not be entirely eliminated. What influence such contamination may have had upon the development of Azotobacter from soils that normally contained no Azotobacter is not known.

Again, it has been shown (*3*) that Azotobacter can exist for varying lengths of time in soils that will not support them indefinitely. Under natural conditions, if Azotobacter exists in the proximity of soils not containing them, almost constant inoculation due to wind, rain, animals, cultivation, etc., is inevitable. The length of time that such introduced organisms may remain in a viable condition apparently depends upon the intensity of the unfavorable influences. It is, therefore, highly probable that Azotobacter may frequently be isolated from soils in which they will not function or even exist for a very long period.

On the other hand, little is known as to how many Azotobacter are necessary to initiate the development of a visible film in laboratory culture media. Unpublished data indicate that appreciable numbers are essential to the development of a characteristic film. Also, nothing is known as to how rapidly they may disappear from a sample of soil removed from its natural environment. It is possible, therefore, that Azotobacter may be present in relatively large numbers in a soil and escape observation by the methods employed in these investigations.

In comparing the reaction with the presence or absence of Azotobacter, and in obtaining a mathematical expression for this association or correlation (the association coefficient), the soils have been divided into two groups; those with a P_H of 6.0 or above and those more acid than P_H 6.0. This division point has been chosen more or less arbitrarily, though the data here presented indicate that it is not far from absolute. The association coefficient was obtained by the use of Yule's (*14*) association coefficient formula, as mentioned elsewhere (*5*). Association coefficients have been calculated both from the data secured by the colorimetric and the electrometric P_H determination. In analyzing the data presented here it will perhaps be best to examine those from each series of soils separately.

SERIES I

The data secured from an examination of the soils of Series I are presented in Table V, rearranged in part in Table VI, and summarized below. These soils together with those of Series II are all local soils and were, perhaps, collected and handled with more care than those of Series III and IV. Samples 1 to 19 were lost through error before the electrometric hydrogen-ion determinations were run. However, in the following summary the electrometric P_H of samples No. 1, 4, 5, 14, and 15 have been regarded as above P_H 6.0 and all other soils below No. 20 as more acid than P_H 6.0. These 19 soils could not be considered in obtaining the numerical average of colorimetric P_H. Likewise samples 85-90, for which no quantitative nitrogen determinations were made, were omitted from the calculations for average nitrogen fixed.

TABLE V.—*Type of growth, nitrogen fixed, and reaction of soils of Series I*

Soil No.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P_H colorimetric.	P_H electrometric.
			Mgm.			
1	Typical Azotobacter	Typical Azotobacter	10.3	+	6.9	.....
2	None	No Azotobacter	2.0	−	5.4	.....
3	do	do	3.0	−	5.6	.....
4	Typical Azotobacter	Typical Azotobacter	6.8	+	6.9	.....
5	do	do	5.4	+	7.1	.....
6	None	No Azotobacter	4.4	−	5.7	.....
7	do	do	4.4	−	5.7	.....
8	do	do	4.6	−	5.6	.....
9	do	do	4.7	−	5.5	.....
10	do	do	−0.6	−	5.6	.....
11	do	do	1.7	−	5.8	.....
12	do	do	4.5	−	5.9	.....
13	do	do	3.2	−	5.6	.....
14	Typical Azotobacter	Typical Azotobacter	8.5	+	7.4	.....
15	do	do	10.3	+	7.4	.....
16	None	No Azotobacter	4.0	−	5.6	.....
17	do	do	3.0	−	5.5	.....
18	do	do	3.7	−	5.6	.....
19	do	do	4.5	−	5.7	.....
20	do	do	4.7	−	5.4	5.10
21	do	do	4.7	−	5.6	5.05
22	do	do	4.7	−	5.6	5.27
23	do	do	4.7	−	5.6	5.10
24	do	do	4.2	−	5.6	4.90
25	Typical Azotobacter	Typical Azotobacter	6.4	+	7.0	6.45
26	do	do	9.9	+	6.6	6.13
27	do	do	7.5	+	6.1	5.88
28	do	do	9.5	+	6.2	5.86
29	do	do	8.8	+	7.6	7.27
30	None	No Azotobacter	4.1	−	5.6	5.39
31	Typical Azotobacter	Typical Azotobacter	10.6	+	7.5	7.71
32	None	No Azotobacter	5.6	−	5.9	5.85
33	do	do	4.0	−	6.7	6.56
34	do	do	4.5	−	6.8	6.74
35	Typical Azotobacter	Typical Azotobacter	6.8	+	7.6	7.62
36	do	do	9.2	+	6.0	6.12
37	None	No Azotobacter	5.4	−	6.2	6.07
38	Nontypical [a]	Azotobacter present [a]	3.9	−	5.6	5.59
39	do	do	8.6	+	6.1	6.05
40	do	do	8.1	+	7.0	6.72
41	do	Typical Azotobacter	7.6	+	6.0	5.98
42	do	do	8.3	+	7.4	7.05
43	Typical Azotobacter	do	9.6	+	7.7	8.75
44	Nontypical	do	9.9	+	7.5	7.71
45	Typical Azotobacter	do	10.1	+	7.4	7.45
46	None	No Azotobacter	1.0	−	5.9	6.03
47	Typical Azotobacter	Typical Azotobacter	10.0	+	7.4	7.52
48	Nontypical	do	6.6	+	6.4	6.52
49	None	No Azotobacter	2.7	−	5.5	5.03
50	do	do	4.0	−	5.8	5.32
51	do	do	3.2	−	5.3	5.41
52	Nontypical	Azotobacter present	5.5	+	7.3	7.27
53	Typical Azotobacter	Tppical Azotobacter	9.0	+	7.7	7.79
54	Nontypical	do	7.4	+	6.0	6.18
55	Typical Azotobacter	do	8.7	+	7.5	7.50
56	Nontypieal	do	7.2	+	7.4	7.18
57	Typical Azotobacter	do	7.6	+	7.5	7.30

[a] P_H not determined electrometrically.

TABLE V.—*Type of growth, nitrogen fixed, and reaction of soils of Series I*—Continued

Soil No.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P_H colorimetric.	P_H electrometric.
			Mgm.			
58	None	No Azotobacter	4.0	−	5.5	5.46
59	Typical Azotobacter	Typical Azotobacter	10.6	+	7.4	7.49
60	None	No Azotobacter	4.6	−	5.8	5.73
61	do	do	3.7	−	5.5	5.51
62	Typical Azotobacter	Typical Azotobacter	7.3	+	7.5	7.55
63	do	do	11.0	+	7.4	7.43
64	do	do	8.3	+	6.1	6.35
65	None	No Azotobacter	5.2	−	5.7	5.94
66	do	do	3.8	−	5.7	5.81
67	do	do	4.1	−	5.5	5.32
68	Nontypical	Typical Azotobacter	6.9	+	6.1	6.05
69	None	No Azotobacter	4.1	−	5.6	5.19
70	Typical Azotobacter	Typical Azotobacter	8.7	+	6.8	6.93
71	Nontypical	do	6.9	+	5.6	5.61
72	do	do	6.8	+	7.0	6.72
73	None	No Azotobacter	4.5	−	5.9	5.88
74	Nontypical [a]	Typical Azotobacter [a]	8.1	+	7.4	7.25
75	Typical Azotobacter	do	6.1	+	7.5	7.30
76	Nontypical [a]	do [a]	3.4	−	5.5	5.63
77	None	No Azotobacter	4.2	−	5.6	5.34
78	Nontypical	Typical Azotobacter	5.5	+	7.7	7.54
79	Typical Azotobacter	do	7.6	+	7.7	8.48
80	Nontypical	Azotobacter present	9.2	+	7.6	7.84
81	Typical Azotobacter	Typical Azotobacter	9.6	+	7.4	7.77
82	do	do	8.0	+	7.7	7.60
83	do	do	9.9	+	7.6	7.72
84	do	do	9.5	+	7.5	7.47
85	do	do	.....	+	7.5	7.62
86	do	do	.....	+	7.5	7.60
87	do	do	.....	+	7.8	7.59
88	do	do	.....	+	6.9	6.88
89	do	do	.....	+	7.5	7.76
90	do	do	.....	+	7.3	7.06

[a] P_H not determined electrometrically.

TABLE VI.—*Correlation between reaction and presence of Azotobacter in soils of Series I*

P_H determined electrometrically.						P_H determined colorimetrically.					
Soil No.	P_H.	Azotobacter.	Soil No.	P_H.	Azotobacter.	Soil No.	P_H.	Azotobacter.	Soil No.	P_H.	Azotobacter.
43	8.75	+	46	6.03	−	87	7.8	+	37	6.2	−
79	8.48	+	41	5.98	+	43	7.7	+	27	6.1	+
80	7.84	+	65	5.94	−	53	7.7	+	39	6.1	+
53	7.79	+	27	5.88	+	78	7.7	+	64	6.1	+
81	7.77	+	73	5.88	−	79	7.7	+	68	6.1	+
89	7.76	+	28	5.86	+	82	7.7	+	36	6.0	+
83	7.72	+	32	5.85	−	29	7.6	+	41	6.0	+
31	7.71	+	66	5.81	−	35	7.6	+	54	6.0	+
44	7.71	+	60	5.73	−	80	7.6	+	12[a]	5.9	−
35	7.62	+	76	5.63	−	83	7.6	+	32	5.9	−
85	7.62	+	71	5.61	+	31	7.5	+	46	5.9	−
86	7.60	+	38	5.59	−	44	7.5	+	73	5.9	−
82	7.60	+	61	5.51	−	55	7.5	+	11[a]	5.8	−
87	7.59	+	58	5.46	−	57	7.5	+	50	5.8	−
62	7.55	+	51	5.41	−	62	7.5	+	60	5.8	−
78	7.54	+	30	5.39	−	75	7.5	+	6[a]	5.7	−
47	7.52	+	77	5.34	−	84	7.5	+	7[a]	5.7	−
55	7.50	+	50	5.32	−	85	7.5	+	19[a]	5.7	−
59	7.49	+	67	5.32	−	86	7.5	+	65	5.7	−
84	7.47	+	22	5.27	−	89	7.5	+	66	5.7	−
45	7.45	+	69	5.19	−	14[a]	7.4	+	3[a]	5.6	−
63	7.43	+	20	5.10	−	15[a]	7.4	+	8[a]	5.6	−
57	7.30	+	23	5.10	−	42	7.4	+	10[a]	5.6	−
75	7.30	+	21	5.05	−	45	7.4	+	13[a]	5.6	−
29	7.27	+	49	5.03	−	47	7.4	+	16[a]	5.6	−
52	7.27	+	24	4.90	−	56	7.4	+	18[a]	5.6	−
74	7.25	+	.....	.....	.....	59	7.4	+	21	5.6	−
56	7.18	+	.....	.....	.....	63	7.4	+	22	5.6	−
90	7.06	+	.....	.....	.....	74	7.4	+	23	5.6	−
42	7.05	+	.....	.....	.....	81	7.4	+	24	5.6	−
70	6.93	+	.....	.....	.....	57	7.3	+	30	5.6	−
88	6.88	+	.....	.....	.....	90	7.3	+	38	5.6	−
34	6.72	−	.....	.....	.....	5[a]	7.1	+	69	5.6	−
40	6.72	+	.....	.....	.....	25	7.0	+	71	5.6	+
72	6.72	+	.....	.....	.....	40	7.0	+	77	5.6	−
33	6.56	−	.....	.....	.....	72	7.0	+	9[a]	5.5	−
48	6.52	+	.....	.....	.....	1[a]	6.9	+	17[a]	5.5	−
25	6.45	+	.....	.....	.....	4[a]	6.9	+	49	5.5	−
64	6.35	+	.....	.....	.....	88	6.9	+	58	5.5	−
54	6.18	+	.....	.....	.....	34	6.8	−	61	5.5	−
26	6.13	+	.....	.....	.....	70	6.8	+	67	5.5	−
36	6.12	+	.....	.....	.....	33	6.7	−	76	5.5	−
37	6.07	−	.....	.....	.....	26	6.6	+	2[a]	5.4	−
39	6.05	+	.....	.....	.....	48	6.4	+	20	5.4	−
68	6.05	+	.....	.....	.....	28	6.2	+	51	5.3	−

[a] P_H not determined electrometrically.

The following summary of Tables V and VI needs little explanation.

Summary of Tables V and VI

Number of soils examined	90
Number of soils containing Azotobacter	51
Number of soils not containing Azotobacter	39
Average mgm. nitrogen fixed, 84 soils	6.25
Average mgm. nitrogen fixed, 45 soils containing Azotobacter	8.32
Average mgm. nitrogen fixed, 39 soils not containing Azotobacter	3.86
Number of soils electrometric P_H 6.0 or above	51
Number of soils electrometric P_H below 6.0	39
Number of soils colorimetric P_H 6.0 or above	53
Number of soils colorimetric P_H below 6.0	37
Number of soils electrometric P_H 6.0 or above containing Azotobacter	47
Number of soils electrometric P_H 6.0 or above not containing Azotobacter	4
Number of soils elcterometric P_H below 6.0 containing Azotobacter	4
Number of soils electrometric P_H below 6.0 not containing Azotobacter	35
Number of soils colorimetric P_H 6.0 or above containing Azotobacter	50
Number of soils colorimetric P_H 6.0 or above not containing Azotobacter	3
Number of soils colorimetric P_H below 6.0 containing Azotobacter	1
Number of soils colorimetric P_H below 6.0 not containing Azotobacter	36
Average electrometric P_H, 71 soils	6.57
Average colorimetric P_H, 90 soils	6.50
Average electrometric P_H, 46 soils containing Azotobacter	7.12
Average electrometric P_H, 25 soils not containing Azotobacter	5.57
Average colorimetric P_H, 51 soils containing Azotobacter	7.12
Average colorimetric P_H, 39 soils not containing Azotobacter	5.70
Association coefficient based on electrometric P_H determinations	0.981
Association coefficient based on colorimetric P_H determinations	0.997

It will be observed that the number of soils more alkaline than P_H 6.0 by either method and not containing Azotobacter and also the number more acid than P_H 6.0 containing Azotobacter are few. There were only four soils by the electrometric and three by the colorimetric method with a P_H of 6.0 or above that did not contain Azotobacter. One of the electrometric P_H 6.0 or above soils (No. 46) not containing Azotobacter was more acid than P_H 6.0 colorimetrically, while soil No. 37 was only slightly more alkaline than P_H 6.0 by both methods. There were four soils with an electrometric and one with a colorimetric reaction more acid than P_H 6.0 recorded as containing Azotobacter. Three of these gave a reaction less acid than P_H 6.0 by one of the methods and the remaining samples did not give a typical Azotobacter growth.

SERIES II

In Table VII are recorded the data collected from the examination of the soils of Series II. Many of these soils were collected near the point where certain soils of Series I were taken and may thus be regarded as controls on the earlier examinations. In Table XIII are recorded in parallel columns the data secured from the two examinations. It will be noted that the data are quite similar even though two years intervened between the two analyses. Part of the data of Table VII are rearranged in Table VIII to show the possible association existing between the presence of Azotobacter and the hydrogen-ion concentration of the soil solution.

TABLE VII.—*Type of growth, nitrogen fixed, and reaction of soils of Series II*

Soil No.	Duplicate soil.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P_H colorimetric.	P_H electrometric.
				Mgm.			
101	1	Typical Azotobacter.	Typical Azotobacter.	10.7	+	6.7	6.71
102	2	None.	No Azotobacter.	6.3	−	5.0	3.78
103	3	do.	do.	5.4	−	5.4	5.27
104	4	Typical Azotobacter.	Typical Azotobacter.	10.5	+	7.1	.....
105	5	do.	do.	11.8	+	7.2	7.08
106	6	None.	No Azotobacter.	6.1	−	5.7	5.25
107	31	Typical Azotobacter.	Typical Azotobacter.	10.2	+	7.4	7.70
108	32	None.	Azotobacter present.	8.5	+	5.6	5.48
109	33	Typical Azotobacter.	Typical Azotobacter.	9.5	+	6.6	6.47
110	34	do.	do.	7.9	+	7.4	7.61
111	35	do.	do.	9.1	+	6.6	6.39
112	.....	Nontypical Azotobacter.	Azotobacter present.	.....	+	5.8	5.48
113	36	Typical Azotobacter.	Typical Azotobacter.	8.9	+	5.6	5.85
114	37	Nontypical Azotobacter.	do.	8.9	+	5.8	5.59
115	38	Typical Azotobacter.	do.	8.6	+	5.4	5.51
116	39	do.	do.	10.0	+	6.0	5.90
117	.....	do.	do.	10.3	+	6.6	6.56
118	.....	do.	do.	8.9	+	7.5	7.28
119	40	do.	do.	9.8	+	7.1	7.00
120	61	Nontypical Azotobacter.	Azotobacter present.	6.0	+	5.8	5.68
121	66	None.	No Azotobacter.	7.6	−	5.7	5.24
122	64	Nontypical Azotobacter.[a]	Azotobacter present.	8.6	+	5.8	5.51
123	65	Typical Azotobacter.	Typical Azotobacter.	8.8	+	5.9	5.64
124	63	do.	do.	9.0	+	6.9	7.06
125	.....	None.	No Azotobacter.	4.7	−	5.5	5.15
126	49	do.	do.	8.0	−	5.5	5.19
127	50	do.	do.	6.6	−	5.5	5.10
128	51	do.	do.	5.1	−	5.9	5.78
129	52	Typical Azotobacter.	Typical Azotobacter.	10.8	+	7.6	7.30
130	53	do.	do.	7.3	+	7.4	7.52
131	54	None.	No Azotobacter.	7.7	−	5.8	5.47
132	.....	Nontypical Azotobacter.	Typical Azotobacter.	9.3	+	5.9	5.54
133	15	Typical Azotobacter.	do.	13.5	+	7.3	7.45
134	77	None.	No Azotobacter.	7.2	−	5.4	4.88
135	.....	Typical Azotobacter.	Typical Azotobacter.	11.7	+	7.2	7.20
136	16	None.	Azotobacter present.	10.5	+	5.4	4.88
137	17	do.	No Azotobacter.	4.3	−	5.8	5.58
138	18	do.	Azotobacter (?).	3.0	−	5.4	5.05
139	.....	do.	do (?)	8.9	+	6.2	5.90
140	.....	Typical Azotobacter.	Typical Azotobacter.	7.3	+	7.0	7.30
141	.....	do.	do.	7.9	+	7.2	7.48
142	82	do.	do.	9.7	+	7.8	7.59
143	79	do.	do.	7.0	+	8.1	8.77
144	80	do.	do.	8.8	+	7.3	7.72
145	83	do.	do.	7.5	+	7.2	7.94
146	.....	do.	do.	8.1	+	6.9	7.16
147	.....	do.	do.	8.8	+	6.8	7.06
148	.....	do.	do.	10.3	+	7.3	7.69
149	.....	do.	do.	8.3	+	7.1	7.62
150	.....	do.	do.	6.4	+	7.3	7.65
151	.....	do.	do.	12.3	+	6.0	6.25
152	.....	do.	do.	9.4	+	6.0	5.96

[a] One sample.

TABLE VII.—*Type of growth, nitrogen fixed, and reaction of soils of Series II*—Continued

Soil No.	Duplicate soil.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	PH colorimetric.	PH electrometric.
				Mgm.			
153	.....	Nontypical Azotobacter.	Azotobacter present.	2.8	—	5.6	5.59
154	.....	Typical Azotobacter.	Typical Azotobacter.	8.3	+	6.0	6.17
155	.....	.....do.....	.....do.....	9.7	+	6.1	6.34
156	.....	None.....	No Azotobacter.....	9.6	—	5.5	5.61
157	.....	Typical Azotobacter.	Typical Azotobacter.	8.0	+	6.0	6.27
158	.....	Nontypical Azotobacter.	Azotobacter present.	8.1	+	6.2	6.51
159	.....	Typical Azotobacter.	Typical Azotobacter.	8.3	+	7.8	7.77
160	.....	.....do.....	.....do.....	10.1	+	6.2	6.89

TABLE VIII.—*Correlation between reaction and presence of Azotobacter in soils of Series II*

PH determined electrometrically.						PH determined colorimetrically.					
Soil No.	PH.	Azotobacter.	Soil No.	PH.	Azotobacter.	Soil No.	PH.	Azotobacter.	Soil No.	PH.	Azotobacter.
143	8.77	+	151	6.25	+	143	8.1	+	155	6.1	+
145	7.94	+	154	6.17	+	142	7.8	+	116	6.0	+
159	7.77	+	152	5.96	+	159	7.8	+	151	6.0	+
144	7.72	+	139	5.90	+	129	7.6	+	152	6.0	+
107	7.70	+	116	5.90	+	118	7.5	+	154	6.0	+
148	7.69	+	113	5.85	+	107	7.4	+	157	6.0	+
150	7.65	+	128	5.78	—	110	7.4	+	123	5.9	+
149	7.62	+	120	5.68	+	130	7.4	+	128	5.9	—
110	7.61	+	123	5.64	+	133	7.3	+	132	5.9	+
142	7.59	+	156	5.61	—	144	7.3	+	112	5.8	+
130	7.52	+	153	5.59	—	148	7.3	+	114	5.8	+
141	7.48	+	114	5.59	+	150	7.3	+	120	5.8	+
133	7.45	+	137	5.58	—	105	7.2	+	122	5.8	+
129	7.30	+	132	5.54	+	135	7.2	+	131	5.8	—
140	7.30	+	122	5.51	+	141	7.2	+	137	5.8	—
118	7.28	+	115	5.51	+	145	7.2	+	106	5.7	—
135	7.20	+	112	5.48	+	104[a]	7.1	+	121	5.7	—
146	7.16	+	108	5.48	+	119	7.1	+	108	5.6	+
105	7.08	+	131	5.47	—	149	7.1	+	113	5.6	+
124	7.06	+	103	5.27	—	140	7.0	+	153	5.6	—
147	7.06	+	106	5.25	—	124	6.9	+	125	5.5	—
119	7.00	+	121	5.24	—	146	6.9	+	126	5.5	—
160	6.89	+	126	5.19	—	147	6.8	+	127	5.5	—
101	6.71	+	125	5.15	—	101	6.7	+	156	5.5	—
117	6.56	+	127	5.10	—	109	6.6	+	103	5.4	—
158	6.51	+	138	5.05	—	111	6.6	+	115	5.4	+
109	6.47	+	134	4.88	—	117	6.6	+	134	5.4	—
111	6.39	+	136	4.88	+	139	6.2	+	136	5.4	+
155	6.34	+	102	3.78	—	158	6.2	+	138	5.4	—
157	6.27	+	.....	.....	.....	160	6.2	+	102	5.0	—

[a] PH not determined electrometrically.

In the following summary it may be observed that there were no soils more alkaline than P_H 6.0 that did not contain Azotobacter. There were 13 soils with an electrometric and 10 with a colorimetric P_H below 6.0 that were recorded as containing Azotobacter. Only 5 of these produced typical Azotobacter film and 10 of the 13 gave a P_H of 5.8 or above by one of the methods. It would appear from the data of this series of soils that P_H 5.8 was nearer the absolute limiting hydrogen-ion concentration than P_H 6.0.

Summary of Tables VII and VIII

Number of soils examined	60
Number of soils containing Azotobacter	46
Number of soils not containing Azotobacter	14
Average mgm. nitrogen fixed, 59 soils	8.41
Average mgm. nitrogen fixed, 45 soils containing Azotobacter	9.16
Average mgm. nitrogen fixed, 14 soils not containing Azotobacter	6.03
Number of soils electrometric P_H 6.0 or above	33
Number of soils electrometric P_H below 6.0	27
Number of soils colorimetric P_H 6.0 or above	36
Number of soils colorimetric P_H below 6.0	24
Number of soils electrometric P_H 6.0 or above containing Azotobacter	33
Number of soils electrometric P_H 6.0 or above not containing Azotobacter	0
Number of soils electrometric P_H below 6.0 containing Azotobacter	13
Number of soils electrometric P_H below 6.0 not containing Azotobacter	14
Number of soils colorimetric P_H 6.0 or above containing Azotobacter	36
Number of soils colorimetric P_H 6.0 or above not containing Azotobacter	0
Number of soils colorimetric P_H below 6.0 containing Azotobacter	10
Number of soils colorimetric P_H below 6.0 not containing Azotobacter	14
Average electrometric P_H, 59 soils	6.37
Average colorimetric P_H, 60 soils	6.39
Average electrometric P_H, 45 soils containing Azotobacter	6.73
Average electrometric P_H, 14 soils not containing Azotobacter	5.21
Average colorimetric P_H, 46 soils containing Azotobacter	6.65
Average colorimetric P_H, 14 soils not containing Azotobacter	5.55
Association coefficient based on electrometric P_H determinations	1.000
Association coefficient based on colorimetric P_H determinations	1.000

The above summary with a few minor exceptions is very similar to that for Series I. The most marked difference is in the average nitrogen fixed by the soils not containing Azotobacter, this being 3.86 mgm. for Series I and 6.03 mgm. for Series II. This difference is due to the much higher fixation of nitrogen by soils not containing Azotobacter in media containing calcium carbonate than in the media without calcium carbonate. This fact has been emphasized in a former article (*4*). No calcium carbonate was added to the media employed in the examination of the soils of Series I, while an excess was added to the media in the examination of all subsequent soils.

SERIES III

The soils of Series III were secured through the county agents of Kansas and did not reach the laboratory in as fresh and possibly as uncontaminated condition as did those collected locally. Samples 138 to 180, inclusive, reached the laboratory while the writer was in the Army and remained several months before being examined. It was believed that the quantity of nitrogen fixed by these soils would not be comparable to that of fresh soils, hence no nitrogen determinations were made. Possibly these soils should be disregarded entirely. The data secured from the examination of the soils of this series are recorded in Tables IX and X, and summarized below.

TABLE IX.—*Type of growth, nitrogen fixed, and reaction of soils of Series III*

Soil No.	County.	Type of film.	Microscopic picture	Nitrogen fixed.	Azotobacter.	P_H colorimetric.	P_H electrometric.
				Mgm.			
161	Pottawatamie.	Typical Azotobacter.	Typical Azotobacter.	8.6	+	6.5	7.21
162	do	Nontypical film.	Azotobacter (?)	7.1	+	5.7	6.02
163	Cloud	do	do	5.8	+	6.0	5.98
164	do	None	No Azotobacter	5.0	−	5.7	5.41
165	do	do	do	4.4	−	5.6	5.54
166	do	do	do	7.0	−	5.5	5.71
167	Wyandotte	Nontypical	Typical Azotobacter.	8.0	+	6.1	6.25
168	Franklin	None	No Azotobacter	6.3	−	5.4	5.17
169	do	Nontypical	Azotobacter present.	7.1	+	5.2	5.10
170	Wilson	do	do	2.9	+	5.9	5.93
171	do	do	do	2.9	+	5.4	5.15
172	do	None	No Azotobacter	5.1	−	5.3	5.02
173	Pawnee	Typical Azotobacter.[a]	Typical Azotobacter.[a]	8.2	+	6.0	6.08
174	Franklin	None	Azotobacter (?)	5.4	−	5.8	5.85
175	do	do	do	3.0	−	5.4	5.29
176	Anderson	Typical Azotobacter.	Typical Azotobacter.	9.1	+	6.5	6.35
177	do	None	Azotobacter (?)	7.2	−	5.6	5.37
178	do	do	No Azotobacter	9.6	−	5.6	5.30
179	Nemaha	do	do	4.7	−	5.8	5.76
180	do	do	do	1.8	−	5.6	5.47
181	Marshall	Nontypical	Azotobacter (?)	7.9	+	5.9	6.10
182	do	None	No Azotobacter	7.8	−	6.2	6.52
183	do	Typical Azotobacter.	Typical Azotobacter.	8.8	+	6.9	7.37
184	do	do	do	8.5	+	6.4	6.77
185	Wyandotte	None	No Azotobacter	4.6	−	5.6	5.44
186	do	Typical Azotobacter.	Typical Azotobacter.	9.9	+	7.2	7.72
187	do	do	do	8.5	+	6.2	6.69
188	Atchinson	Nontypical	Azotobacter present.	7.4	+	5.6	5.52
189	do	None	No Azotobacter	0.8	−	5.6	5.52
190	do	do	Azotobacter present.[a]	5.5	−	5.6	5.34
191	do	do	No Azotobacter	5.3	−	5.7	5.32
192	Wilson	do	do	7.2	−	5.6	5.22
193	Jewell	Nontypical	Typical Azotobacter.	9.5	+	6.6	6.84
194	do	Typical Azotobacter.	do	8.1	+	6.0	6.15
195	Chase	None	No Azotobacter	7.7	−	5.8	5.69
196	do	do	do	6.6	−	5.4	4.90
197	do	Typical Azotobacter.	Typical Azotobacter.	6.9	+	6.6	7.57
198	Lyon	None	Azotobacter (?)	4.6	−	5.9	5.49
199	do	Nontypical	Typical Azotobacter.	5.1	+	5.9	6.07
200	Osborne	Typical Azotobacter.	do	7.9	+	7.6	8.28
201	do	None	No Azotobacter	6.1	−	6.1	5.98
202	Kearney	Typical Azotobacter.	Typical Azotobacter.	7.5	+	7.4	8.19
203	do	do	do	7.0	+	7.0	7.25

[a] One sample.

TABLE IX.—*Type of growth, nitrogen fixed, and reaction of soils of Series III*—Contd.

Soil No.	County.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P_H colorimetric.	P_H electrometric.
				Mgm.			
204..	Washington.	None.....	No Azotobacter..	2.7	−	5.8	5.10
205..	Doniphan...	Typical Azotobacter.	Typical Azotobacter.	7.9	+	6.1	6.02
206..	.....do.....	.....do.[a].....	.....do.[a].....	5.4	−	5.7	5.32
207..	.....do.....	.....do.....	.....do.....	.....	+	7.1	7.94
208..	Rush.....	.....do.....	.....do.....	11.6	+	7.2	7.60
209..	Greenwood.	None.....	No Azotobacter..	0.3	−	5.7	5.53
210..	.....do.....	.....do.....	.....do.....	5.7	−	5.3	5.05
211..	.....do.....	.....do.....	.....do.....	4.0	−	5.3	5.10
212..	.....do.....	.....do.....	.....do.....	5.7	−	5.6	5.39
213..	Rawlins....	Typical Azotobacter.	Typical Azotobacter.	10.5	+	7.6	8.30
214..	.....do.....	.....do.....	.....do.....	10.0	+	7.3	7.98
215..	.....do.....	.....do.....	.....do.....	9.3	+	6.7	7.28
216..	.....do.....	.....do.....	.....do.....	9.6	+	6.9	7.40
217..	Greenwood.	.....do.....	.....do.....	11.1	+	5.6	5.15
218..	Jewell.....	.....do.....	.....do.....	10.6	+	6.6	7.28
219..	.....do.....	None.....	No Azotobacter..	6.0	−	6.6	7.25
220..	Pottawatamie.	Typical Azotobacter.	Typical Azotobacter.	11.2	+	6.4	6.34
221..	Ford.....	.....do.[a].....	.....do.[a].....	8.2	+	6.6	6.66
222..	.....do.....	.....do.[a].....	.....do.[a].....	11.8	+	6.5	6.69
223..	.....do.....	.....do.....	.....do.....	9.5	+	6.7	6.63
224..	.....do.....	.....do.....	.....do.....	9.3	+	6.9	7.52
225..	McPherson..	Nontypical.....	.....do.....	7.4	+	5.9	5.91
226..	.....do.....	Typical Azotobacter.	.....do.....	3.6	+	6.7	7.13
227..	.....do.....	None.....	No Azotobacter..	5.0	−	5.8	5.66
228..	.....do.....	.....do.....	.....do.....	5.1	−	5.6	5.20
229..	Burbon.....	Typical Azotobacter.	Typical Azotobacter.	10.2	+	7.1	7.60
230..	Osborne....	.....do.....	.....do.....	8.9	+	6.6	6.98
231..	Kearney....	Nontypical.....	.....do.....	7.0	+	7.4	8.25
232..	.....do.....	Typical Azotobacter.	.....do.....	10.1	+	7.4	8.31
233..	Greenwood.	Nontypical.....	.....do.....	7.0	+	7.0	7.47
234..	Meade.....	Typical Azotobacter.	.....do.....	8.1	+	7.4	8.15
235..	.....do.....	.....do.....	.....do.....	8.7	+	6.5	6.66
236..	Chautauqua.	None.....	No Azotobacter..	4.5	−	5.6	5.30
237..	.....do.....	.....do.....	Azotobacter (?)...	3.7	−	5.5	5.02
238..	Morris.....	.....do.....	No Azotobacter..	.....	−	5.7	5.54
239..	Pawnee.....	Typical Azotobacter.	Typical Azotobacter.	.....	+	6.1	6.08
240..	.....do.....	.....do.....	.....do.....	.....	+	6.6	7.03
241..	Meade.....	Nontypical.....	.....do.....	.....	+	6.4	6.18
242..	Elk.....	.....do.[a].....	.....do.[a].....	.....	−	5.9	5.51
243..	Meade.....	None.....	No Azotobacter..	.....	−	6.7	6.96
244..	Chautauqua	.....do.....	Azotobacter (?)..	.....	−	5.1	5.22
245..	Rush.....	.....do.....	.....do.....	.....	−	6.1	6.54
246..	Douglas....	.....do.....	No Azotobacter..	.....	−	5.8	5.90
247..	.....do.....	.....do.....	.....do.....	.....	−	5.5	5.37
248..	.....do.....	.....do.....	.....do.....	.....	−	5.7	5.47
249..	.....do.....	.....do.....	.....do.....	.....	−	5.4	5.12
250..	Coffey.....	Nontypical.....	Typical Azotobacter.	.....	+	5.9	6.00
251..	.....do.....	None.....	No Azotobacter..	.....	−	6.0	6.10

[a] One sample.

TABLE IX.—*Type of growth, nitrogen fixed, and reaction of soils of Series III*—Contd.

Soil No.	County.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P_H colorimetric.	P_H electrometric.
				Mgm.			
252..	Coffey	None.....	No Azotobacter..	.....	−	5.6	5.37
253..	Pawnee.....	Typical Azotobacter.	Typical Azotobacter.	.....	+	6.4	6.25
254..	Greenwood .	None.....	No Azotobacter..	.....	−	5.4	4.68
255..	Wichita....	Typical Azotobacter.	Typical Azotobacter.	.....	+	7.2	7.79
256..	.....do.....	None.....	Azotobacter (?)...	.....	−	6.2	6.35
257..	.....do.....	Nontypical.....	Typical Azotobacter	.....	+	7.8	8.13
258..	.....do.....	Typical Azotobacter.	.....do.....	.....	+	7.6	8.26
259..	Logan.....	.....do.....	.....do.....	.....	+	6.5	6.49
260..	Thomas.....	None.....	Azotobacter (?)...	.....	−	7.0	7.48
261..	Pratt.....	.....do.....	No Azotobacter..	.....	−	5.9	5.81
262..	...do.....	.....do.....	.....do.....	.....	−	6.9	7.23
263..	Lyon.....	.....do.....	Azotobacter (?)...	.....	−	5.5	4.93
264..	Hodgeman..	Typical Azotobacter.	Typical Azotobacter.	.....	+	7.4	7.52
271..	Woodson....	None.....	No Azotobacter...	.....	−	5.6	5.41
272..	Pratt.....	Typical Azotobacter.	Typical Azotobacter.	.....	+	7.4	7.72
273..	.....do.....	None.....	No Azotobacter..	.....	−	6.5	6.42
274..	Finney.....	Nontypical.....	Typical Azotobacter.	.....	+	6.5	6.96
275..	.....do.....	Typical Azotobacter.	.....do.....	.....	+	7.4	8.06
276..	Jackson.....	None.....	No Azotobacter..	.....	−	5.7	5.27
277..	.....do.....	.....do.....	.....do.....	.....	−	5.4	5.10
278..	Hodgeman..	Nontypical.....	Azotobacter (?)...	.....	+	7.4	7.99
279..	.....do.....	None.....	No Azotobacter..	.....	−	6.4	6.81
280..	.....do.....	Typical Azotobacter.	Typical Azotobacter.	.....	+	5.7	6.51

TABLE X.—*Correlation between reaction and presence of Azotobacter in soils of Series III*

PH determined electrometrically.						PH determined colorimetrically.					
Soil No.	PH.	Azotobacter.	Soil No.	PH.	Azotobacter.	Soil No.	PH.	Azotobacter.	Soil No.	PH.	Azotobacter.
232...	8.31	+	239...	6.08	+	257...	7.8	+	173...	6.0	+
213...	8.30	+	173...	6.08	+	200...	7.6	+	194...	6.0	+
200...	8.28	+	199...	6.07	+	213...	7.6	+	251...	6.0	−
258...	8.26	+	162...	6.02	+	258...	7.6	+	170...	5.9	+
231...	8.25	+	205...	6.02	+	202...	7.4	+	181...	5.9	+
202...	8.19	+	250...	6.00	+	231...	7.4	+	198...	5.9	−
234...	8.15	+	201...	5.98	−	232...	7.4	+	199...	5.9	+
257...	8.13	+	163...	5.98	+	234...	7.4	+	225...	5.9	+
275...	8.06	+	170...	5.93	+	264...	7.4	+	242...	5.9	−
278...	7.99	+	225...	5.91	+	272...	7.4	+	250...	5.9	+
214...	7.98	+	246...	5.90	−	275...	7.4	+	261...	5.9	−
207...	7.94	+	174...	5.85	−	278...	7.4	+	174...	5.8	−
255...	7.79	+	261...	5.81	−	214...	7.3	+	179...	5.8	−
272...	7.72	+	195...	5.80	−	186...	7.2	+	195...	5.8	−
186...	7.72	+	179...	5.76	−	208...	7.2	+	204...	5.8	−
208...	7.60	+	166...	5.71	−	255...	7.2	+	227...	5.8	−
229...	7.60	+	227...	5.66	−	207...	7.1	+	246...	5.8	−
197...	7.57	+	238...	5.54	−	229...	7.1	+	162...	5.7	+
264...	7.52	+	165...	5.54	−	203...	7.0	+	164...	5.7	−
224...	7.52	+	209...	5.53	−	233...	7.0	+	191...	5.7	−
260...	7.48	−	188...	5.52	+	260...	7.0	−	206...	5.7	−
233...	7.47	+	189...	5.52	−	183...	6.9	+	209...	5.7	−
216...	7.40	+	242...	5.51	−	216...	6.9	+	238...	5.7	−
183...	7.37	+	198...	5.49	−	224...	6.9	+	248...	5.7	−
218...	7.28	+	248...	5.47	−	262...	6.9	−	276...	5.7	−
215...	7.28	+	180...	5.47	−	215...	6.7	+	280...	5.7	+
219...	7.25	−	185...	5.44	−	223...	6.7	+	165...	5.6	−
203...	7.25	+	164...	5.41	−	226...	6.7	+	177...	5.6	−
262...	7.23	−	271...	5.41	−	243...	6.7	−	178...	5.6	−
161...	7.21	+	212...	5.39	−	193...	6.6	+	180...	5.6	−
226...	7.13	+	177...	5.37	−	197...	6.6	+	185...	5.6	−
240...	7.03	+	252...	5.37	−	218...	6.6	+	188...	5.6	+
230...	6.98	+	247...	5.37	−	219...	6.6	−	189...	5.6	−
243...	6.96	−	190...	5.34	−	221...	6.6	+	190...	5.6	−
274...	6.96	+	206...	5.32	−	230...	6.6	+	192...	5.6	−
182...	6.90	−	191...	5.32	−	240...	6.6	+	212...	5.6	−
193...	6.84	+	236...	5.30	−	161...	6.5	+	217...	5.6	+
279...	6.81	−	178...	5.30	−	176...	6.5	+	228...	5.6	−
184...	6.77	+	175...	5.29	−	222...	6.5	+	236...	5.6	−
222...	6.69	+	276...	5.27	−	235...	6.5	+	252...	5.6	−
187...	6.69	+	192...	5.22	−	259...	6.5	+	271...	5.6	−
235...	6.66	+	244...	5.22	−	273...	6.5	−	166...	5.5	−
221...	6.66	+	228...	5.20	−	274...	6.5	+	237...	5.5	−
223...	6.63	+	168...	5.17	−	184...	6.4	+	263...	5.5	−
245...	6.54	−	217...	5.15	+	220...	6.4	+	247...	5.5	−
280...	6.51	+	171...	5.15	+	241...	6.4	+	168...	5.4	−
259...	6.49	+	249...	5.12	−	253...	6.4	+	171...	5.4	+
273...	6.42	−	277...	5.10	−	279...	6.4	−	175...	5.4	−
256...	6.35	−	204...	5.10	−	182...	6.2	−	196...	5.4	−
176...	6.35	+	211...	5.10	−	187...	6.2	+	249...	5.4	−
220...	6.34	+	169...	5.10	+	256...	6.2	−	254...	5.4	−
253...	6.25	+	210...	5.05	−	167...	6.1	+	277...	5.4	−
167...	6.25	+	237...	5.02	−	201...	6.1	−	172...	5.3	−
241...	6.18	+	172...	5.02	−	205...	6.1	+	210...	5.3	−
194...	6.15	+	263...	4.93	−	239...	6.1	+	211...	5.3	−
251...	6.10	−	196...	4.90	−	245...	6.1	−	169...	5.2	+
181...	6.10	+	254...	4.68	−	163...	6.0	+	244...	5.1	−

Summary of Tables IX and X

Number of soils examined	114
Number of soils containing Azotobacter	60
Number of soils not containing Azotobacter	54
Average mgm. nitrogen fixed, 76 soils	6.95
Average mgm. nitrogen fixed, 44 soils containing Azotobacter	8.28
Average mgm. nitrogen fixed, 32 soils not containing Azotobacter	5.10
Number of soils electrometric P_H 6.0 or above	63
Number of soils electrometric P_H below 6.0	51
Number of soils colorimetric P_H 6.0 or above	60
Number of soils colorimetric P_H below 6.0	54
Number of soils electrometric P_H 6.0 or above containing Azotobacter	53
Number of soils electrometric P_H 6.0 or above not containing Azotobacter	10
Number of soils electrometric P_H below 6.0 containing Azotobacter	7
Number of soils electrometric P_H below 6.0 not containing Azotobacter	44
Number of soils colorimetric P_H 6.0 or above containing Azotobacter	49
Number of soils colorimetric P_H 6.0 or above not containing Azotobacter	11
Number of soils colorimetric P_H below 6.0 containing Azotobacter	11
Number of soils colorimetric P_H below 6.0 not containing Azotobacter	43
Average electrometric P_H, 114 soils	6.32
Average colorimetric P_H, 114 soils	6.21
Average electrometric P_H, 60 soils containing Azotobacter	6.94
Average electrometric P_H, 54 soils not containing Azotobacter	5.63
Average colorimetric P_H, 60 soils containing Azotobacter	6.62
Average colorimetric P_H, 54 soils not containing Azotobacter	5.77
Association coefficient based on electrometric P_H determinations	0.942
Association coefficient based on colorimetric P_H determinations	0.891

The data here summarized vary but slightly from those for the two former series. There were 10 soils with an electrometric and 11 with a colorimetric P_H of 6.0 or above that failed to show Azotobacter. Of these 11, 8 were among those that remained in the laboratory for several months before being examined. One of the remaining 3 reacted more acid and 1 only slightly less acid than P_H 6.0 by one of the methods.

There were 7 soils that reacted electrometrically and 11 colorimetrically more acid than P_H 6.0, recorded as containing Azotobacter. Six of these reacted less acid than P_H 6.0 and 2 of the remaining less acid than PH 5.9 by one of the methods. Only 1 soil more acid than P_H 5.9 produced a typical Azotobacter growth accompanied by good fixation of nitrogen.

SERIES IV

In Table XI and XII data collected from the soils of Series IV are presented. These soils were collected in a large number of instances from experimental plots where the effect of lime upon acid soils was being studied. Such plots are usually located upon areas of acid soil. From observation such soils do not contain Azotobacter. The data here presented show that many of these limed soils should be, as far as reaction is concerned, favorable to the growth of Azotobacter, yet no Azotobacter were found. In other words, the addition of lime has changed the hydrogen-ion from an unfavorable to a favorable concentration for Azotobacter without apparently influencing the Azotobacter flora. There are many limed soils, however, to which the quantity of lime added apparently has not been sufficient to change appreciably the hydrogen-ion concentration.

TABLE XI.—*Type of growth, nitrogen fixed, and reaction of soils from different States, Series IV*

Soil No.	State.	Limed.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P_H colorimetric.	P_H electrometric.
					Mgm.			
301	Michigan...	o	None.....	No Azotobacter.	4.2	−	5.9	5.76
302	.....do.....	o	Typical Azotobacter.	Typical Azotobacter.	7.1	+	5.6	6.64
303	.....do.....	o	None.....	No Azotobacter.	3.7	−	7.4	7.38
304	.....do.....	o	Typical Azotobacter.	Typical Azotobacter.	11.4	+	6.3	5.73
305	.....do.....	o	None.....	No Azotobacter.	4.8	−	5.9	5.69
306	California..	o	Nontypical...	Typical Azotobacter.	8.1	+	6.1	5.61
307	.....do.....	o	None.....	No Azotobacter.	6.5	−	5.9	5.49
308	.....do.....	o	.....do.....	Typical Azotobacter.[a]	5.6	−	6.3	5.81
309	.....do.....	o	Typical Azotobacter.	.....do.....	10.8	+	7.8	8.14
310	.....do.....	o	None.....	.....do.....	5.4	+	6.3	7.08
311	Ohio.....	+	.....do.....	No Azotobacter.	0.3	−	6.6	7.01
312	.....do.....	+	.....do.....	.....do.....	3.6	−	7.0	7.33
313	.....do.....	+	.....do.....	.....do.....	4.7	−	4.8	5.05
314	.....do.....	o	.....do.....	.....do.....	4.8	−	5.9	6.03
315	.....do.....	o	.....do.....	.....do.....	4.3	−	5.9	6.07
316	North Carolina.	o	.....do.....	.....do.....	4.5	−	4.6	5.00
317	.....do.....	+	.....do.....	.....do.....	6.0	−	6.4	6.78
318	.....do.....	+	.....do.....	.....do.....	5.0	−	5.9	6.01
319	New Jersey.	+	.....do.....	Azotobacter (?)	5.3	−	6.8	6.71
320	.....do.....	o	.....do.....	Azotobacter present.	4.8	−	4.9	4.80
321	.....do.....	+	.....do.....	Azotobacter (?)	6.0	−	6.4	6.52
322	.....do.....	+	.....do.....	Azotobacter present.	4.4	−	7.0	6.93
323	.....do.....	o	.....do.....	No Azotobacter.	4.4	−	4.9	4.90
324	Iowa.....	o	Typical Azotobacter.	Typical Azotobacter.	11.6	+	7.0	7.11
325	.....do.....	o	None.....	No Azotobacter.	4.9	−	4.9	4.78
326	.....do.....	o	.....do.....	.....do.....	4.8	−	4.8	4.83
327	.....do.....	o	Typical Azotobacter.[a]	Typical Azotobacter.[a]	6.5	+	4.9	4.93
328	.....do.....	o	None.....	No Azotobacter.	4.5	−	5.3	5.88
329	New York..	+	Typical Azotobacter.	Typical Azotobacter.	7.5	+	7.2	7.20
330	.....do.....	+	None.....	.....do.[a].....	5.5	+	7.0	6.84
331	.....do.....	+	Nontypical...	.....do.....	6.6	+	6.9	6.79
332	.....do.....	+	None.....	Azotobacter (?)	6.5	−	6.5	6.23
333	.....do.....	o	.....do.....	.....do. (?)....	5.7	−	5.2	5.47
334	Arkansas....	o	.....do.....	.....do. (?)....	7.8	+	7.0	6.96
335	.....do.....	o	.....do.....	.....do. (?)....	5.4	−	4.9	5.05
336	.....do.....	o	.....do.....	.....do. (?)....	5.0	−	5.5	5.58
337	Oregon....	o	.....do.....	No Azotobacter.	2.7	−	5.2	5.27
338	.....do.....	o	.....do.....	.....do.....	3.5	−	5.4	5.39
339	.....do.....	o	.....do.....	.....do.....	3.3	−	5.0	5.24
340	.....do.....	o	.....do.....	.....do.....	3.1	−	5.6	5.93

[a] One sample only.

TABLE XI.—*Type of growth, nitrogen fixed, and reaction of soils from different States, Series IV*—Continued.

Soil No.	State.	Limed.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P_H colorimetric.	P_H electrometric.
					Mgm.			
341	Oregon.....	o	None.....	Azotobacter...	3.5	—	5.5	5.66
342	Alabama...	o	.....do.....	.....do.....	1.0	—	4.6	4.44
343	.....do.....	o	.....do.....	.....do.....	1.6	—	5.9	5.83
344	.....do.....	o	.....do.....	.....do.....	0.3	—	4.8	5.05
345	.....do.....	+	.....do.....	Azotobacter (?)	3.0	—	5.9	5.66
346	.....do.....	+	.....do.....	No Azotobacter.	1.7	—	5.9	5.51
347	Mississippi..	o	Typical Azotobacter.	Typical Azotobacter.	8.0	+	6.2	6.17
348	.....do.....	o	None.....	Azotobacter (?)	2.5	—	4.6	4.21
349	.....do.....	o	Typical Azotobacter.	Typical Azotobacter.	8.3	+	6.6	6.76
350	.....do.....	o	.....do.....	.....do.....	8.6	+	6.2	6.20
351	.....do.....	o	.....do.....	.....do.....	9.5	+	7.4	7.74
352	Illinois.....	+	.....do.....	.....do.....	.....	+	6.3	7.20
353	.....do.....	o	None.....	No Azotobacter.	.....	—	4.8	5.00
354	.....do.....	+	Typical Azotobacter.	Typical Azotobacter.	.....	+	5.8	6.51
355	.....do.....	o	None.....	No Azotobacter.	.....	—	4.6	4.19
356	.....do.....	o	.....do.....	.....do.....	.....	—	4.7	4.70
357	.....do.....	+	.....do.....	.....do.....	.....	—	5.2	5.73
358	Indiana....	o	.....do.....	.....do.....	4.0	—	4.3	3.80
359	.....do.....	+	.....do.....	.....do.....	6.0	—	5.0	4.93
360	Georgia....	+	.....do.....	.....do.....	5.8	—	6.6	6.69
361	.....do.....	+	.....do.....	.....do.....	6.1	—	5.8	6.05
362	.....do.....	o	.....do.....	.....do.....	6.6	—	5.0	5.24
363	.....do.....	o	.....do.....	.....do.....	6.6	—	6.1	5.85
364	.....do.....	o	.....do.....	.....do.....	5.8	—	4.8	4.76
365	Ohio.....	+	.....do.....	.....do.....	6.2	—	6.2	5 88
366	.....do.....	+	Nontypical...	Typical Azotobacter.	6.8	+	6.8	6.84
367	.....do.....	+	None.....	No Azotobacter.	5.1	—	6.2	6.18
368	.....do.....	o	Nontypical...	Typical Azotobacter.	9.0	+	7.1	7.48
369	.....do.....	o	.....do.....	.....do.....	10.4	+	6.7	7.05
370	.....do.....	o	None.....	Azotobacter present.	9.6	+	7.0	7.45
371	New Jersey.	o	.....do.....	No Azotobacter.	5.3	—	4.8	4.70
372	.....do.....	+	.....do.....	.....do.....	5.1	—	5.7	5.37
373	.....do.....	o	.....do.....	.....do.....	4.7	—	5.0	4.22
374	.....do.....	+	.....do.....	.....do.....	5.3	—	6.5	6.44
375	.....do.....	o	.....do.....	.....do.....	5.0	—	4.8	5.12
376	.....do.....	+	.....do.....	.....do.....	5.4	—	6.8	6.86
377	North Carolina.	o	.....do.....	.....do.....	5.2	—	5.0	4.98
378	.....do.....	o	Nontypical[a]	Typical Azotobacter.[a]	5.4	—	4.8	4.90
379	.....do.....	o	None.....	No Azotobacter.	5.4	—	4.4	3.73
380	.....do.....	o	.....do.....	.....do.....	5.2	—	5.0	4.93
381	.....do.....	o	.....do.....	.....do.....	4.4	—	5.4	5.09
382	.....do.....	o	.....do.....	.....do.....	5.8	—	5.0	4.63
383	Massachusetts.	+	Nontypical...	Azotobacter (?)	4.0	—	6.5	6.29
384	.....do.....	+	.....do.....	.....do.(?).....	4.3	—	6.6	6.27

[a] One sample only.

TABLE XI—*Type of growth, nitrogen fixed, and reaction of soils of different States, Series IV*—Continued.

Soil No.	State.	Limed.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P_H colorimetric.	P_H electrometric.
					Mgm.			
385	Massachusetts.	+	None	No Azotobacter.	4.0	−	5.7	5.31
386	do	+	do	do	4.1	−	5.6	5.24
387	do	o	do	do	4.4	−	4.4	4.10
388	do	o	do	do	3.5	−	4.6	4.01
389	Maryland	o	do	do	6.4	−	4.8	4.70
390	do	+	do	do	4.0	−	4.8	4.90
391	do	+	do	do	4.2	−	6.6	6.77
392	do	o	do	do	5.2	−	4.7	4.48
393	do	o	do	do	4.5	−	4.7	4.51
394	do	+	do	do	4.4	−	6.0	6.18
395	Rhode Island.	o	do	do	4.6	−	4.7	4.58
396	do	+	do	do	2.7	−	5.6	5.64
397	do	o	do	do	3.9	−	4.9	5.30
398	do	+	do	do	4.6	−	6.6	6.77
399	do	o	do	do	3.8	−	6.5	5.88
400	do	+	do	do	3.9	−	4.6	4.31
401	Virginia	+	Typical Azotobacter.	Typical Azotobacter.	6.0	+	5.6	6.15
402	do	+	do	do	6.8	+	6.7	7.03
403	do	+	do	do	6.8	+	7.0	7.30
404	do	o	None	No Azotobacter.	4.2	−	5.0	4.70
405	do	o	do	do	5.8	−	4.9	4.75
406	do	o	do	do	1.3	−	5.2	4.83
407	Kentucky	o	do	do	6.6	−	4.9	4.82
408	do	+	do	do	4.8	−	6.2	6.12
409	do	o	do	do	4.2	−	5.8	5.19
410	do	+	do	do	5.2	−	6.8	7.06
411	do	o	do	do	7.0	−	4.8	4.82
412	do	+	do	do	5.4	−	6.7	6.62
413	North Carolina.	o	do	do	3.9	−	6.0	6.28
414	do	+	do	do	4.3	−	5.2	4.97
415	do	o	do	do	3.9	−	4.9	4.81
416	do	+	do	do	2.8	−	5.9	6.07
417	do	o	do	do	3.5	−	4.8	4.66
418	Tennessee	o	Nontypical	Typical Azotobacter.	3.2	+	4.9	4.68
419	do	+	do	do	2.5	+	5.5	5.69
420	do	o	None	Azotobacter (?)	1.0	−	4.8	4.59
421	do	+	Nontypical	Typical Azotobacter.	4.0	+	5.4	5.42
422	do	o	do	do	3.2	+	4.8	4.53
423	do	+	do	do	5.4	+	5.5	5.68
424	West Virginia.	o	None	No Azotobacter.	4.8	−	4.6	4.09
425	do	+	do	do	6.6	−	4.8	4.66
426	do	o	do	do	4.2	−	4.6	4.02
427	do	+	do	do	4.1	−	5.0	4.90
428	do	o	do	do	3.3	−	4.6	3.99
429	do	+	do	do	4.6	−	5.5	5.51
430	Indiana	o	do	do	5.7	−	4.8	4.58
431	do	+	Typical Azotobacter.[a]	Typical Azotobacter.[a]	8.3	+	5.6	5.03
432	New York	+	do	do	6.9	+	6.7	7.30
433	do	+	Nontypical[a]	Azotobacter present.	5.1	−	6.6	6.78

[a] One sample only.

TABLE XI.—*Type of growth, nitrogen fixed, and reaction of soils from different States, Series IV*—Continued.

Soil No.	State.	Limed.	Type of film.	Microscopic picture.	Nitrogen fixed.	Azotobacter.	P_H colorimetric.	P_H electrometric.
					Mgm.			
434	New York..	+	Nontypical..	Typical Azotobacter.	6.1	+	6.2	6.84
435	.....do.....	+	None.....	Azotobacter (?)	7.3	−	5.7	6.23
436	.....do.....	o	.....do.....	No Azotobacter.	4.4	−	5.0	4.98
437	.....do.....	+	Typical Azotobacter.	Typical Azotobacter.	8.3	+	6.3	6.72
438	South Carolina.	o	Nontypical...	.....do.....	6.4	+	5.1	4.98
439	.....do.....	o	Typical Azotobacter.	.....do.....	8.9	+	7.6	8.14
440	.....do.....	+	.....do.....	.....do.....	6.8	+	6.8	7.84
441	.....do.....	o	Nontypical [a]	.....do.....	5.3	−	4.9	4.90
442	.....do.....	+	None.....	No Azotobacter.	4.2	−	4.9	5.15
443	.....do.....	+	.....do.....	.....do.....	4.4	−	5.2	5.34
444	Pennsylvania.	o	Typical Azotobacter.	Typical Azotobacter.	7.6	+	7.0	7.20
445	.....do.....	o	None.....	No Azotobacter.	5.6	−	5.2	5.59
446	.....do.....	o	.....do.....	Azotobacter (?)	6.3	−	4.8	5.07
447	.....do.....	+	Typical Azotobacter.	Typical Azotobacter.	8.0	+	7.4	8.41
448	.....do.....	+	.....do.....	.....do.....	8.1	+	8.2	8.25
449	.....do.....	o	None.....	No Azotobacter.	4.7	−	4.6	4.24
265	Missouri....	o	.....do.....	.....do.....	5.0	−	4.7	4.36
266	.....do.....	o	.....do.....	.....do.....	4.5	−	5.2	4.97
267	.....do.....	+	Typical Azotobacter	Typical Azotobacter.	5.8	+	6.9	7.10
268	.....do.....	o	.....do.....	.....do.....	9.1	+	7.1	7.67
269	.....do.....	o	.....do.....	.....do.....	7.5	+	6.7	7.18

[a] One sample only.

TABLE XII.—*Correlation between reaction and presence of Azotobacter in soils of Series IV*

P_H determined electrometrically.						P_H determined colorimetrically.					
Soil No.	P_H.	Azotobacter.	Soil No.	P_H.	Azotobacter.	Soil No.	P_H.	Azotobacter.	Soil No.	P_H.	Azotobacter.
447...	8.41	+	445...	5.59	−	448...	8.2	+	401...	5.6	+
448...	8.25	+	336...	5.58	−	309...	7.8	+	431...	5.6	+
439...	8.14	+	429...	5.51	−	439...	7.6	+	429...	5.5	−
309...	8.14	+	346...	5.51	−	303...	7.4	−	336...	5.5	−
440...	7.84	+	307...	5.49	−	351...	7.4	+	341...	5.5	−
366...	7.84	+	333...	5.47	−	447...	7.4	+	419...	5.5	+
391...	7.77	−L [a]	421...	5.42	+	329...	7.2	+	423...	5.5	+
351...	7.74	+	338...	5.39	−	368...	7.1	+	338...	5.5	−
268...	7.67	+	372...	5.37	−	268...	7.1	+	381...	5.4	−
368...	7.48	+	443...	5.34	−	312...	7.0	−L	421...	5.4	+
370...	7.45	+	385...	5.31	−	322...	7.0	−L	328...	5.3	−
303...	7.38	−	397...	5.30	−	324...	7.0	+	443...	5.2	−
312...	7.33	−L	337...	5.27	−	330...	7.0	+	445...	5.2	−
403...	7.30	+	386...	5.24	−	334...	7.0	+	226...	5.2	−
432...	7.30	+	339...	5.24	−	370...	7.0	+	333...	5.2	−
352...	7.20	+	362...	5.24	−	403...	7.0	+	337...	5.2	−
444...	7.20	+	409...	5.19	−	444...	7.0	+	357...	5.2	−

[a] L=Limed soil.

TABLE XI.—*Type of growth, nitrogen fixed, and reaction of soils from different States, Series IV*—Continued.

P_H determined electrometrically.						P_H determined colorimetrically.					
Soil No.	P_H.	Azotobacter.	Soil No.	P_H.	Azotobacter.	Soil No.	P_H.	Azotobacter.	Soil No.	P_H.	Azotobacter.
329...	7.20	+	442...	5.15	−	331...	6.9	+	406...	5.2	−
269...	7.18	+	375...	5.12	−	267...	6.9	+	414...	5.2	−
324...	7.11	+	381...	5.09	−	319...	6.8	−L	438...	5.1	+
267...	7.10	+	344...	5.05	−	366...	6.8	+	436...	5.0	−
310...	7.08	+	335...	5.05	−	376...	6.8	−L	339...	5.0	−
410...	7.06	−L	313...	5.05	−	410...	6.8	−L	359...	5.0	−
369...	7.05	+	431...	5.03	+	440...	6.8	+	362...	5.0	−
402...	7.03	+	353...	5.00	−	369...	6.7	+	373...	5.0	−
311...	7.01	−L	316...	5.00	−	402...	6.7	+	377...	5.0	−
334...	6.96	+	438...	4.98	+	432...	6.7	+	380...	5.0	−
322...	6.93	−L	436...	4.98	−	269...	6.7	+	382...	5.0	−
376...	6.86	−L	377...	4.98	−	412...	6.7	−L	404...	5.0	−
434...	6.84	+	414...	4.97	−	311...	6.6	−L	427...	5.0	−
330...	6.84	+	266...	4.97	−	349...	6.6	+	320...	4.9	−
331...	6.79	+	446...	4.97	−	360...	6.6	−L	323...	4.9	−
317...	6.78	−L	327...	4.93	+	384...	6.6	−L	325...	4.9	−
433...	6.78	−L	380...	4.93	−	391...	6.6	−L	327...	4.9	+
398...	6.77	−L	359...	4.93	−	398...	6.6	−L	335...	4.9	−
349...	6.76	+	441...	4.90	−	433...	6.6	−L	397...	4.9	−
437...	6.72	+	427...	4.90	−	332...	6.5	−L	405...	4.9	−
319...	6.71	−L	323...	4.90	−	374...	6.5	−L	407...	4.9	−
360...	6.69	−L	378...	4.90	−	383...	6.5	−L	415...	4.9	−
302...	6.64	+	390...	4.90	−	399...	6.5	−L	418...	4.9	+
412...	6.62	−L	326...	4.83	−	317...	6.4	−L	441...	4.9	−
321...	6.52	−L	406...	4.83	−	321...	6.4	−L	442...	4.9	−
354...	6.51	+	407...	4.82	−	304...	6.3	+	313...	4.8	−
374...	6.44	−L	411...	4.82	−	308...	6.3	−	326...	4.8	−
383...	6.29	−L	415...	4.81	−	310...	6.3	+	344...	4.8	−
413...	6.28	−	320...	4.80	−	352...	6.3	+	353...	4.8	−
384...	6.27	−L	325...	4.78	−	437...	6.3	+	364...	4.8	−
435...	6.23	−L	364...	4.76	−	347...	6.2	+	371...	4.8	−
332...	6.23	−L	405...	4.75	−	330...	6.2	+	375...	4.8	−
350...	6.20	+	404...	4.70	−	365...	6.2	−L	378...	4.8	−
394...	6.18	−L	389...	4.70	−	367...	6.2	−L	446...	4.8	−
367...	6.18	−L	371...	4.70	−	408...	6.2	−L	430...	4.8	−
347...	6.17	+	356...	4.70	−	434...	6.2	+	425...	4.8	−
401...	6.15	+	418...	4.68	+	306...	6.1	+	422...	4.8	+
408...	6.12	−L	425...	4.66	−	363...	6.1	−	420...	4.8	−
416...	6.07	−L	417...	4.66	−	394...	6.0	−L	417...	4.8	−
315...	6.07	−	382...	4.63	−	413...	6.0	−	411...	4.8	−
361...	6.05	−L	420...	4.59	−	301...	5.9	−	390...	4.8	−
314...	6.03	−	395...	4.58	−	305...	5.9	−	389...	4.8	−
318...	6.01	−L	430...	4.58	−	307...	5.9	−	356...	4.7	−
340...	5.93	−	422...	4.53	+	314...	5.9	−	392...	4.7	−
328...	5.88	−	393...	4.51	−	315...	5.9	−	393...	4.7	−
365...	5.88	−	392...	4.48	−	318...	5.9	−	395...	4.7	−
399...	5.88	−	342...	4.44	−	343...	5.9	−	265...	4.7	−
363...	5.85	−	265...	4.36	−	345...	5.9	−	316...	4.6	−
343...	5.83	−	400...	4.31	−	346...	5.9	−	342...	4.6	−
308...	5.81	−	449...	4.24	−	416...	5.9	−	348...	4.6	−
301...	5.76	−	373...	4.22	−	354...	5.8	+	355...	4.6	−
357...	5.73	−	348...	4.21	−	361...	5.8	−	388...	4.6	−
304...	5.73	+	355...	4.19	−	409...	5.8	−	400...	4.6	−
305...	5.69	−	387...	4.10	−	435...	5.7	−	424...	4.6	−
419...	5.69	+	424...	4.09	−	385...	5.7	−	426...	4.6	−
423...	5.68	+	426...	4.02	−	372...	5.7	−	428...	4.6	−
341...	5.66	−	388...	4.01	−	302...	5.6	+	449...	4.6	−
345...	5.66	−	428...	3.99	−	340...	5.6	−	379...	4.4	−
396...	5.64	−	358...	3.80	−	386...	5.6	−	387...	4.4	−
306...	5.61	+	379...	3.73	−	396...	5.6	−	358...	4.3	−

It could not be expected that the mere correcting of the reaction of an acid, Azotobacter-free soil would bring about the establishment of an Azotobacter flora, unless accompanied by natural or artificial inoculation. Artificial inoculation with Azotobacter has been practiced to a very limited extent and it is probable that none of the soils examined have received such treatment. Natural inoculation would probably eventually occur in all cases. The rapidity with which it would take place would depend primarily upon the proximity of soils containing Azotobacter and upon the activity of the agencies operating in the transfer of soil from one to the other. Among these agencies would be wind, drainage, animals, insects, birds, etc., and the more or less artificial transfer upon agricultural tools and machinery. If soils containing Azotobacter are in close proximity, and the means of transfer are active, inoculation may occur very rapidly. For, in an effort to study the effect of inoculation upon adjacent plots, the writer found it impossible to prevent inoculation under certain conditions, while under other conditions such inoculation has not appeared during a period of three years. Some of the limed Azotobacter-free soils here recorded have been receiving lime for many years. As previously mentioned, many of the plots to which lime has been applied are located on rather large areas of strongly acid soils. Such plots may be some distance from areas that contain Azotobacter and, hence, chances for natural inoculation are poor. On the other hand, the writer repeatedly cultured the fertility plots of the Missouri Agricultural Experiment Station for Azotobacter during the years 1912–1914 with negative results. During 1914–15 certain of these plots received an addition of lime. When cultured for Azotobacter again in 1919 a vigorous flora was found to be present. At this last examination the reaction of the soil from a plot that had been limed was found to be neutral (soil No. 267, Table XI). Soil from adjacent unlimed plots was found to be strongly acid (soils Nos. 265 and 266, Table XI). In other words, decreasing the hydrogen-ion concentration of this soil to a point below 1×10^{-6} has resulted in the establishment of a vigorous Azotobacter flora in a comparatively short time. Other soils not far from these plots have been found to contain Azotobacter and it may be assumed that natural inoculation readily took place. It is not uncommon to find adjacent plots or areas of natural soil one of which contains Azotobacter while the other does not.

Summary of Tables XI and XII

Number of soils examined	154
Number of soils containing Azotobacter	42
Number of soils not containing Azotobacter	112
Average mgm. nitrogen fixed, 148 soils	5.30
Average mgm. nitrogen fixed, 40 soils containing Azotobacter	7.35
Average mgm. nitrogen fixed, 108 soils not containing Azotobacter	4.53
Number of soils electrometric P_H 6.0 or above	60
Number of soils electrometric P_H below 6.0	94
Number of soils colorimetric P_H 6.0 or above	57
Number of soils colorimetric P_H below 6.0	97
Number of soils electrometric P_H 6.0 or above containing Azotobacter	32
Number of soils electrometric P_H 6.0 or above not containing Azotobacter	28
Number of limed soils electrometric P_H 6.0 or above not containing Azotobacter	24
Number of normal soils electrometric P_H 6.0 or above not containing Azotobacter	4
Number of soils electrometric P_H below 6.0 containing Azotobacter	10
Number of soils electrometric P_H below 6.0 not containing Azotobacter	84

Number of soils colorimetric P_H 6.0 or above containing Azotobacter	31
Number of soils colorimetric P_H 6.0 or above not containing Azotobacter	26
Number of limed soils colorimetric P_H 6.0 or above not containing Azotobacter	22
Number of normal soils colorimetric P_H 6.0 or above not containing Azotobacter	4
Number of soils colorimetric P_H below 6.0 containing Azotobacter	11
Number of soils colorimetric P_H below 6.0 not containing Azotobacter	86
Average electrometric P_H, 154 soils	5.72
Average colorimetric P_H, 154 soils	5.70
Average electrometric P_H, 42 soils containing Azotobacter	6.70
Average electrometric P_H, 112 soils not containing Azotobacter	5.35
Average colorimetric P_H, 42 soils containing Azotobacter	6.46
Average colorimetric P_H, 112 soils not containing Azotobacter	5.42
Association coefficient based on electrometric P_H determinations	0.971
Association coefficient based on colorimetric P_H determinations	0.968

Liming is an artificial method by which the reaction of soils can suddenly be changed from an unfavorable to a favorable condition for the growth of Azotobacter. Unless accompanied by inoculation with Azotobacter, soils that have thus been rendered favorable and do not contain Azotobacter should not be classed with soils that normally have a favorable reaction and do not contain Azotobacter. For these reasons the limed soils with reactions of P_H 6.0 or above and not containing Azotobacter have been separated from the others and are not considered in the association coefficient caluclations.

In Series IV there were 28 soils tested by the electrometric and 26 by the colorimetric method (32 different soils) with a reaction of P_H 6.0 or above that did not contain Azotobacter. Twenty-four of the former and 22 of the latter are known to have been limed more or less recently. There were, therefore, only 6 unlimed soils with P_H 6.0 or above by one of the methods that did not contain Azotobacter. Five of the 6 reacted more acid than P_H 6.0 by one of the methods, leaving only 1 unlimed soil definitely more acid than P_H 6.0 and not containing Azotobacter. It is probable that lime has been applied to some of the soils of which no record is available. In a few instances the data indicate that some unknown factor has been influencing the reaction. (Compare soils 413 with 414, 438 with 439, 399 with 400, and 363 with 364.) It is believed that the data on history were incorrectly recorded in a few instances. There were 10 soils that reacted electrometrically and 11 colorimetrically (13 different soils) more acid than P_H 6.0 in which no Azotobacter were recorded. Five of these were less acid than P_H 6.0 by one of the methods; not one of the remaining 8 had a typical Azotobacter growth accompanied by good fixation of nitrogen in both samples.

GENERAL SUMMARY AND DISCUSSION

Number of soils examined	418
Number of soils containing Azotobacter	199
Number of soils not containing Azotobacter	219
Average mgm. nitrogen fixed, 367 soils	6.36
Average mgm. nitrogen fixed, 174 soils containing Azotobacter	8.30
Average mgm. nitrogen fixed, 193 soils not containing Azotobacter	4.61
Number of soils electrometric P_H 6.0 or above	207
Number of soils electrometric P_H below 6.0	211
Number of soils colorimetric P_H 6.0 or above	206
Number of soils colorimetric P_H below 6.0	212
Number of soils electrometric P_H 6.0 or above containing Azotobacter	165
Number of soils electrometric P_H 6.0 or above not containing Azotobacter	42
Number of limed soils electrometric P_H 6.0 or above not containing Azotobacter	24

Number of normal soils electrometric P_H 6.0 or above not containing Azotobacter	18
Number of soils electrometric P_H below 6.0 containing Azotobacter	34
Number of soils electrometric P_H below 6.0 not containing Azotobacter	177
Number of soils colorimetric P_H 6.0 or above containing Azotobacter	166
Number of soils colorimetric P_H 6.0 or above not containing Azotobacter	40
Number of limed soils colorimetric P_H 6.0 or above not containing Azotobacter	22
Number of normal soils colorimetric P_H 6.0 or above not containing Azotobacter	18
Number of soils colorimetric P_H below 6.0 containing Azotobacter	33
Number of soils colorimetric P_H below 6.0 not containing Azotobacter	179
Average electrometric P_H, 398 soils	6.09
Average colorimetric P_H, 418 soils	6.11
Average electrometric P_H, 193 soils containing Azotobacter	6.88
Average electrometric P_H, 205 soils not containing Azotobacter	5.44
Average colorimetric P_H, 199 soils containing Azotobacter	6.72
Average colorimetric P_H, 219 soils not containing Azotobacter	5.56
Association coefficient based on electrometric P_H determinations	0.959
Association coefficient based on colorimetric P_H determinations	0.961

In this summary there are a few points to which the writer wishes to call special attention. Little is known of the practical significance of Azotobacter in the nitrogen economy of soils. After carefully reviewing all the available literature and data on the subject Greaves (*8*) says:

> In conclusion, it may be stated that, although the part played by Azotobacter in maintaining the nitrogen of the soil has not been definitely measured, it is nevertheless an important factor. It is, therefore, conservative to state that these organisms under favorable conditions add from 15 to 40 pounds of available nitrogen to each acre of soil yearly.

If these figures are a conservative estimate of the quantity of nitrogen fixed by these organisms, the presence or absence of such a flora in soils is of enormous economic importance. Yet, more than half the soils examined during the past few years apparently do not contain Azotobacter. There are organisms present in practically all soils capable of fixing some nitrogen under laboratory conditions. The quantity of nitrogen fixed under the conditions of these experiments is only approximately half as great in the absence of Azotobacter as when they are present. It is believed that under actual soil conditions the differences are much more marked than these figures indicate. If this important group of organisms is absent from such a large per cent of soils, what are the factors controlling their presence and absence and how can the unfavorable conditions be remedied?

In a former publication (*2*) an apparent close correlation between the absolute reaction of the soil solution and the presence of Azotobacter in soils was shown. The present data demonstrate the existence of such a correlation. By using Yule's association formula the very high coefficient of 0.96 is obtained, indicating an unmistakable correlation. Very few soils in which the hydrogen-ion concentrations exceed 1×10^{-6} contain Azotobacter, while practically 11 soils with a hydrogen-ion concentration less than 1×10^{-6} contain an active Azotobacter flora. The per cent of soils of different hydrogen-ion concentrations containing Azotobacter is found in Table XIV and shown graphically in figure 1.

TABLE XIII.—*Comparison of the presence of Azotobacter, nitrogen fixed, and reaction of same soils collected 2 years apart*

Soil No.		Azotobacter.		Nitrogen fixed.		Colorimetric P_H.		Electrometric P_H.	
1918	1920	1918	1920	1918	1920	1918	1920	1918	1920
				Mgm.	*Mgm.*				
1	101	+	+	10.3	10.7	6.9	6.7	.....	6.71
2	102	−	−	2.0	6.3	5.4	5.0	.....	3.78
3	103	−	−	3.0	5.4	5.6	5.4	.....	5.27
4	104	+	+	6.8	10.5	6.9	7.1	.....	.....
5	105	+	+	5.4	11.8	7.1	7.2	.....	7.08
6	106	−	−	4.4	6.1	5.7	5.7	.....	5.25
15	133	+	+	10.3	13.5	7.4	7.3	.....	7.45
16	136	−	+?	4.0	10.5	5.6	5.4	.....	4.88
17	137	−	−	3.0	4.3	5.5	5.8	.....	5.58
18	138	−	−	3.7	3.0	5.6	5.4	.....	5.05
31	107	+	+	10.6	10.2	7.5	7.4	7.71	7.70
32	108	−	+?	5.6	8.5	5.9	5.6	5.85	5.48
33	109	−	+	4.0	9.5	6.7	6.6	6.56	6.47
34	110	−	+	4.5	7.9	6.8	7.4	6.74	7.61
35	111	+	+	6.8	9.1	7.6	6.6	7.62	6.39
36	113	+	+	9.2	8.9	6.0	5.6	6.12	5.85
37	114	−	+	5.4	8.9	6.2	5.8	6.07	5.59
38	115	−	+	3.9	8.6	5.6	5.4	5.59	5.51
39	116	+	+	8.6	10.0	6.1	6.0	6.05	5.90
40	119	+	+	8.1	9.8	7.0	7.1	6.72	7.00
49	126	−	−	2.7	8.0	5.5	5.5	5.03	5.19
50	127	−	−	4.0	6.6	5.8	5.5	5.32	5.10
51	128	−	−	3.2	5.1	5.3	5.9	5.41	5.78
52	129	+	+	5.5	10.8	7.3	7.6	7.27	7.30
53	130	+	+	9.0	7.3	7.7	7.4	7.79	7.52
54	131	+	−	7.4	7.7	6.0	5.8	6.08	5.47
61	120	−	+	3.7	6.0	5.5	5.8	5.51	5.68
63	124	+	+	11.0	9.0	7.4	6.9	7.43	7.06
64	122	+	+?	8.3	8.6	6.1	5.8	6.35	5.51
65	123	−	+	5.2	8.8	5.7	5.9	5.94	5.64
66	121	−	−	3.8	7.6	5.7	5.7	5.81	5.24
77	134	−	−	4.2	7.2	5.6	5.4	5.34	4.88
79	143	+	+	7.6	7.0	7.7	8.1	8.48	8.77
80	144	+	+	9.2	8.8	7.6	7.3	7.84	7.72
82	142	+	+	8.2	9.7	7.7	7.8	7.60	7.59
83	145	+	+	9.9	7.5	7.6	7.2	7.72	7.94

TABLE XIV.—*The number and percentage of soils of various reactions containing Azotobacter*

P_H reaction.	Electrometric.					Colorimetric.				
	Number of soils.	Azotobacter.				Number of soils.	Azotobacter.			
		Present.		Absent.			Present.		Absent.	
		No.	*Per cent.*	*No.*	*Per cent.*		*No.*	*Per cent.*	*No.*	*Per cent.*
Above 7.50	57	57	100	0	0	32	32	100	0	0
7.00 to 7.49	49	45	92	4	8	60	58	97	2	3
6.50 to 6.99	33	27	82	6	18	45	39	90	6	10
6.00 to 6.49	38	30	80	8	20	47	37	80	10	20
5.50 to 5.99	64	22	34	42	66	122	24	20	98	80
5.00 to 5.49	75	7	9	68	91	43	6	14	37	86
4.50 to 4.99	42	5	12	37	88	44	3	7	41	93
Below 4.50	16	0	0	16	100	3	0	0	3	100
Total	374	193	...	181	...	396	199	...	197	...

The existence in soils of an excess of hydrogen over hydroxyl-ions is no longer questioned. In 75 per cent of the 418 soils tested an excess of hydrogen over hydroxyl-ions was found whether the concentration was measured electrometrically or colorimetrically. A large number of examples are on record showing the toxic effect of a high concentration of hydrogen-ions upon bacteria. The writer has recently shown (*6*) that pure cultures of Azotobacter will not grow in laboratory media with a hydrogen-ion concentration appreciably in excess of 1×10^{-6}.

It is quite generally accepted that soil acidity is indirectly responsible for certain plant root diseases, and it is possible that the influence of the absolute reaction of the soil solution upon Azotobacter here indicated is also indirect. However, since the same identical limiting hydrogen-ion concentration has been found with pure cultures, in laboratory media (*6*) as in soil solutions, it is believed that the recorded absence of this group

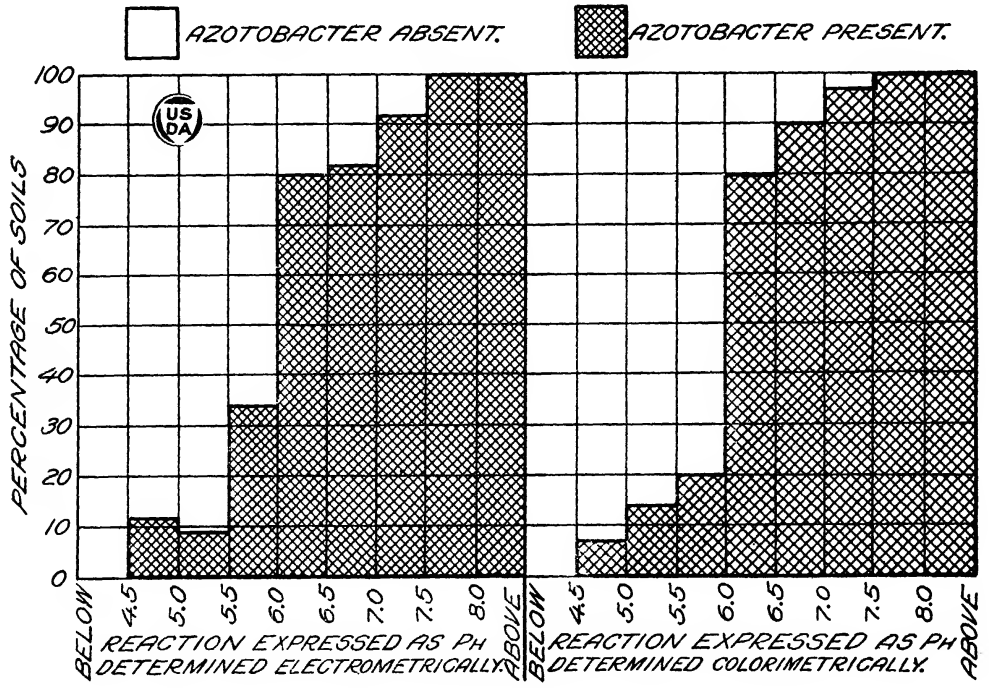

FIG. 1.—Percentage of soils of different reactions containing Azotobacter

of organisms in the soils examined is in most instances correct and is directly due to the toxic effect of the high concentration of hydrogen-ions existing in the soil solution. Furthermore, it is believed that the maximum hydrogen-ion concentration tolerated by this group of organisms is very near 1×10^{-6} in soils as well as in laboratory media. Whether or not the hydrogen-ions are the ultimate limiting agent, the fact has been clearly established that the two are definitely associated. It is also evident from the data accumulated in this laboratory and shown elsewhere (*3*) that the same factors that will raise the hydrogen-ion concentration of a soil solution appreciably above, or lower it below, 1×10^{-6} will also render the soil capable or incapable, as the case may be, of supporting an Azotobacter flora.

How can the unfavorable conditions for the existence and growth of Azotobacter in soils be corrected? Attention has been called to the fact that a reduction in the hydrogen-ion concentration is probably the

one essential condition to be fulfilled, in rendering a soil capable of supporting Azotobacter. This has been accomplished experimentally in a number of different ways. Perhaps the simplest and most economical one is the addition of sufficient lime to reduce the hydrogen-ion concentration to a point below 1×10^{-6}. Attention has also been called (*3*) to the ease with which this can be done in the laboratory. In a forthcoming publication it will be shown that the same is true under natural soil conditions. The most acid soil that has been encountered locally required approximately 18,000 pounds of calcium carbonate to reduce the acidity to below P_H 6.0. Three years ago sufficient lime to accomplish this was added under natural conditions accompanied by inoculation both with soil containing Azotobacter and with cultures grown in the laboratory. These plots still contain a vigorous Azotobacter flora and will fix, in laboratory media, two to three times as much nitrogen as will adjacent, similarly inoculated, unlimed plots from which the Azotobacter disappeared within a few days following inoculation.

It is not the purpose of this paper to advocate the use of lime and Azotobacter inoculation solely as a means of aiding the maintenance of the nitrogen supply of soils. For, while it is believed that this factor alone would justify the expense, the economic phases of the proposition have not been sufficiently investigated.

Experiments are now under way that promise to solve this phase of the problem locally. However, it is generally agreed that permanent agriculture is impossible without a soil sufficiently supplied with lime. Nature has provided ample means for maintaining the nitrogen supply of native soils. The American farmer has so ignored nature's methods that many soils have become depleted of their nitrogen supply and are productive only when supplied artificially with nitrogen at an enormous expense. One of nature's methods is through the agency of different types of nitrogen-fixing bacteria. One essential requirement of these organisms is a low hydrogen-ion concentration in the soil. While the quantity of nitrogen fixed by these organisms might not alone justify the expense of maintaining a low degree of acidity, this factor, coupled with the other recognized physicochemical benefits to the soil and physiological benefits to the growing plants, would unquestionably justify such a practice. The possible influence of adequate liming upon the nitrogen economy of the soil is mentioned as an added stimulus to the use of lime.

Another point to be noted is the general similarity of results, whether based upon the electrometric or the colorimetric method of determining the hydrogen-ion concentration of the soil solution. The individual determinations frequently do not agree as closely as might be desired. Yet the conclusions, so far as this study is concerned, would be identical regardless of data upon which they were based. In a general way the farther removed from neutrality, the wider the variation between the two methods. The electrometric method usually recorded a higher or lower hydrogen-ion concentration, as the case might be, than the colorimetric method if the soil were appreciably acid or alkaline. It is believed, however, that the general agreement between the two methods is sufficiently close to justify the use of the much quicker and less expensive colorimetric method as an aid in the solution of many soil problems, particularly where the initial expense prohibits the purchase of the necessary expensive electrometric apparatus.

The possibility of a biological means of determining the lime requirements of soils is indicated by the data here presented. If a series of groups or of individual microorganisms with definite limiting hydrogen-ion concentrations could be isolated from soils it would only be necessary to determine the presence or absence of certain species in order to establish the absolute reaction of the soil solution in situ. Furthermore, it would be comparatively simple to ascertain how much lime to add in order to render the soil capable of supporting the various species. It has been possible to determine with an accuracy of a few hundred pounds the quantity of lime necessary to add to soils more acid than P_H 6.0 in order to render them capable of supporting Azotobacter. It is believed that not until the plant physiologist has established the optimum and limiting hydrogen-ion concentrations of the soil solution for the various agricultural plants, and the bacteriologist has associated these limits with definite groups of microorganisms, will a true and accurate physiological basis for determining and correcting the reaction of soils be possible. Azotobacter can now be used as a biological means of separating soils into two groups, depending upon reaction; and the quantity of lime necessary to change the reaction of the more acid group into the less acid group can be definitely determined.

CONCLUSIONS

(1) Seventy-five per cent of the 418 soils examined were found to be acid. Fifty per cent were more acid than P_H 6.0 and 50 per cent apparently did not contain Azotobacter.

(2) In general the electrometric and colorimetric methods of determining the hydrogen-ion concentration of the soil solution agreed.

(3) A definite and very close correlation has been established between the absolute reaction of the soil solution and the presence or absence of Azotobacter in the soil.

(4) Very few soils more acid than indicated by a hydrogen-ion concentration of 1×10^{-6} contain Azotobacter, while this group of organisms is in most instances present in soils with a lower hydrogen-ion concentration. Since a very similar limiting hydrogen-ion concentration has been found for pure cultures of Azotobacter in laboratory media, it is believed that the very close correlation existing between the reaction and the presence of Azotobacter indicates that the absolute reaction is of paramount importance, if not the actual limiting factor, in controlling the presence of this group of organisms in soils.

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A STUDY OF FACTORS AFFECTING THE NITROGEN CONTENT OF WHEAT AND OF THE CHANGES THAT OCCUR DURING THE DEVELOPMENT OF WHEAT[1]

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INTRODUCTION

The subject matter of this paper pertains to a study of the nitrogen content of wheat produced under various experimental conditions and a study of changes that occur during the development of wheat. The investigation had its inception in studies of nitrogen content of wheat by Thatcher (*31*, *32*, *33*).[3]

In the States of California, parts of Idaho, Montana, Oregon, Utah, and Washington, wheat, irrespective of variety, generally develops large, plump kernels of comparatively soft texture. The nitrogen or gluten content of these soft wheats is variable, with localized districts apparently more favorable to the production of wheat with high percentages of nitrogen or gluten than is observed in others. Farther east, in the States of North Dakota, South Dakota, Nebraska, Kansas, Minnesota, Missouri, and Iowa the wheat kernels, generally, are of smaller size. These smaller-sized kernels, however, may be plump yet the texture of the grain may be comparatively harder. The percentage of nitrogen or gluten in this harder type of wheat varies but, generally speaking, is comparatively higher than in the softer wheats.

The influence of exchange of seed, breeding new varieties, selections for nitrogen content, soil types, soil treatment, fertilizers, water, and temperature on the percentage of nitrogen in wheat have been studied by various investigators. From the results of investigation, it is held that the variation in composition of wheat is due, chiefly, to variation in environmental conditions, such as rain, sunshine, humidity of the atmosphere, methods of harvesting, methods of cultivations, and amount of manure. The effect of continuous cropping, rotation, the method and time of sowing seed, including the quantity and quality of the seed sown, have also been studied. With the exception of irrigation, the first three of these conditions are wholly uncontrollable; the remainder of them can be regulated, and it is to these that the investigational work has been and should be directed to determine whether or not the nitrogen content of wheat can be increased.

HISTORICAL REVIEW

EFFECT OF CLIMATE ON NITROGEN CONTENT OF WHEAT

Over 60 years ago, Lawes and Gilbert (*7*) concluded from their experiments that long periods of growth produced plump kernels of low nitrogen content. Later these men (*8*) stated that—

> a season favorable for long and continuous growth after heading, produced well developed kernels and larger yields; the mature grain developed under the most favorable conditions contained a high amount of starch and a low amount of potash, phosphoric acid, and nitrogen.

[1] Accepted for publication Oct. 17, 1921.

[2] The author desires to thank Alexander Carlyle, L. F. Seneco, and Sam Bockman for their assistance in looking after the field work.

[3] Reference is made by number (italic) to "Literature used," p. 951–953.

Richardson (*18*) and Brewer (*3*) concurred that climate exerted the greatest influence. According to Wiley (*39, p. 244; 40*) a cool climate and short periods of growth are favorable for high protein, while a warm climate and long periods of growth are favorable for low protein content. Jensen (*6*) expressed the opinion that a "continental climate" was favorable to the formation of a small grain of high nitrogen content. Schindler (*19*) concluded that a warm, moist climate prolonged the kernel development, resulting in a high starch and low protein wheat. Melikov (*13*) gave heavy rainfall as the cause for low nitrogen content in wheat. LeClerc (*9*) deduced that the differences in composition for any one locality from season to season was due to varying weather conditions. The differences in composition of wheat analyzed by him for different localities were pronounced. Williams (*41*) stated that variation in protein was independent of variety and of soil treatment. He ascribed it to seasonal influences. When the wheat was small or shrunken the protein content was high and vice versa. Thatcher, in a study correlating the protein content of wheat with the rainfall in various districts from which samples were taken, observed that the protein content varied inversely with the total rainfall (*35*). According to Deherain (*5*), slow ripening was favorable to a heavy yield of wheat with average gluten content, while hot summer conditions were favorable to rapid ripening and high gluten content.

LeClerc (*9, p. 202*), in making a comparative study of the composition of wheat grown under arid and humid conditions, concluded from his experiments that the nitrogen content is higher in the wheat grown on the arid and semiarid land than it is found in wheat grown on humid and irrigated land. In other words—

an excessive amount of rainfall or irrigation is always accompanied by a crop containing a very low percentage of protein.

IRRIGATION WATER

Investigations pertaining to the influence of water on composition of grain have been carried on over a comparatively long period. The irrigation investigations in charge of the Utah Agricultural Experiment Station are especially valuable, and show how the nitrogen content of the grain is increased through the judicious use of water. The report of a progressive study of the nitrogen content of grain by Widstoe (*37*) in 1921 shows that large applications of water may increase slightly the percentage of protein in wheat.. The percentage of nitrogen in spring wheat decreases with the increased use of water, ranging from 15.26 per cent protein for 20 inches to 26.7 per cent protein for 4.63 inches. With applications of water ranging from 8.89 inches to 40 inches, the variation in nitrogen content is found to be irregular, but the 40-inch application of water shows higher protein than is found in the wheat receiving either 21 or 30 inches of water.

The time of applying the water appears to be very important, since Widstoe and Stewart (*38*) find that the protein content of wheat decreased from 18.05 per cent to 15.98 with the increased use of water after the middle of July. When water is applied before the first of July, the protein content of the wheat is increased. Stewart and Greaves (*28*) concur with LeClerc (*9, p. 202*) that wheat grown on arid nonirrigated land contains more protein than is found in wheat grown on adjoining irrigated land. Prianischnikov (*17*) finds that high per-

centages of water in the soil lowers the nitrogen content of the grain. He also finds that the growing period for the wheat, undoubtedly due to a weaker soil solution and especially the concentration of nitrates which are inadequate for the best development, is shortened with the use of an increased water supply. Von Seelhorst and Krzymowski (*21*) also find that the ripening period may be shortened by keeping the water content of the soil up to 85 per cent of its capillary capacity.

Preul (*16*) studied the influence of variable amounts of water on the composition of wheat grown on soil rich or poor in fertility. He found that the nitrogen content of the wheat was high when the application of water was kept constantly low. A lack of water during the late stages of growth caused a comparatively low nitrogen content in the grain.

Shutt (*24, 25*) states that the wheat grown on newly cleared scrub land compared with wheat grown on fallow or cultivated land is physically softer, "piebald," more starchy, and lower in nitrogen content. This finding, he believed is due to the larger amount of water contained in the newly cleared scrub land, causing a prolongation of the growing period of the plant.

SOIL AND FERTILIZERS

The soil as a factor in influencing the composition of wheat has been studied. Thatcher (*32*), LeClerc (*11*), and Shaw and Walters (*22*) are of the opinion that the composition of soil has very little, if any, influence on the composition of grain. Shaw and Walters state that the soil nitrogen content has little, if any, direct influence upon the nitrogen content of grain and that the factor of climate is sufficient to entirely overshadow the soil factor. In connection with these experiments, the work of other investigators on the influence of fertilizers should not be overlooked. Snyder (*26*) states that—

> increasing the amount of nitrogen in the soil, increases the amount of nitrogen in the grain.

Ames (*1*) shows that, without exception, application of nitrogen fertilizer increased the nitrogen content of the grain. The proportion of phosphorus, potassium, and nitrogen in the wheat plant is increased by the addition of these elements to the soil. Whitson, Wells, and Vivian (*36*) believe that under the same seasonal conditions the most important factor in causing variation in the composition of crops is the amount of nitrates in the soil. On the other hand, Soule and Van Atter (*27*) state that the use of fertilizers on the growing of wheat in a rich soil did not appreciably increase the protein content.

INVESTIGATIONAL WORK

I. FACTORS AFFECTING THE NITROGEN CONTENT OF WHEAT

EFFECT OF WIDTH OF ROWS ON NITROGEN CONTENT OF WHEAT

In the earlier investigational work undertaken at this experiment station on the influence of length of growing season, soil, or climatic condition on the percentage of nitrogen in wheat, methods of preparing the soil and seeding were followed which were not considered a part of the investigation but which undoubtedly played a very important part in the results obtained.

It was found that the percentage of nitrogen in the fall-sown wheats was only slightly lower than that in the spring-grown wheat. These similarities in nitrogen content have indicated that there are some factors, beside the length of the growing season, which have influenced the formation of wheat of high nitrogen content. It was further found that wheat grown in the nursery plots contained more nitrogen than was found in wheat grown in variety-test plots or under practical farming conditions. The wheat grown in the nursery contained 18 per cent more nitrogen than was found in the wheat grown in the variety-test plots, and this nitrogen content was considerably higher than was found in the wheat grown under field conditions. These outstanding differences in the percentages of nitrogen pointed to the influence of the method of preparing the soil on the composition of the wheat. It was observed that the wheat grown in the nursery plots was sown in rows 18 inches apart, while the wheat grown in the variety-test plots was seeded 6 inches apart. Furthermore, the nursery plots received a very thorough cultivation, a practice which was not followed in the variety-test plots. These differences in soil treatment and seeding undoubtedly played a very important part in the development of wheat containing different amounts of nitrogen and also indicated the possible reason for the similarity of the percentages of nitrogen in the fall-sown and spring-sown wheats.

The study of the influence of cultivation on wheat sown in rows different distances apart was, therefore, undertaken. The wheat was sown in rows 6, 9, 12, 15, and 18 inches apart. That sown 6 and 9 inches apart could not be properly cultivated but represented farm methods on well-prepared soil. A sample of the same wheat grown on another plot was also analyzed for the purpose of making a comparison of the nitrogen content of the wheat grown in the nursery with that under field conditions. The results are given in Table I.

TABLE I.—*Percentage of nitrogen in wheat (hybrid 143) sown in rows various distances apart and cultivated*

Treatment.	Fall wheat.		Spring wheat.	
	Nitrogen.	Increase over field.	Nitrogen.	Increase over field.[a]
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Field grown	2.11	.....	.....	.....
Nursery grown:				
6 inches apart, end rows	2.30	9.0	2.50	18.5
6 inches apart, middle rows	2.35	11.4	2.56	21.3
9 inches apart, end rows	2.38	12.8	2.46	16.6
9 inches apart, middle rows	2.32	10.0	2.53	19.9
12 inches apart, end rows	2.54	20.4	2.58	22.2
12 inches apart, middle rows	2.48	17.5	2.68	27.0
15 inches apart, end rows	2.53	19.9	2.55	20.8
15 inches apart, middle rows	2.47	17.1	2.56	24.6
18 inches apart, end rows	2.66	26.1	2.56	21.3
18 inches apart, middle rows	2.66	26.1	2.77	31.2

[a] Calculated using (2.11 per cent) field sample of fall wheat as a basis.

It will be noted that the smallest increase of nursery-grown over field-grown wheat is 9 per cent, while the largest increase is 31.2 per cent. The fall wheat grown in rows 18 inches apart in the nursery shows 15

per cent increase in nitrogen over that grown in rows 6 inches apart. The same comparison with spring wheat shows a 10.8 per cent increase. The amount of nitrogen in the spring wheat containing the lowest percentage of nitrogen is 6.95 per cent greater than the corresponding fall wheat. Comparing the highest percentages in spring and fall wheats, it is found that the amount in the spring wheat is greater by 4.1 per cent.

From the results in Table I it will be noted that the nitrogen content of the wheat was increased by increasing the distance between the rows. This is more noticeable in the fall than in the spring seeding. Omitting the rows of 6 and 9 inch seeding, the percentage of nitrogen in the fall and spring is only slightly in favor of the latter. Owing to the fact that the extremes of variation in nitrogen are not as marked in the spring as in the fall seeding, it is of interest to note that the fall-sown wheat shows a slightly higher nitrogen content for the wheat grown in the rows next to the end than in the middle rows, with the exception of that grown 18 inches apart, where there is no difference observed. The spring wheat shows higher results for nitrogen in the middle rows than it does for those next the end.

EFFECT OF VARYING AMOUNTS OF WATER AND WIDTH OF ROWS ON NITROGEN CONTENT

In a subsequent experiment, a study of the influence of varying amounts of water on the nitrogen content of wheat was made. The field was divided into two series of plots, and two varieties of wheat were grown on them. The rows were sown 6, 9, 12, 15, and 18 inches apart. The amount of water used in the experiment ranged from 8.78 inches rainfall to 12, 16, and 20 inches. The land was cultivated at various times during the growing period in order to remove weeds and maintain a mulch. The average results for nitrogen content of the wheat are recorded in Table II.

TABLE II.—*Average percentage of nitrogen in wheat grown on cultivated plots receiving varying amounts of water*

Variety.	Distance.	Plot I, 8.78 inches of water.[a]	Plot II, 12 inches of water.	Plot III, 16 inches of water.	Plot IV, 20 inches of water.
	Inches.				
Sonora	6	2.37	2.36	2.23	2.22
	9	2.13	2.13	2.15	2.03
	12	2.01	2.21	2.21	2.17
	15	2.26	2.21	2.12	2.30
	18	2.53	2.46	2.31	2.48
Average		2.26	2.27	2.20	2.24
Hybrid 143	6	2.21	2.13	2.29	2.26
	9	2.03	1.88	1.88	2.12
	12	2.03	2.06	2.04	2.16
	15	2.02	2.39	1.89	2.08
	18	2.17	2.42	2.20	2.30
Average		2.09	2.18	2.06	2.18

[a] Total precipitation at Grandview in 1914 was 8.78 inches.

There are some abnormal results which undoubtedly are due to the washing of the plant food from some of the plants toward others, because the lowest results for nitrogen were obtained where the irrigation water entered the plots while the highest results were found in the adjacent rows.

In general, the composition of the wheat grown on plots receiving 20 inches of total water shows approximately as high nitrogen content as was found on any of the other plots. The spreading of the rows 18 inches apart appears to have influenced the nitrogen content of the wheat.

The following year the experiment was repeated with the exception of distributing the irrigation water, especially for the 16 and 20 inch irrigated plots, over a longer period. The purpose of this change was to prevent an excessive dilution and washing of the plant food. The results for nitrogen are recorded in Table III.

TABLE III.—*Average percentage of nitrogen in wheat grown on cultivated plots receiving varying amounts of water*

Variety.	Distance.	Plot I, rainfall only.[a]	Plot II, 12 inches of water.	Plot III, 16 inches of water.	Plot IV, 20 inches of water.
	Inches.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Sonora	6	1.96	2.29	2.41	2.22
	9	1.91	2.05	2.17	2.10
	12	1.97	2.12	2.08	2.20
	15	2.08	2.10	2.15	2.17
	18	2.44	2.21	2.21	2.29
Average		2.07	2.15	2.20	2.20
Hybrid 143	6	1.83	2.16	2.30	2.21
	9	1.81	1.81	1.93	2.10
	12	1.81	1.97	2.00	2.08
	15	2.01	2.20	1.98	2.06
	18	2.21	2.44	2.14	2.24
Average		1.93	2.12	2.08	2.14

[a] Total precipitation at Grandview in 1914 was 8.78 inches.

The wheat sown in rows 18 inches apart has evidently been benefited, since it contains more nitrogen than was found in wheat sown in rows 6 and 12 inches apart.

II. CHANGES THAT OCCUR DURING THE DEVELOPMENT OF WHEAT

DISTRIBUTION OF NITROGEN IN KERNEL AND PLANT

In order to know more definitely what conditions are beneficial to the production of high percentages of nitrogen in the grain it is of considerable importance to know something with regard to the amount of nitrogen in the whole plant as well as different parts of the plant, especially after the development of the kernel has commenced. For this study, plants of nearly equal lengths and weights were selected at 7-day intervals. Nitrogen determinations were made on the stems and leaves between the joints or nodes, above the top node, and on kernel and chaff. The data in Table IV show the percentages of nitrogen in the plant exclusive

of kernels, percentage of nitrogen in kernels, average weight of kernel, total and average weight of nitrogen in kernel. The data in Table V show the grams of nitrogen in the chaff, above the top nodes, below the top node, below the second node from top, and in some cases (when present) below third node, as well as total weight of nitrogen in the plants.

TABLE IV.—*Percentage distribution of nitrogen in plant and kernels, Hegnauer tract*

Date.	Nitrogen in plant, not kernel.	Nitrogen in kernel.	Average weight of kernel.	Total nitrogen in kernels.	Average nitrogen in kernels.
	Per cent.	*Per cent.*	*Gm.*	*Gm.*	*Gm.*
July 1	0.871	2.23	0.00402	0.00709	0.0000885
8	.800	2.12	.00976	.0155	.000207
15	.571	1.98	.01305	.0155	.000258
22	.529	1.87	.0298	.0484	.000557
29	.460	1.51	.0356	.0334	.000528

TABLE V.—*Weight and distribution of nitrogen in wheat plant, Hegnauer tract*

Date.	Chaff.	Above top node.	Below top node.	Below second node.	Below third node.	Total plant.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
July 1	0.0074	0.0196	0.00569	0.00540	0.00239	0.04757
8	.0076	.0102	.0092	.0042	.0029	.0496
15	.00401	.0091	.0056	.0042	.0020	.04041
22	.0048	.0089	.0065	.0022	.....	.0408
29	.0022	.0038	.0012	.0014	.....	.0420

It is a well-established fact that the percentage of nitrogen in the plant proper decreases with the continuous growth of the plant. This is clearly seen from the results given in Table IV, which also shows the nitrogen percentage of the kernel decreasing with the progressive development and increased weights of the kernel when grown under field conditions.

The weight distribution of the nitrogen in the several parts of the plant furnishes us some interesting data regarding the changes that take place as the kernels develop in size. It will be seen from the data given in Table V that the nitrogen (reading down the column) moves from the lowest to uppermost part of the plant, and the nitrogen is evidently the first to be drawn upon for the formation of the kernels. At the time of the filling of the kernel the highest percentage of nitrogen is found in the part of the plant above the top node and in the chaff, and the lowest percentage of nitrogen is found in the part below the lowest node. As the nitrogen enters into the kernel, the quantity in the chaff appears to be depleted first, followed by the final draft from the straw above the top node. Therefore, if the conditions are favorable for normal development, all of the excess nitrogen in the different parts of the plant will be moved upward and transferred into the kernel.

EFFECT OF IRRIGATION WATER ON WEIGHT OF KERNEL AND VARIATION OF NITROGEN DURING GROWTH

It is generally believed that the final filling of the kernel takes place some time immediately after the grain has been cut. This belief has been based almost entirely on theory, and there is nothing in literature of a scientific nature that informs us as to its correctness. The importance of water in assisting the transference of the nitrogen into the grain has been studied, so that we are aware of the influence of water in increasing the weight and nitrogen content of the grain in the early stages of kernel development.

The plan for the first year of the experiment was to study the nitrogen composition of the developing grain at intervals of one week. The land was divided into five different plots as follows: Plot I, receiving rainfall amounting to approximately 8.78 inches; plot II, irrigated sufficiently to make the total rain and irrigation water equal to 15 inches; plot III, given 20 inches of water; plot IV, given 25 inches of water; and plot V, given 30 inches of water. Blocks of entire plants were pulled at each period and stored. Afterwards the grain was threshed out and analyzed. The results for average weight of kernels are recorded in Table VI, and average results for percentage of nitrogen are recorded in Table VII.

TABLE VI.—*Weight of grain grown with various amounts of water*

Date.	Plot I, rainfall only.	Plot II, 15 inches of water.	Plot III, 20 inches of water.	Plot IV, 25 inches of water.	Plot V, 30 inches of water.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
July 1	0.0069	0.0052	0.0061	0.0051	0.0064
8	.0151	.0148	.0144	.0136	.0117
15	.0262	.0263	.0262	.0238	.0230
22	.0322	.0335	.0375	.0347	.0346
29	.0333	.0356	.0407	.0379	.0398

TABLE VII.—*Nitrogen in grain grown with various amounts of water*

Date.	Plot I, rainfall only.	Plot II, 15 inches of water.	Plot III, 20 inches of water.	Plot IV, 25 inches of water.	Plot V, 30 inches of water.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
July 1	3.00	3.21	3.04	2.95	2.63
8	2.53	2.38	2.21	1.95	2.60
15	2.42	2.29	1.82	1.92	1.97
22	2.56	2.31	2.07	1.97	2.04
29	2.73	2.20	2.14	1.92	2.02

The highest average weight of the kernels for the first two weeks of development was obtained in the nonirrigated plot I. At the end of the third week the weight of the kernels were equally as high in plots II and III as in plot I. For the fourth week period and at maturity, the weight of the kernels in plots II, III, IV, and V exceeded those of plot I.

With reference to nitrogen content for the first week, plots I, II, and IV have about the same values. Wheat from plot II tested the highest and wheat from plot V analyzed the lowest for nitrogen content. In the

second week the results for nitrogen were in favor of wheat grown on plot II with plot I wheat showing nearly as much nitrogen. At the third, fourth, and maturity periods the highest nitrogen content was found in plot I wheat. Wheat from plots II, III, and IV did not change appreciably after the drop in nitrogen from the first to the second week. With the exception of the wheat grown on nonirrigated land, it should be noted that the varying amounts of water applied had not appreciably affected the nitrogen content of the mature grain.

A subsequent trial was made with two varieties of wheat for the purpose of studying the influence of varying amounts of water when distributed over a longer period of growth than in the previous experiment. The average weights of kernels in the Sonora and Hybrid 143 wheats are recorded in Table VIII.

TABLE VIII.—*Weight of kernels as influenced by applying varying amounts of water over a wide period and then cultivating after water application*

Variety.	Distance.	Plot I, rainfall only.	Plot II, 12 inches of water.	Plot III, 16 inches of water.	Plot IV, 20 inches of water.
	Inches.	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Sonora	6	0.0506	0.0503	0.0503	0.0506
	9	.0499	.0516	.0507	.0520
	12	.0507	.0515	.0507	.0517
	15	.0505	.0514	.0520	.0505
	18	.0515	.0524	.0523	.0503
Hybrid 143	6	.0332	.0331	.0344	.0338
	9	.0329	.0326	.0335	.0327
	12	.0332	.0334	.0336	.0344
	15	.0327	.0330	.0349	.0346
	18	.0343	.0346	.0350	.0350

Apparently, the variation in amount of water used or the distance of the rows has not had any effect on the Sonora wheat, since the weights of the kernels are fairly uniform, regardless of conditions of the experiments. Hybrid No. 142 wheat increased slightly in weight in the 15 and 18 inch rows, where 20 inches of water were applied.

EFFECT OF WATER ON TRANSLOCATION OF NITROGEN

For the purpose of learning whether or not the placing of the wheat plant in water would affect the nitrogen content of the wheat, uniform wheat plants were selected and brought to the laboratory. One-half of the wheat heads and the grain were removed from three-fourths of the samples, and each plant and the grain removed were given corresponding numbers. The heads in the other one-fourth of the samples were not touched. The plants were divided into two lots, one-half of which were placed upright in beakers of water and the other one-half were left standing near but were not placed in water. Following the termination of the experiment, determinations of the weight and the nitrogen content of the grain in both samples were made. The results are recorded in Table IX.

TABLE IX.—*Influence of water in moving nitrogenous and nonnitrogenous material from the stems into the wheat kernel*

	Average weight per kernel.	Average percentage of nitrogen.	Average weight of nitrogen per kernel.
	Gm.		*Gm.*
Out of water:			
Whole heads	0.0071	2.62	0.000186
Half heads	.0077	2.38	.000183
In water:			
Whole heads	.012	3.24	.000389
Half heads	.015	3.23	.000487

The average weight of the kernels at the beginning of the experiment was between 0.0071 and 0.0077 gm. and the nitrogen content varied from 2.38 to 2.62 per cent. At the close of the experiment, whereas the percentages of nitrogen were higher in the whole heads than in the kernels of the half heads, the total weight of nitrogen in both instances was practically the same. The weights of the kernels and percentage of nitrogen in the kernels of the plants which were placed in water had increased. The increase in the weight of kernels and the weight of nitrogen in the one-half of the kernels left on the head was found to be proportionally greater than was obtained for the average of all the kernels left intact or, as it is termed in Table IX, the whole heads. In other words, the increased amount of material in kernels left on one-half the head if distributed to double the number of kernels would have given results similar to those found in all the kernels left intact on the head. Translocation of plant food material has been brought about, and, because of its more highly nitrogeneous character, it is believed that larger quantities of water are required to move the nitrogenous matter than are required to move the nonnitrogenous matter into the kernels. Accordingly, water should prove beneficial to high rather than low nitrogen content. The protein of wheat depends first upon the supply of available nitrogen (*4, v. 10; 5*) for the plant, and second upon movement of the nitrogenous material into the grain.

RELATION OF PERCENTAGE OF MOISTURE IN THE KERNELS TO FILLING

In addition to investigational work mentioned above, the weight of the grain, moisture, starch content, etc., were determined at the various stages of the development of the kernels. The samples were collected every other day, and observations for maximum and minimum temperature and relative humidity were recorded. The results for weight and moisture content of the kernels have been plotted in figure 1.

Apparently no filling occurs in grain having a moisture content of 40 per cent or less. The vertical parts of the curves represent measurements made during periods of desiccation.

The figures for maximum and minimum temperatures and relative humidity have not been submitted because it was impossible to show conclusive relationship between these figures and those for changes of weight of kernels during the period of development. There were periods in the development, however, where the cooler nights were favorable for greater increases in weight than was found to be the case where the warmer nights prevailed. Considering the changes in weight

of the kernel, it should be noted that there were times when very little increase in weight was obtained while at other periods considerable gain was observed. On the whole, it should be noted that the kernels of wheat kept on increasing in weight up to the period when the percentage of moisture dropped to approximately 40. This period is sometimes spoken of as the period of desiccation. The slight fluctuations on the downward curve are probably due to variations occurring in method of sampling

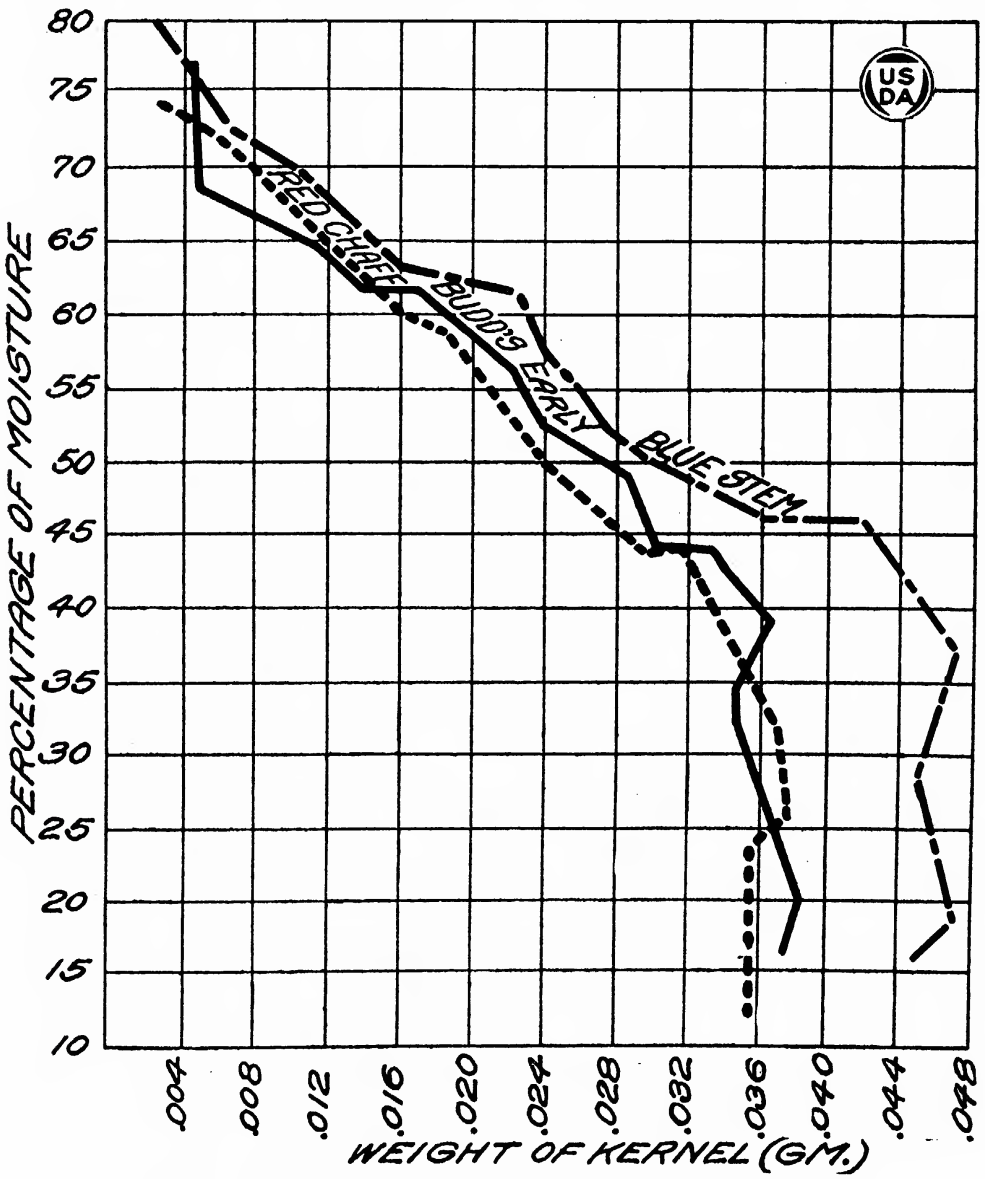

FIG. 1.—Relative moisture content of wheat kernels during growth

which at this period would be more noticeable, because the kernels were heavier, even though the errors were probably not any greater than those during the preceding periods of sampling. Any increase in nitrogen content must precede a period when the kernels contain 40 per cent or more moisture. In the period of embryo development there is considerable moisture in the grain. We also know that in the period of 60 per cent or more of moisture, the simpler forms of nitrogen material are

moving into the kernels at a very rapid rate (*15*) and during the period of decline in moisture content of the grain synthesize into the material known as gluten.

It is contended by Brenchley and Hall (*2*) that in the filling of the endosperm part of the kernel the material is uniform and always possesses the same ratio of nitrogenous to nonnitrogenous material and ash. Thatcher (*33*, *34*,) found that the ratio of protein to carbohydrates decreased with the progress of the development of the kernel. Regardless of this difference in the findings, we should not ignore the possibility that changes in composition might be brought about by abnormal conditions.

RELATION OF NITROGEN TO PHOSPHORUS

In the investigations by Brenchley and Hall (*2*) it has been shown that the ash and the phosphoric acid enter the grain simultaneously

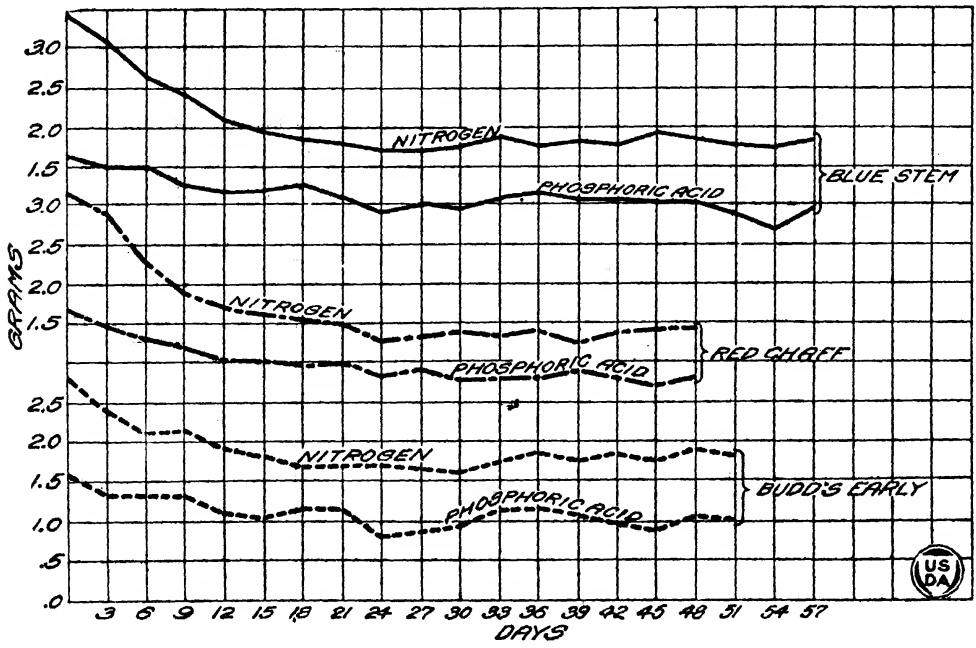

FIG. 2.—Relative time of entrance of phosphoric acid and nitrogen into three varieties of wheat

with the nitrogen. These results have been confirmed, and the graphs for nitrogen and phosphoric acid are shown in figure 2.

It is evident that not only nitrogen but phosphoric acid as well must be available in sufficient quantities in the soil if high-nitrogen wheat is to be obtained. It should be further noted that the amount of nitrogen entering into the kernels at the earliest stages of development is proportionally larger than is the case in subsequent stages of development. The embryo is developed and perfected in this early stage and undoubtedly the larger amount of nitrogen required at this period is for the construction of the embryo. Following the development of the embryo we observe that the phosphoric acid runs parallel with the nitrogen content of the kernel.

SUMMARY

(1) Increasing the distance between the rows seemed to increase the nitrogen content of wheat grown in the nursery under nonirrigated conditions at Pullman.

(2) Hybrid 143 showed a higher percentage of nitrogen when spring-sown than when fall-sown.

(3) Increasing the distance between the rows did not seem to affect the nitrogen content of wheat under irrigated conditions at Grandview.

(4) The percentage of nitrogen in the kernel decreased as the grain matured.

(5) The nitrogen in the plant moved toward the kernel as the grain approached maturity.

(6) Irrigation did not affect the nitrogen content of wheat.

(7) Placing the wheat plant in water moved nitrogen into the kernel.

(8) Wheat kernels showed little increase in weight after the moisture declined to 40 per cent.

(9) Phosphorus entered the grain simultaneously with nitrogen except in the early stages of development.

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RELATIVE SUSCEPTIBILITY OF CITRUS FRUITS AND HYBRIDS TO CLADOSPORIUM CITRI MASSEE [1]

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INTRODUCTION

During the course of citrus-canker investigations by the authors in Alabama, only one other Citrus disease has appeared naturally with any frequency. This disease is citrus scab, caused by *Cladosporium citri* Massee (*5*).[2]

So far as the writers are aware, no one has ever attempted to make any extensive observations to determine the relative susceptibility of Citrus plants to scab. All reports so far published on this subject have been based on occasional field observations of commercial varieties. A summary of the scattered literature reveals the fact that, with a few exceptions, the Citrus plants mentioned as subject to scab by all writers can be arranged into three groups, according to their susceptibility, as follows:

1. Severely attacked.
   - Sour orange.
   - Rough lemon.
   - Lemon.
2. Moderately attacked.
   - Satsuma and other mandarin oranges.
   - Lime (*8, p. 82*).
   - Grapefruit.
   - Trifoliate orange (*2, p. 244*).
3. Rarely attacked.
   - Some few varieties of sweet orange (*1; 7; 6, p. 115*).

Observations on the amount of scab were made by the authors at times during the growing season over a period of four years on a representative lot of Citrus plants growing under approximately the same conditions in the canker isolation field near Loxley, Ala. They were made in connection with the authors' citrus-canker work, accounts of which have appeared (*3, 4*). In these publications, the type, age, and number of plants grown are given in some detail. Owing to the fact that only few of the plants were large enough to set and bear fruit, the degree of susceptibility to scab of the various plants is based on the amount of scab present on the leaves and angular wood.

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[2] Reference is made by number (italic) to "Literature cited," p. 959.

RELATIVE SUSCEPTIBILITY TO SCAB

With the exception of *Poncirus trifoliata* (L.) Raf., all the wild relatives of Citrus so far grown in the isolation field are nonsusceptible to scab. With this exception, scab appears to be strictly limited to Citrus fruits and their hybrids. In this respect it differs from citrus-canker, which can attack a large number of Rutaceous plants.

In Table I an attempt has been made to list the majority of Citrus fruits and their hybrids grown in the isolation field, and to indicate the average relative susceptibility of these plants to scab, as has been observed at times over a period of four years under various conditions. The relative susceptibility of the various species and varieties to scab as given in the table is tentative. Many plants are quite susceptible to scab in the early spring when the first growth pushes out, while no scab appears on the foliage of the second or succeeding growths. Some plants, on the other hand, present a more or less scabby appearance during the whole season. Whether the plants placed under the first heading are nonsusceptible in a strict sense, only direct inoculations will show.

TABLE 1.—*Relative susceptibility of Citrus fruits and hybrids to scab*

Genus and species.	Scab not observed.	Slightly susceptible.	Susceptible.	Very susceptible.
Poncirus trifoliata, trifoliate orange		×	×	
Citrus hystrix, round leaf form		×		
C. hystrix, pointed leaf form				×
Citrus sp., Ichang lemon		×		
C. grandis, grapefruit seedlings		×		
Duncan		×		
Sullivan		×		
Shaddock, Fla.				×
Pummelo, Hirado Buntan	×			
Chinese, Orangedale				×
Marks				×
Indian, Roeding			×	
Siamese		×		
C. sinensis, orange, navel	×			
Temple	×			
Japanese No. 1	×			
Japanese No. 2	×			
Sekkan	×			
Pankan	×			
Tankan	×			
C. nobilis, orange, King		×		
C. nobilis var. *deliciosa*, tangerine, Cleopatra		×		
C. nobilis var. *unshiu*, Satsuma			×	
C. mitis. Calamondin				×
Citrus sp. Kanzu				×
Citrus sp. Natsu-mikan	×			
Citrus sp. orange, Naranja				×
Citrus sp. orange, Narute				×
Citrange (*P. trifoliata* × *C. sinenses*)		×	×	
Citrandarin (*P. trifoliata* × *C. nobilis*)		×	×	
Citrunshu (*P. trifoliata* × *C. nobilis* var. *unshiu*)				×
Citrangequat (citrange × *Fortunella margarita*)				×
Citranguma (citrange × *C. nobilis* var. *unshiu*)			×	
Citrangarin (citrange × *C. nobilis* var. *deliciosa*)		×		
Citrangedin (citrange × *C. mitis*)		×	×	
Limequat (*C. aurantifolia* × *F. margarita*)	×			
Bigaraldin (*C. aurantium* × *C. mitis*)	×			
Orangelo (*C. sinensis* × *C. grandis*)	×			
Oranguma (*C. sinensis* × *C. nobilis* var. *unshiu*)	×			
Orangequat (*C. sinensis* × *F. margarita*)		×	×	
Satsumelo (*C. nobilis* var. *unshiu* × *C. grandis*)		×	×	
Clemelo (*C. nobilis* var. *deliciosa* × *C. grandis*)		×		
Siamelo (*C. nobilis* × *C. grandis*)	×			
Tangelo (*C. nobilis* var. *deliciosa* × *C. grandis*)		×		
Siamor (*C. nobilis* × *C. sinensis*)	×			
Sopomaldin (*C. grandis* × *C. mitis*)	×			
Calashu (*C. mitis* × *C. nobilis* var. *unshiu*)		×		
Citraldin (*P. trifoliata* × *C. mitis*)	×			
Calarin (*C. mitis* × *C. nobilis* var. *deliciosa*)	×			
False hybrids		×	×	

Poncirus trifoliata varies from slightly susceptible to susceptible, depending on the season. As a rule, very little scab occurs after primary infection takes place on the unfolding buds and the young shoots in the spring.

The round leaf form of *Citrus hystrix* DC. which is susceptible to canker is only occasionally attacked by scab, while the pointed leaf form which is semi-resistant to canker is very susceptible to scab. Primary infection is usually severe on young leaves and shoots, although the plants present a scabby appearance throughout the growing season.

The fact that *Citrus hystrix*, native of the Philippine Islands, where no scab has ever been found, is susceptible under our Gulf Coast conditions, leads one to assume that *Cladosporium citri* is unable to persist under Philippine conditions, which is one reason why scab has never been reported from the islands, rather than the fact that scab has never been introduced.

The Ichang lemon, possibly a natural hybrid between a lemon and pummelo, appears to be more susceptible to scab than the sour and commercial varieties of lemons.

The behavior of the plants belonging to the *Citrus grandis* group in their susceptibility to scab is quite variable, ranging from nonsusceptible to very susceptible. Whether there is a difference in the varietal resistance is not known. The fact that plants of all ages, conditions, and types were employed makes it doubly hard to draw any conclusions. Judging from observations of other investigators, the grapefruit has only within recent years been reported susceptible to scab. On the whole, it can be said that the ordinary Florida varieties, as represented by Duncan and Sullivan, are only slightly susceptible; the Florida Shaddock, very susceptible; the Indian and Chinese pummelos, susceptible; and the Siamese pummelos, slightly susceptible. The Hirado Buntan pummelo has never developed scab in the field. While there is a decided difference in scab susceptibility of the various plants in the *C. grandis* group, we do not know whether the differences are varietal or due to their individual reaction to environmental conditions which in turn influences scab susceptibility.

No scab has ever been noted on any of the plants belonging to the *Citrus sinensis* group. These observations are in common with those made by most investigators. Stevens (7) reports the Lue Gim Gong as attacked by scab in Florida, and it is the only variety of sweet orange so recorded in America. Earle (1) in Porto Rico finds that some of the round oranges are occasionally attacked by scab. Reinking (6) also reports a plant of the *C. sinensis* group subject to scab in China. On the whole, it can be stated that the sweet orange group, with an occasional exception, is nonsusceptible to scab.

Of the *C. nobilis* group, both the King orange and Satsuma are slightly susceptible to susceptible, depending on weather conditions. Most of the scab occurs during the early spring on the young leaves and small fruits. During late seasons, such as prevailed in 1915 and 1920, Satsumas are badly attacked by scab, resulting in considerable damage to the fruit. The Cleopatra tangerine, which is semiresistant to canker, evidently does not scab. It is one of the few plants promising as canker resistant which is not attacked by scab.

Citrus mitis Blancho is another example of a plant native to the Philippine Islands which is very susceptible to scab under our Gulf Coast conditions. Scab is usually quite severe in the early spring on the

unfolding leaves and twigs. These plants present a more or less scabby appearance throughout the season.

Yuzu or Kansu orange is a plant native of North China; in fact, it grows farther north than any other Citrus. Like *C. mitis*, it is quite resistant to canker, but very susceptible to scab. It is rather peculiar that there are here two plants, one native to the tropics, the other to North China, both somewhat resistant to canker, but susceptible to scab under Gulf Coast conditions. Scab is found on the Kansu plants throughout the growing season.

The *Natsu-Mikan*, possibly a natural hybrid similar to the tangeloes, has so far remained free from scab. The Naranja and Narute oranges, introductions from the Far East, are among the most susceptible plants in the field. These plants present an extremely scabby appearance throughout the growing season.

As a rule, all trifoliate orange hybrids are attacked to some extent in the early spring, varying in intensity, and depending somewhat on the second parent. Thus, citrunshu, with Satsuma as a second parent, is more susceptible to scab than citrange with the common orange as the second parent.

The citrange hybrids vary still more in their susceptibility to scab, ranging from slightly susceptible to very susceptible. The behavior of the citrangequat is extremely interesting. Kumquat, the second parent of this cross belonging to the wild relatives, is outside the range of scab susceptibility. However, when crossed with the citrange, the resulting hybrid is very susceptible to scab, and the plants are generaly scabby throughout the growing season in the field. On the other hand, it is the most promising canker-resistant hybrid that has yet been found.

Limequat has so far remained free from scab. The orangelo and oranguma have also remained clean. The orangequat is a plant similar in many respects to the Satsuma. Thus, in the type of plant and its susceptibility to canker and scab, it behaves like the Satsuma. However, why should it equal the Satsuma in its susceptibility to scab when both of its parents have been so far reported free from scab? Its susceptibility to scab must be closely related to the reaction of this plant to environmental conditions favoring scab attacks.

The bigaraldin presents the opposite extreme in that no scab has been observed on this hybrid, although both its parents, the sour orange and calomondin, are very susceptible to scab.

The mandarin orange-grapefruit hybrids as represented by satsumelo, clemelo, and tangelo vary from slightly susceptible to susceptible. No scab has been observed on siamelo. The hybrids having *Citrus mitis* as one parent either have remained free from scab or are only slightly susceptible, notwithstanding the fact that *C. mitis* is very susceptible to scab.

All the false hybrids vary from slightly susceptible to susceptible, depending on the condition of the plants.

SUMMARY

The range of Citrus plants susceptible to scab has been extended much beyond the few well-known groups of commercial varieties, heretofore reported. No doubt some of the plants reported as nonsusceptible may later be found to be susceptible, while many species and varieties which were not tested will also be found to be susceptible to scab.

All evidences from our observations show that scab is limited to the Citrus fruits and their hybrids, with the exception of *Poncirus trifoliata.* The species and varieties within a group vary in their susceptibility to scab. A good example of this occurs in the *Citrus grandis* or grapefruit group, and is further accentuated among the hybrids. Susceptibility to scab varies not only from season to season, depending on weather conditions, but varies even within the same season. Thus a bad scab year is determined largely by weather conditions just at the time of the formation of the leaves and fruit. In most cases, scab susceptibility narrows down to the reaction of the host plant to environmental conditions, essential to scab infection and the development of the disease. This does not necessarily mean conditions favorable to the best and most normal development of the plants, as under these conditions few of the plants are susceptible to scab. It is extremely interesting to note that in this connection a number of plants which are promising as canker resistant are as a whole susceptible to scab.

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AN IMPROVED METHOD FOR THE DETERMINATION OF NICOTINE IN TOBACCO AND TOBACCO EXTRACTS [1]

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HISTORICAL REVIEW

The two methods which are now more generally used in this country for the estimation of nicotine in tobacco and tobacco extracts are those of Kissling and the silicotungstic acid method as perfected by Chapin.[3] They are the only ones for this determination which have been adopted as official by the Association of Official Agricultural Chemists.[4] It is of interest to note in this connection that while Chapin's procedure for extracts was adopted by the association, a material change appears in the association method. This change is in the amount and manner of adding the alkali preparatory to the distillation; it is opportune, even if made unwittingly, because it tends to increase the accuracy of the method when applied to tobacco. In this connection it is important that a sufficient quantity of alkali be added in the distillation to liberate combined nicotine. The association method should emphasize this point more clearly, since it is possible in following their directions in the distillation to have the solution test alkaline due to free nicotine and yet an insufficient quantity of alkali may have been added to liberate that in combination.

Chapin describes the various procedures which have been proposed for the estimation of nicotine and the defects he found in their application, so it is not necessary to mention them here. Unfortunately, however, Chapin, in his work on the silicotungstic acid method, did not include tobacco in his studies, otherwise he probably would have found that errors may often occur in the use of this method on tobacco, as will be mentioned later. The method as outlined by him was proposed for tobacco extracts and when so applied in the present work has been found to be more satisfactory than for tobacco itself.

While it is generally recognized that the Kissling method will give good results in the hands of an experienced analyst, provided no interfering substances are present in the determination, it is nevertheless, conceded that the silicotungstic acid method will probably give more reliable and concordant results in ordinary use.

After the work reported in this paper was practically completed, the writer learned for the first time, through a mutual acquaintance, that a similar method had been published by Rasmussen,[5] in Denmark. As no reference to it could be found in the literature at hand, a copy was obtained

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[3] CHAPIN, R. M. THE DETERMINATION OF NICOTINE IN NICOTINE SOLUTIONS AND TOBACCO EXTRACTS. *In* U. S. Dept. Agr. Bur. Anim. Indus. Bul. 133, p. 21. 1911.

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[5] RASMUSSEN, HANS BAGGESGAARD. OM BESTEMMELSE AF NIKOTIN. *In* K. Danske Vidensk. Selsk. Skr. Naturvidensk. og Math. Afd., Ser. 8, Bd. I, p. 66. 1916.

and it was found that while Rasmussen's method is somewhat similar in principle, it is entirely different in technic from the one described in this paper. As no determinations by the former method have been made here, its accuracy is not known. Certain features of it, however, are mentioned for comparison with the writer's procedure.

The extraction of nicotine from the tobacco to which alcoholic soda has been previously added is carried on in a flask containing a mixture of equal parts of ethyl ether and petroleum ether, by frequent shaking for 5 hours. The solvent is then filtered from the tobacco through a folded filter covered with a glass plate. An aliquot is taken for acid extraction. With extracts, 3 or 4 gm. of the sample are placed in a 100 cc. flask together with 5 cc. concentrated caustic soda, 5 cc. water, and 50 cc. of the above mixed ethers. The mixture is allowed to stand 4 or 5 hours with frequent shaking, then 25 cc. of the solvent is drawn off and treated as before. In both cases the nicotine is precipitated with silicotungstic acid. Doubts might be raised as to the complete extraction of the nicotine, especially from extracts handled in the manner indicated, as compared with a Soxhlet extraction of the sample as prepared for analysis here. Furthermore, errors may arise due to evaporation when the solvents are filtered and aliquots withdrawn in the manner described. Finally, no provision is made for the prevention of emulsions often obtained in the acid washing of the ether extract of some samples and without which no method of this character is practical.

EXPERIMENTAL WORK

The writer, having occasion to determine the nicotine content of a large number of various grades and varieties of tobacco grown in Kentucky, found that it would be very convenient to have a method which would be more rapid than either of the official methods and equally accurate. As several determinations were to be made at the same time and facilities for making simultaneous steam distillations were not at hand, it was desirable to have a method that would, if possible, eliminate such distillations. A steam distillation of nicotine is objectionable inasmuch as it is often a long, tedious procedure that requires careful attention in order that the boiling liquid shall be maintained at a low volume to remove all nicotine.

The writer's previous experience with the Kissling method has shown that there are two principal errors to be avoided: First, the presence of ammonia in the nicotine distillate, due to its incomplete separation from the ether extract of the sample, and, second, the possible loss of nicotine in the evaporation of this ether extract previous to its distillation. The first error will, of course, give high results, due to the ammonia being titrated and calculated as nicotine, whereas the other causes low results; consequently, satisfactory figures obtained by the method may be sometimes the result of a balancing of errors. On the other hand, experience with the silicotungstic acid method in this work has shown that while it is decidedly preferable to the Kissling, nevertheless certain points in its use have to be carefully supervised in order to obtain satisfactory results. The writer's experiments show that this method as outlined by Chapin gives more satisfactory results on nicotine extracts than on tobacco. The reasons for this will be mentioned later.

Experience here has shown that the distillation as prescribed in the silicotungstic acid method, even though carried on under proper condi-

tions, is sometimes a long, tedious procedure; and, if not so conducted, will often give inaccurate results on tobacco. Accordingly, it was thought possible to substitute an ether extraction of the sample for the steam distillation of it in the silicotungstic acid method, in order to obtain the nicotine for precipitation. Afterwards, the nicotine could be extracted from the ether by acid and determined in the manner prescribed in this method. Accordingly, considerable work has been carried on with this in view, and, after many preliminary determinations, a method has been devised which has usually been found to be more rapid than either of the official methods and, with some samples at least, gives more accurate results than the silicotungstic acid method unless the latter is carried on under carefully controlled conditions. It combines the good features of both official methods and eliminates an undesirable one common to both, namely, the steam distillation. The method is as follows:

REAGENTS

Alcoholic sodium hydroxid solution: Dissolve 6 gm. of sodium hydroxid in 40 cc. of water and 60 cc. of 90 per cent alcohol.

Silicotungstic acid solution: Prepare a 12 per cent solution of the silicotungstic acid having the formula $4H_2O$, SiO_2, $12WO_3$, $22H_2O$. (There are several silicotungstic acids. The acids $4H_2O$, SiO_2, $10WO_3$, $3H_2O$ and $4H_2O$, SiO_2, $12WO_3$, $20H_2O$, do not give crystalline precipitates with nicotine and should not be used.)

Ethyl ether of quality equal to U. S. P. concentrated.

Dilute hydrochloric acids (1 to 4) and (1 to 1,000).

DETERMINATION

Weigh 1 gm. of tobacco extract (high grade 40 per cent) or more of extracts containing less nicotine; or 5 gm. of finely powdered tobacco which has been previously dried at room temperature or slightly above, to permit powdering, and put into a beaker or, preferably, a porcelain dish. Add 2.5 cc. of the alcoholic sodium hydroxid to the tobacco powder or about 5 cc. to the extract; it is necessary that enough alcoholic sodium hydroxid be added to liberate the nicotine. Follow in the case of extracts with a sufficient quantity of pure powdered calcium carbonate to form a moist but not lumpy mass. Mix thoroughly with a pestle or spatula, transfer to a suitable container in a Soxhlet extractor and exhaust for about 5 hours with ether. (Probably another solvent could be substituted for this purpose.) It is important that the temperature of the cooling water during the extraction should not be much over 20° C., and sufficient solvent should be used in order that its volume in the extraction flask should never be less than about 25 cc. After the extraction is made, the exhausted sample is removed and the excess of ether recovered, but the volume of ether extract should not be permitted to go below the above minimum or more if necessary to keep the extractive matter in solution at ordinary temperature. (If the temperature of the cooling water is too high and the volume of solvent in the flask becomes too low during the extraction, there may be a small loss of nicotine from samples of high percentage.)

The ether extract is transferred to a separatory funnel, preferably with a short stem, the extraction flask washed with a little ether and finally about twice with hydrochloric acid (1 to 4) to remove all nicotine.

The extract is shaken with four to six portions (10 cc. each) of cold dilute hydrochloric acid (1 to 4). If an emulsion forms during the successive extractions with the acid, this can be prevented by adding a small amount of 95 per cent alcohol, about 1 cc. being sufficient for each separate extraction. (When it is found necessary to use alcohol, it is essential that it be added after the shaking has been completed. Further shaking should be avoided as much as possible, as one or two inversions of the funnel will break the emulsion. A minimum amount of alcohol and shaking are best, since it has been found that when alcohol is added along with the acid, followed by vigorous shaking, it has some solvent action on extraneous material that precipitates with the reagent. Consequently, alcohol should not be used unless necessary, and only in the manner described.) The successive extractions are run through a funnel into a 100 cc. graduated flask. A small ball of fine glass wool may be placed in the neck of the funnel. The extraction should be continued until a few drops of the acid solution give no turbidity with the reagent. The funnel is carefully washed with water and the volume made to 100 cc. An aliquot corresponding to about 0.25 gm. or more of extract or 1 to 2 gm. of tobacco, depending on the amount of nicotine present, is placed in a beaker and diluted to 400 to 500 cc. volume with distilled water. A sufficient quantity of dilute hydrochloric acid (1 to 4) is then added so as to have at least 3 cc. or more present, to each 100 cc. of liquid, including the quantity of acid contained in the aliquot. At this point, the solution should test distinctly acid with a few drops of methyl orange; if not acid, more should be added. There is then added, with constant stirring, 1 cc. of the silicotungstic acid reagent for each 0.01 gm. of nicotine supposed to be present. Stir thoroughly for about 3 to 5 minutes or until the precipitate is crystalline and settles quickly. To insure an excess of the reagent, a few drops are added to the clear supernatant liquid and the absence of any precipitate noted. The solution is allowed to stand at least 18 or preferably about 24 hours. The precipitate is filtered on an ashless filter (C. S. & S. No. 590 or similar quality) and preferably through a Hirsch or Buchner funnel by decanting first the clear liquid and testing a few drops of it with a little nicotine solution to insure an excess of reagent. By decanting practically all the clear solution the filtration is more quickly made. (In case of very small precipitates, the addition of a small amount of ashless filter paper pulp to the precipitate at the time it is to be filtered will insure a clear filtrate.) When the precipitate is transferred to the filter, if the filtrate should be turbid, it is filtered again until clear. If, at the beginning, the precipitate is made crystalline by proper stirring, the filtration is made quickly and without trouble. The precipitate is thoroughly washed with cold dilute hydrochloric acid (1 to 1,000). Transfer the precipitate, without removing from the paper, to a weighed platinum crucible, dry carefully at low heat until the paper is charred, then ignite over a Bunsen burner until all carbon is eliminated. Finally, heat over a Teclu or Meker burner for not over 10 minutes, or over a moderate blast for about 5 minutes. It is not advisable to prolong the final heating beyond the time mentioned, because of a very small but continued loss at this temperature. The weight of the residue multiplied by 0.114 gives the weight of nicotine present in the aliquot used.

In the development of this method, the following experiments were made to test important features of the work:

ABSENCE OF FOREIGN MATERIAL IN THE ACID SOLUTION OBTAINED FROM THE ETHER EXTRACT WHICH MIGHT PRECIPITATE WITH THE REAGENT

As experiments had shown that such material would more likely be obtained from tobacco than from extracts, aliquots of the same acid solution of tobacco were compared. One aliquot was distilled and determined by the silicotungstic acid method while the other aliquot was handled at this stage as described in the method above. The results are given in Table 1.

TABLE I.—*Percentage of nicotine in tobacco (moisture free)*

No.	Chapin's silicotungstic acid method.	Improved method.
61422	1.29	1.33
61423	2.17	2.23
61426	0.70	0.75
61436	7.26	7.30
61437	5.47	5.51
Average	3.38	3.42

It is apparent from Table I that in the acid solution obtained from the ether extract of these samples, no foreign material is present which might precipitate with the reagent and materially affect the results, unless it is volatile with steam.

THE AMOUNT OF ALCOHOL USED IN THE MANNER PRESCRIBED IN THE METHOD APPARENTLY DOES NOT HAVE ANY APPRECIABLE DETRIMENTAL EFFECT

Duplicate determinations were made on different tobaccos, in one of which alcohol was used as prescribed, while in the other it was omitted. The results are given in Table II.

TABLE II.—*Percentage of nicotine in tobacco (moisture free)*

No.	Alcohol used.	No alcohol used.
80236	6.94	6.80
80237	5.98	6.02
80238	6.35	6.30
80239	5.72	5.82
80273	2.98	2.87
80274	2.10	2.05
80275	1.79	1.77
80276	0.97	0.9
80382	7.77	7.7
80383	7.04	6.94
80384	5.78	5.70
Average	4.86	4.82

Apparently alcohol used in the quantity and manner prescribed in the method has very little, if any, effect on the results. The use of alcohol would be more likely to affect the results in the analysis of tobacco than in that of extracts. Experience has shown that it is not usually required for extracts and is not always necessary for tobacco.

Alcohol has been found, however, to have a solvent action for extraneous matter contained in the ether extract of some tobaccos if it is added with the acid and thoroughly shaken, as is done in the acid extraction. For example, two of the samples in Table II, when alcohol was used in this manner, gave the following results: No. 80236 = 7.37 per cent and No. 80237 = 6.32 per cent nicotine. On the other hand, it has been repeatedly found that when as much as 5 cc. alcohol is added to an aliquot after the acid extraction is made, identical results are obtained as compared with another aliquot containing no alcohol. However, it should not be used except where it is necessary and then only in the manner prescribed.

When a comparison was made of this method with Chapin's silicotungstic acid method, it was found that higher, and in some samples of tobacco abnormally higher, results were obtained by the latter method. Much better agreement, however, was shown with extracts. Steam distillations showed that whereas not over 750 cc. of distillate was usually sufficient to obtain the nicotine from extracts, a much larger volume was generally required for tobacco, notwithstanding the fact that a smaller amount of nicotine might be present in the larger distillate. Again, it was found essential that the solution in the distilling flask should be maintained at about 15 to 25 cc. in order to distill over the nicotine in a minimum volume of distillate. If the volume of liquid greatly exceeds this extreme difficulty is met with in distilling the nicotine, especially from tobacco. For example, tests with negative results have been made for nicotine in a distillate coming from a comparatively large solution, although further concentration to the optimum volume showed its presence. Another interesting fact observed in the steam distillation was that the nicotine results obtained by its use, especially with tobacco, were largely influenced by the amount of alkali used in the distillation. For instance, it was found that where 2 gm. of sodium hydroxid was used in the distillation higher results were always obtained than when smaller amounts were employed, despite the fact that in the former case the nicotine usually came over in a smaller volume of distillate. In all distillations the liquid in the distilling flask was alkaline, and sufficient alkali was present to liberate combined nicotine. It has been shown in this connection that the larger amount of alkali liberates some volatile compound other than nicotine which comes over in the distillate and is precipitated by the reagent. This substance occurs in variable quantity in tobacco, and a distillate containing it generally has some odor and color. The compound does not give the characteristic test for nicotine, but forms with silicotungstic acid a dirty white precipitate which becomes pinkish on standing. Where this substance occurs in appreciable quantity it can be obtained by distilling with an excess of alkali (2 gm.) the exhausted tobacco obtained from the ether extraction, or, after a weak alkaline distillation is completed, a further addition of alkali up to the above amount and continued distillation will show its presence. There is no doubt but that the excess of alkali and heat used in the distillation acts to a greater or less extent on the tobacco residue

contained in the small volume necessary to liberate the nicotine, and forms a volatile product or products which are found in the distillate, precipitate with the reagent and affect the results. Further work will be done to determine, if possible, the nature of this reaction.

The following precautions are therefore necessary in the distillation: (1) Use the minimum amount of alkali necessary to liberate all the nicotine; (2) keep the volume of liquid in the flask at about 15 to 25 cc. to facilitate the distillation of the nicotine; (3) continue the distillation until a few cubic centimeters of the distillate shows no opalescence when treated with a drop of dilute hydrochloric acid and a drop of the silicotungstic acid. Under such conditions, however, a large volume of distillate will sometimes be obtained, as shown in Table III.

TABLE III.—*Percentage of nicotine in tobacco (moisture free); effect of different amounts of NaOH in distillation of 5 gm. sample on the volume of distillate necessary to liberate the nicotine.*

No.	2 gm. NaOH.	Volume of distillate.	Final test for nicotine.	Less than 2 gm. NaOH.	Volume of distillate.	Final test for nicotine.
61469	7.55	900	Absent.	[1] 7.38	1,500	Absent.
				[1] 7.29	1,500	Do.
				[2] 6.90	2,900	Do.
61537	6.33	900	.....do.	[2] 5.67	1,800	Present.
				5.98	2,400	Absent.
80073	7.85	1,800	.....do.	[1] 6.76	1,000	Do.
	7.83	1,800	.....do.	[1] 6.65	1,000	Do.
80144	.....	.....	.....	[2] 7.32	2,100	Present.
				[2] 7.51	2,700	Absent.

[1] NaOH used in distillation, 0.25 gm.
[2] NaOH used in distillation, 0.15 gm.

DIRECT PRECIPITATION OF NICOTINE

It was thought that it might be possible with some extracts, provided no interfering substances were present, to make a direct precipitation of the nicotine and thereby eliminate the distillation and ether extraction necessary in the other methods. Experiments were made, therefore, by taking a weighed sample of extract, diluting it to a definite volume and directly precipitating the nicotine in an aliquot with the reagent in the manner prescribed in the improved method as to volume, acidity, and handling of the precipitate. While the precipitates were often colored, very satisfactory results were obtained on most of the extracts, although precipitable matter in others proved to be detrimental. The results obtained are given in Table V.

This method was also tried on tobacco by making an aqueous or weak akaline extraction, filtering and using an aliquot as described in the preceding paragraph. It was not found applicable, however, as shown by the determinations below, on two samples from Table III. No. 61469 gave 9.13 per cent nicotine by an akaline extraction and No. 61537 gave 7.53 per cent by an akaline and 7.37 per cent nicotine by an aqueous extraction.

The results obtained by the improved method on tobacco and extracts in comparison with Chapin's silicotungstic acid method, together with those found by the latter when variable amounts of alkali were used in the distillation, are given in Tables IV and V.

TABLE IV.—*Percentage of nicotine in tobacco (moisture free).*

No.	Chapin's silicotungstic acid method.		Improved method.
	2 gm. NaOH used in steam distillation of 5 gm. sample.	Less than 2 gm NaOH used in steam distillation of 5 gm. sample.	
61469	7.55	[1] 7.38	6.99
		[1] 7.29	7.01
		[2] 6.90	7.00
61537	6.33	7.19	
		[2] 5.98	5.98
			6.18
			6.08
80073	7.85	[1] 6.76	6.70
	7.83		6.88
	7.84	[1] 6.65	6.79
		6.71	
80074	6.37	[1] 6.19	6.01
80076	5.32	[1] 5.09	4.92
80117	3.67	.....	3.37
80121	3.97	.....	3.68
80126	2.71	.....	2.48
80129	4.57	.....	4.43
80130	4.50	.....	4.24
80134	1.75	.....	1.55
80144	.....	[2] 7.51	7.67
80195	.....	[2] 7.77	7.83
80263	.....	[1] 2.56	2.57
			2.58
			2.58

[1] NaOH used in distillation, 0.25 gm.
[2] NaOH used in distillation, 0.15 gm.

TABLE V.—*Percentage of nicotine in tobacco extracts and products*

No.	Brand.	Guaranty.	Direct precipitation method.	Chapin's silico-tungstic acid method.[1]	Improved method.
43889[2]		16.42	18.77	17.31	15.96
				[3] 17.44	16.71
				17.38	16.34
80054	"Nico-Fume"	40.00	41.60	40.78	40.77
80055	"Hammond's Tobacco Extract"	4.00	4.26	4.27	4.25
80056	"Nikoteen"	30.00	31.67	30.85	30.78
80064	"Thompson's Rose-Nicotine"	5.00	5.13	5.18	[4] 4.89
				[5] 5.26	[4] 4.89
				5.22	4.89
80065		.18	.72	.13	.13
80066		3.57	4.78	3.70	[4] 3.51
				[5] 3.82	[4] 3.51
				3.76	3.51
80356	"Hall's Nicotine Solution"	40.00	40.43	[3] 40.62	39.69
80357	"Hall's Nicotine Sulfate"	40.00	40.15	[3] 39.82	39.88
40358	"Hall's Nicotine Fumigator"	12.50	.....	[3] 13.26	13.18
80823	"Black Leaf 40"	40.00	40.54	40.00	40.13
80824		.....	1.64	[6] 2.47	1.65
				2.50	
				2.49	
80826		.....	94.63	[7] 93.73	93.42
			94.70	[7] 93.75	93.60
				[7] 94.60	94.14
			94.67		
				94.03	93.72
80828		40.00	40.61	[8] 39.74	40.39
			40.73	[8] 40.19	40.88
			40.67	39.97	40.64
80829	"Black Leaf 40"	40.00	42.21	[8] 41.87	42.09

[1] In this method, the following weights of sample were distilled with 2 gm. NaOH unless otherwise indicated:
Samples containing 50 per cent or more of nicotine, 0.6 gm.
Samples containing 30 to 50 per cent of nicotine, 1 gm.
Samples containing 3 to 30 per cent of nicotine, 2 gm.
Samples containing less than 3 per cent of nicotine, 5 gm.

[2] This extract had been prepared for several years and was supposed to contain 16.42 per cent of nicotine, 2.78 per cent of pyridin and 2.37 per cent of ammonium chlorid. The nicotine guaranty is probably only approximate. The results obtained for nicotine by all methods include any pyridin that may have been present in the washed precipitate.

[3] NaOH used in distillation, 0.60 gm.

[4] Duplicates from same ether extraction.

[5] NaOH used in distillation, 0.15 gm.

[6] NaOH used in distillation, 0.24 gm.

[7] NaOH used in distillation, 0.90 gm.

[8] NaOH used in distillation, 0.30 gm.

SUMMARY

(1) A method has been devised for the determination of nicotine in tobacco and tobacco extracts which has been found to be as rapid and as accurate as the silicotungstic acid method and more satisfactory on tobacco. The other standard method commonly used for nicotine is the Kissling.

(2) The new procedure eliminates an undesirable feature, namely, the steam distillation, common to both of the above methods.

(3) It has been shown that special precautions should be taken in carrying on the distillation in the silicotungstic acid method. Under proper conditions very good results were usually obtained by its use; otherwise, serious errors were often found when working on tobacco.

(4) A direct precipitation method which obviates a preliminary ether extraction or steam distillation to obtain the nicotine, has also been tried and fairly satisfactory results were obtained on most of the tobacco extracts employed. It was not found, however, to be applicable to tobacco and a few extracts.

NUTRITIVE VALUE OF MIXTURES OF PROTEINS FROM CORN AND VARIOUS CONCENTRATES[1]

By D. BREESE JONES, *Chemist in Charge;* A. J. FINKS, *formerly Associate Biological Chemist;* and CARL O. JOHNS, *formerly Chemist in Charge, Protein Investigations Laboratory, Bureau of Chemistry, United States Department of Agriculture*

INTRODUCTION

The nutritive value of mixtures of peanut and soy-bean flours as supplements to wheat-flour proteins has been shown in previous publications (*4*, *5*) [2] from this laboratory. This paper is a continuation of these studies with whole yellow corn. In addition to the peanut and soy-bean flours as protein supplements to corn are also included meal made from tomato-seed press cake and coconut meal made by both the solvent and expression processes. Data on both the chemical and nutritional studies of the proteins from tomato seed and coconut have already been published (*3*, *6*, *7*, *8*, *9*). Each of the concentrates mentioned has been found adequate for the normal growth of albino rats as the sole source of protein in otherwise complete diets.

It has been shown by several investigators that whole corn is not adequate for normal growth. Feeding experiments with zein, a protein which constitutes more than one-half of the total proteins in maize, have shown it to be deficient in lysine and tryptophane—amino acids which are essential for the growth of animals. Experiments also have been recorded which show that when corn proteins are fed at a high enough intake level—as, for example, in corn gluten—normal growth can be secured.

Whole corn constitutes a large part of the ration of many animals and it is of practical importance to the feeder to know whether the addition of small quantities of protein concentrates such as coconut meal, tomato-seed, press cake, soy-bean and peanut flour will result in a protein mixture which will meet the nutritional requirements for normal growth. Our experiments with these concentrates show that a mixture of 25 parts of tomato-seed press cake, soy-bean flour, or peanut flour with 75 parts of corn satisfies the protein requirement for the normal growth of white rats. In the case of the coconut meal, equal parts were necessary to secure the same result. This may not have been due, however, to inferiority in the nutritional value of the coconut-meal proteins, but rather to the bulky character of the meal caused by its higher content of crude fiber. Chemical studies of the proteins of these concentrates have shown that they are relatively high in lysine and tryptophane, and are therefore well suited to supplement the corn proteins which are deficient in these amino acids.

EXPERIMENTS WITH WHOLE YELLOW CORN MEAL

Two diets containing 7.2 per cent of protein in which the proteins came solely from whole yellow corn were used. Diet 1 contained 16 per cent of butter fat. Diet 2 was made up of the same composition, with

[1] Accepted for publication Nov. 24, 1922.
[2] Reference is made by number (italic) to "Literature cited," p. 977–978.

the exception that lard was used in the place of butter fat. The growth curves in Chart 1 show that about equally poor growths resulted with both of these diets, an average gain in weight of only 0.73 gm. per gram of protein intake being obtained. Although diet 2 contained no source of vitamin A other than the yellow corn (unless the lard may have contained some), growth was obtained at approximately the same rate as when diet 1 with the butter fat was used. No indication of xerophthalmia was observed. These facts indicate the presence of vitamin A

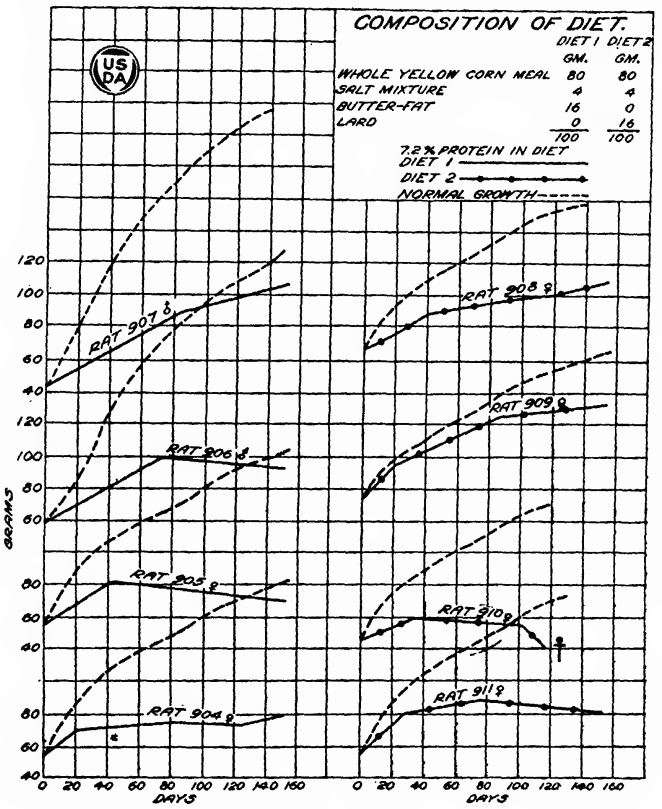

CHART 1.—Curves showing the poor rate of growth of young albino rats fed on a diet the proteins of which came solely from whole yellow corn.

in yellow corn, as has previously been shown by Steenbock and Boutwell (*13*).

EXPERIMENTS WITH CORN AND TOMATO-SEED PRESS CAKE

Studies made in this laboratory (*8*) have shown that tomato-seed press cake contains nearly 37 per cent of protein and that the nutritionally essential amino acids are well represented in these proteins, being relatively high in arginine, lysine, and cystine. Qualitative tests also show the presence of tryptophane. The nutritional adequacy of these proteins, as shown by chemical studies, has been confirmed by feeding experiments (*3*).

On a diet in which the proteins were furnished by 75 parts of whole yellow corn meal and 25 parts of tomato-seed press cake, growth at the normal rate was secured (Chart 2). On such a diet, the tomato-seed press cake furnished 7.4 per cent, and the corn 5.4 per cent of protein.

Decreasing the protein level to 7.2 per cent, but keeping the proportion of tomato-seed press cake to corn the same, resulted in a rate of growth somewhat below the normal. This was better, however, than the diet in which corn alone was used to furnish the same amount of protein. An average gain in weight of 0.94 gm. per gram of protein intake was obtained during an 11-week period on this mixture. With the corn alone, an average gain of only 0.73 gm. was secured. The low level of the protein intake was responsible for a rate of growth somewhat below normal. By using a diet somewhat below the minimum requirement of protein for normal growth, the maximum gain in weight per gram of protein intake is obtained. Such a diet affords a better basis for a comparative study of the growth-promoting value of different proteins.

EXPERIMENTS WITH CORN AND COCONUT PRESS CAKE

Diets in which the proteins were obtained from mixtures of equal parts of coconut meal and whole yellow corn enabled albino rats to

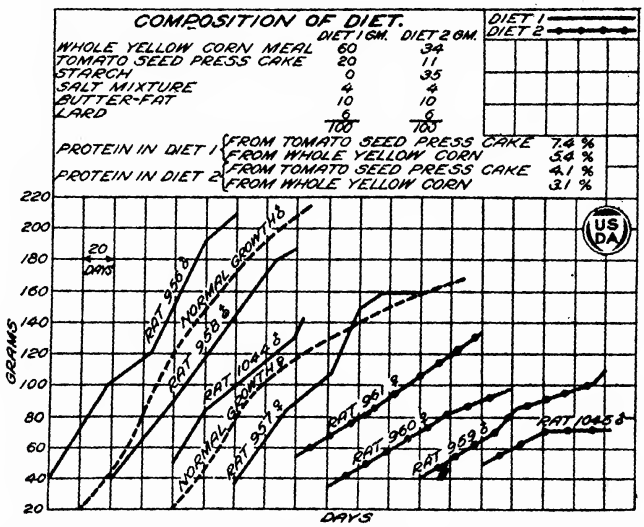

CHART 2.—The plain continuous lines represent the rate of growth of rats on diet 1, containing 5.4 per cent of protein from corn, supplemented with 7.4 per cent of protein from tomato-seed press cake; the growth represented by the continuous dotted lines was made on diet 2, of which 3.1 per cent of the proteins came from corn, and 4.1 per cent from tomato-seed press cake.

grow at the normal rate. On such a diet, the coconut meal furnished 8.4 and the corn 3.6 per cent protein, respectively. This experiment shows that the coconut proteins supplement corn proteins, since better than normal growth was obtained with 12 per cent of the mixture of proteins, while 13.1 per cent of coconut proteins alone, in an otherwise adequate diet, just barely sufficed for normal growth (7). When coconut meal furnished 4.2 per cent and corn 5.4 per cent protein in the diet, growth was somewhat subnormal. The coconut meal used in these diets was made both by the expression process (A) and by the solvent process (B). The processes seemed to be of equal value as regards the nutritive properties of the meal produced. The results of this experiment are recorded in Chart 3. The efficiency of a coconut-corn protein mixture for promoting growth, as shown by these experiments, is in agreement with the results reported by Maynard and Fronda (*11*), who found that "a mixture of corn meal protein and coconut-oil-meal protein was of

slightly higher quality than the former alone, but much better than the latter alone."

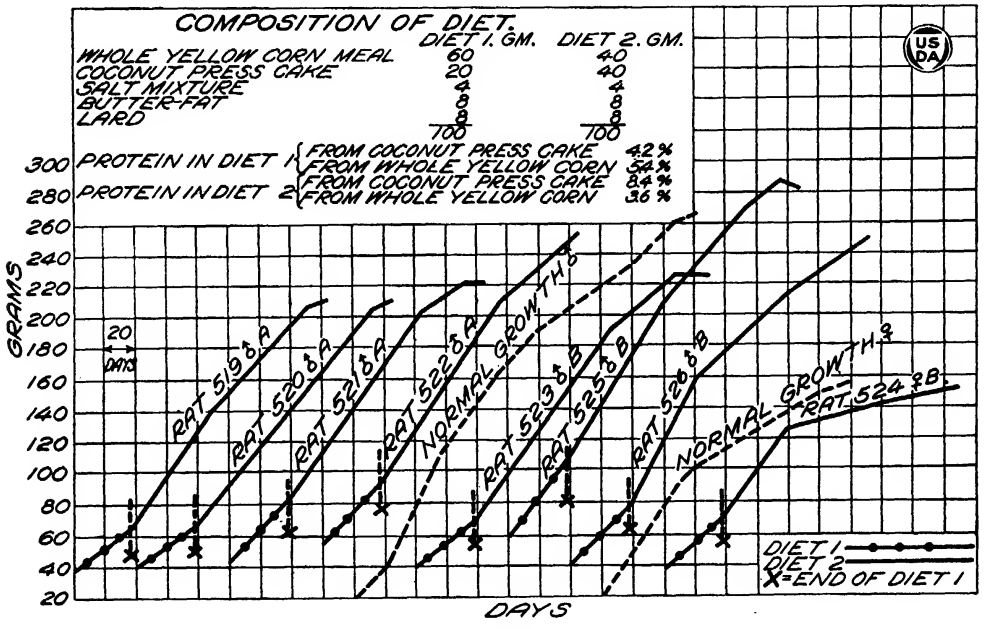

CHART 3.—Curves showing the growth-promoting efficiency of a coconut-corn protein mixture. The compositions of the diets are given on the chart.

EXPERIMENTS WITH CORN AND PEANUT MEAL

When peanut meal forms the sole source of protein at intake levels of from 15 to 18 per cent in diets which are otherwise complete, Daniels and

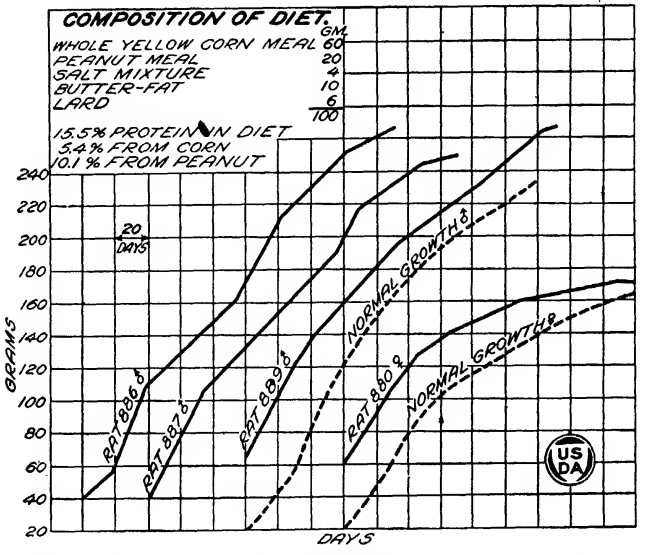

CHART 4.—These curves represent the growth-promoting value of a protein mixture, 5.4 per cent of which was furnished by corn meal, and 10.1 per cent by peanut meal.

Loughlin (*1*) have shown that normal growth can be secured. In our experiments normal growth was obtained with mixtures consisting of 75 parts of corn meal and 25 parts of peanut flour at a protein level of 15.5 per cent. From such a protein mixture two-thirds of the protein was

furnished by the peanut flour (Chart 4.) By keeping the proportion of the concentrate to corn the same, but lowering the protein level of the diet to 7.2 per cent, a better rate of growth was obtained than when corn

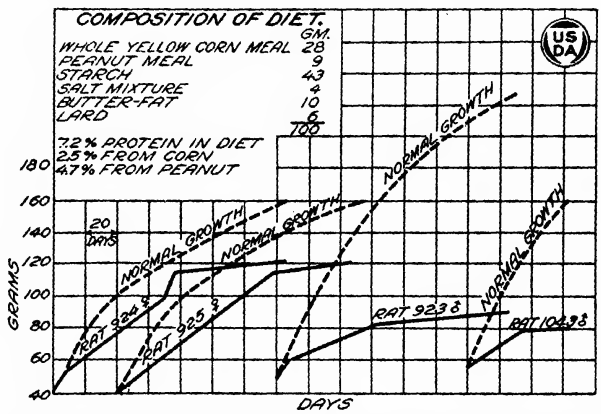

CHART 5.—Growth curves showing the supplementary value of peanut proteins when fed with corn proteins, at a low protein intake level.

alone furnished the protein in a diet otherwise adequate at the same level (Chart 5).

The average gain in weight per gram of protein in this mixture during an eleven-week period was 1.23 gm. compared with 0.73 gm. on corn alone. Rat 1043, in the above lot, was not included in the average.

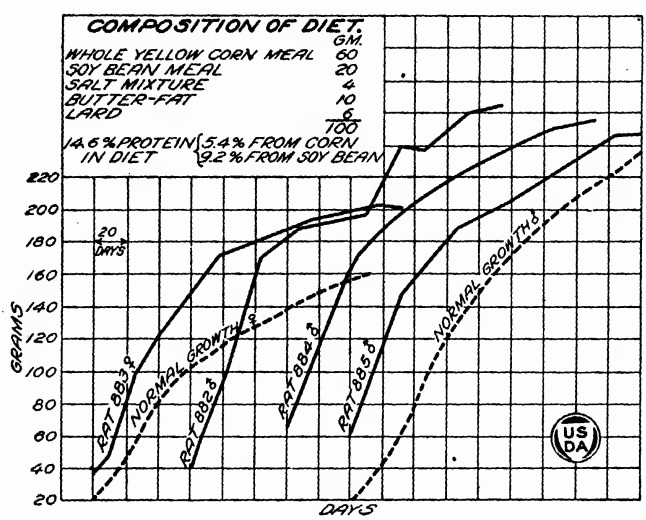

CHART 6.—These curves show the excellent growths obtained on a diet 5.4 per cent of the proteins of which was furnished by corn, and 9.2 per cent by soy-bean meal.

EXPERIMENTS WITH SOY BEAN AND CORN

It has been shown (*12*, *2*) that soy-bean proteins are adequate for the normal growth of albino rats. Our experiments were made with a diet in which 25 parts of soy-bean flour replaced an equal quantity of corn meal. This diet contained 14.6 per cent of protein. Better growth than at the normal rate was obtained (Chart 6) when to such a mixture was added a suitable inorganic salt mixture, lard and butter fat. In this diet, 9.2 per cent of the protein was furnished by soy beans and 5.4 per

cent by the corn. McCollum (*10*) has shown that a supplementary relationship exists between corn and soy-bean proteins in the ratio of 6 per cent of the former to 3 per cent of the latter.

When the protein level of the corn-soy-bean mixture was reduced to 7.2 per cent, so that the corn furnished 2.6 per cent and soy bean 4.6 per cent of the protein of the diet, decidedly better growth resulted than when corn at the same protein level in an otherwise adequate diet was used. Rat 926 of this lot showed even a better than normal rate of growth at the end of about 95 days (Chart 7).

During an eleven-week period, the rats of this lot (excluding rat 1041, which for some reason other than a deficiency in the diet made practically

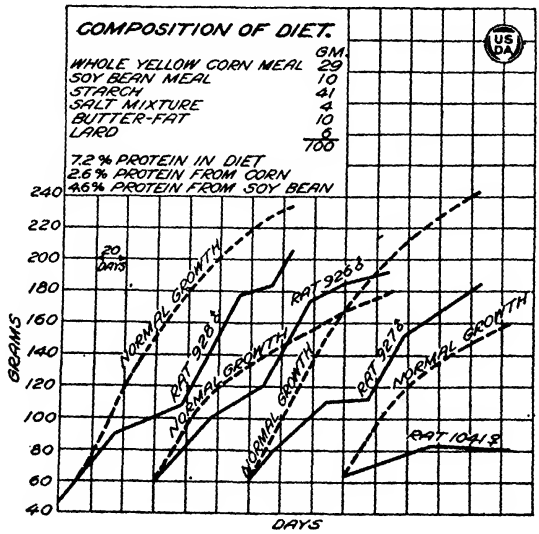

CHART 7.—Curves showing the supplementary value of soy-bean proteins when fed with corn proteins, at a low protein intake level.

no growth) made an average gain of 1.48 gm. per gram of protein consumed.

TABLE I.—*Gain of body weight per gram of ingested protein*

Diet.[1]	Protein in—			Gain per gram (11 weeks).
	Corn.	Concentrate.	Total.	
	Per cent.	*Per cent.*	*Per cent.*	*Gm.*
Corn	7.2	.....	7.2	0.73
Corn+tomato-seed press cake	3.1	4.1	7.2	.94
Corn+peanut meal	2.5	4.7	7.2	1.23
Corn+soy-bean meal	2.6	4.6	7.2	1.48

[1] These diets were made adequate with respect to the nutritionally essential factors other than proteins. (See charts.)

Although the ratio of the percentage of concentrate protein to corn protein is not exactly the same in these diets (Table 1), it can safely be concluded that the growth-promoting value of the proteins of these concentrates, as supplements to the proteins of corn, is in the following order: Soy bean, peanut, and tomato seed. In the experiments with the coco-

nut press cake, no diets containing as low a protein content as 7.2 per cent were used. Therefore, a direct comparison of the nutritive value of the proteins of this concentrate with the other concentrates studied, cannot easily be made.

SUMMARY

Mixtures consisting of 25 parts of tomato-seed press cake, soy-bean flour, or peanut flour, and 75 parts of yellow corn meal, which contained from 12 to 15 per cent of protein, have been found to furnish proteins adequate for the normal growth of albino rats when incorporated in a diet made nutritionally adequate with respect to the dietary factors other than protein.

A mixture of equal parts of corn meal and coconut meal at a protein level of 12 per cent was found efficient for growth at the normal rate. The growth was somewhat subnormal when the proportion of protein was reduced to 9.6 per cent.

From experiments in which the mixtures of corn meal and concentrates furnished 7.2 per cent of protein, it is concluded, from the gain in weight per gram of protein consumed, that the comparative growth-promoting value of the proteins of tomato seed, peanut and soy bean, as a supplement to corn proteins, is in the order: Soy bean, peanut, and tomato seed.

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THE MODE OF INHERITANCE OF RESISTANCE TO PUCCINIA GRAMINIS WITH RELATION TO SEED COLOR IN CROSSES BETWEEN VARIETIES OF DURUM WHEAT [1]

By J. B. HARRINGTON, *Graduate Student in Plant Breeding, University of Minnesota,* and O. S. AAMODT, *Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

It has long been recognized that the production of economically desirable varieties of wheat resistant to *Puccinia graminis tritici* Erikss. & Henn. would be of great value. Until recently, hybridization experiments with this end in view were based upon a very incomplete knowledge of both the exact nature of the pathogene and the nature of the inheritance of resistance, and consequently, satisfactory results were not obtained. A knowledge of the mode of inheritance of certain desired economic characters of wheat together with the discovery that there are several biologic forms of stem rust of wheat has been of much importance in placing breeding studies on a definite basis. Several varieties belonging to different species or subspecies of wheat have been found to be resistant to certain biologic forms. It is hoped that by hybridization, resistance to all biologic forms may be combined in one variety. The present problem was attacked chiefly for the purpose of determining whether the resistance of two varieties, having different factors for resistance, may be combined in the progeny of a cross between them.

REVIEW OF PREVIOUS INVESTIGATIONS

Since 1894, when Eriksson (*7*) [3] discovered biologic specialization in the black stem rust, numerous investigators (*6*, *9*, *25*) have corroborated his work. The reports of Ward, Evans, and others (*32*, *8*, *9*) indicated that biologic forms might change rather rapidly as a result of host influence but more recent work by Stakman and others (*27*, *30*) showed that biologic forms were apparently quite stable. Previous to 1916, it was generally believed that only one form of black rust attacked wheat.

[1] Accepted for publication Feb. 20, 1923. Cooperative investigations between the Sections of Plant Breeding and Plant Pathology, Minnesota Agricultural Experiment Station, and the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

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[2] The authors wish to express their appreciation for the assistance rendered by Dr. H. K. Hayes and Dr. E. C. Stakman, both of the Minnesota Agricultural Experiment Station, and to thank Mr. M. N. Levine, Assistant Pathologist, Office of Cereal Investigations, U. S. Department of Agriculture, for certain biologic forms of wheat rust which he furnished.

[3] Reference is made by number (italic) to "Literature cited," p. 995-996.

However, the investigations of Stakman and others (*28*, *29*, *21*, *20*, *26*) have proved that there are a number of biologic forms of this rust.

A total of 37 different biologic forms of *Puccinia graminis tritici* have been isolated at the Minnesota station by Stakman and Levine (*25a*). No one form has been found to attack all varieties of Triticum species and no wheat variety has been found to be resistant to all rust forms, except Khapli emmer, a variety of *T. dicoccum*. The exact distribution of each biologic form is not known (*19*). Perhaps not half of the 37 forms are present in any one wheat-growing district. For example, only 14 forms had been collected up to December, 1921, in western Canada (*23*). However, as it is possible that some forms may spread into new districts, the problem of breeding for resistance means working for a variety resistant to all known forms of rust.

The study of disease resistance in wheat dates back over a century. According to Biffen (*3*), Thomas Knight, in 1815, suggested that disease-resistant varieties might be raised, and Farrar, in 1889, stated that susceptibility to rust was hereditary in wheat. Biffen (*3*), in 1905, crossed Red King, a variety susceptible to *Puccinia glumarum* (Schm.) Erikss. & Henn., with Burt, a resistant variety. The F_1 progeny was susceptible and the F_2 progeny segregated into susceptible and resistant in a 3: 1 ratio. Later, Biffen (*4*) stated that the relatively immune hybrid forms of wheat bred true and that the immunity was independent of any discernible morphological character, that is, the factors for resistance were inherited independently of other factors. More recently, Biffen (*5*) reported the production of a rust-resistant wheat which likewise possessed other desirable economic characters.

Some excellent results in breeding for disease resistance in crop plants have been reported by various workers, but the problem is rarely as difficult as in the case of stem rust of wheat. Gaines (*12*) crossed two varieties of wheat highly resistant to bunt and obtained in the F_2 some plants which were more susceptible than either parent and a large percentage which were immune. He concluded that the two varieties had different factors for resistance. Garber (*13*), in 1921, found that resistance to *P. graminis avenae* Erikss. & Henn. was a dominant character, a ratio of 3 resistant to 1 susceptible being obtained in F_2.

A definite grouping of the wheat subspecies according to their reaction to *P. triticina* Erikss. was made by Vavilov (*31*) in 1914. The use of interspecific crosses in breeding for rust resistance is liable to some complication owing to the linkage of certain factors. In 1917, Freeman (*10*) reported cases of linked quantitative characters in wheat crosses. In 1919, Hayes, Parker, and Kurtzweil (*16*) found that in crosses of varieties of *T. vulgare* with varieties of *T. dicoccum*, resistance was dominant. In crosses of *T. vulgare* with *T. durum* susceptibility was dominant and in F_2 there was strong linkage between rust resistance and the durum character. The significant fact, however, was that some crossing-over occurred. It was apparent, therefore, that there was a possibility of transferring the resistance of durums or emmers to common wheats.

Puttick (*24*), in 1920–21, studied the reaction of the F_2 generation of a cross between a common and a durum wheat to two biologic forms of *P. graminis tritici* to which these varieties reacted reciprocally. F_2 seedlings were inoculated with one rust and later the infected leaves were cut off and the plants were inoculated with a second rust form. All gradations between complete susceptibility and immunity to both

forms of rust were obtained. Some of the progeny appeared to be resistant to both forms.

More recently Melchers and Parker (*22*) found that a single factor was responsible for the resistance in crosses of Kanred with Marquis, Preston, and Haynes Bluestem, which were inoculated with a single form of rust. Segregation in F_2 was in a 3:1 ratio of resistant and susceptible plants. The results were verified by carrying the study through the third generation.

Aamodt (*1*, *2*), in 1921, studied the inheritance of resistance to several biologic forms of *P. graminis tritici* in a cross between Kanred, a winter wheat, and Marquis, a spring-sown variety. Kanred is resistant to a number of biologic forms whereas Marquis is susceptible to most of them. F_3 seedling plants inoculated with a form of rust to which Kanred was immune and Marquis susceptible proved either resistant or susceptible. Families, homozygous for spring habit of growth and for resistance to all the forms of rust to which Kanred was resistant, were obtained in the F_3. Here a single factor apparently determined the reaction to several biologic forms, to 11 of which Kanred is immune.

Of the 37 known biologic forms of stem rust 21 were found in the area where hard red spring wheats are grown.[4] The most recent study in disease-resistance breeding at the Minnesota station shows the possibility of synthetically producing a desirable *vulgare* variety of wheat that will be resistant to all of these biologic forms (*17*). This is to be accomplished by means of several crosses and double crosses between varieties having the desired resistance and those having the necessary bread-wheat qualities for yield, milling, baking, etc.

In the present investigation use was made of a cross between two varieties of durum wheat which react reciprocally to two biologic forms of rust, each being resistant to the form to which the other is susceptible. It was hoped that some F_3 families would prove resistant to both forms of rust. In addition to a study of the genetic factors concerned with resistance the relation of seed color to rust resistance was also considered. That resistance to several biologic forms is governed by a single factor, in the Kanred × Marquis cross, indicates the importance of learning as much as possible about the inheritance of resistance to various rust forms in different crosses.

MATERIALS AND METHODS

The F_3 families[5] of two *T. durum* crosses, Kubanka 8 (C. I. 4063) × Pentad (C. I. 3322) and Mindum (Minn. No. 470) × Pentad, were used. Kubanka No. 8 is a white-seeded selection from common Kubanka (C. I. 1440). Pentad is a red-seeded variety selected at the North Dakota station. Mindum is a white-seeded selection of the Arnautka type. Seedling plants from each F_3 family were inoculated in the greenhouse with two biologic forms of *P. graminis tritici*. The crosses were made in 1920, and the F_1 generation was grown in the greenhouse at Washington, D. C., during the winter of 1920–21. The F_2 generation was grown in the plant-breeding nursery at University Farm, St. Paul, Minn., during the 1921 season, the plants being harvested individually.

[4] Unpublished results of Stakman and Levine.

[5] The term "F_3 family" is used in this article for the seedling plants grown from the seeds of a single F_2 plant.

The material was a part of that which was being used for the purpose of obtaining resistant wheat for growing in the Northwest. This problem was being cooperatively investigated by the Sections of Plant Breeding and Plant Pathology of the Minnesota Agricultural Experiment Station, and the Office of Cereal Investigations of the United States Department of Agriculture. The individually harvested F_2 plants were furnished to the present writers. The biologic forms of rust were some of those which were being studied by M. N. Levine.

Plants of each F_3 family were grown in 4-inch pots in the greenhouse, one family in each pot. All pots of soil were steam sterilized immediately before use and the seed was sown at a uniform depth. A large proportion of the hybrid seed as well as of the parental seed was infected with Helminthosporium and consequently the number of plants per pot varied between 8 and 20. The seedlings were grown in a seedling section of the greenhouse in which no rust-infected plants were kept. When they were about 3 inches high they were inoculated with fresh urediniospores of one of the two forms used. Only one rust form was worked with at one time. The pots were then placed in an incubation chamber for 40 to 48 hours with a number of control pots of the parental varieties inoculated in the same way. The two forms of rust were kept on well-separated benches to avoid accidental contamination of one with the other. An attempt was made to keep the temperature, moisture, and light condition the same for all plants. The methods used were essentially the same as those described by Stakman and Piemeisel (*29*).

Notes on the character of infection with both forms were taken from 15 to 19 days after the date of inoculation. The amount and rapidity of rust development was found to vary in response to environmental conditions. However, the character of infection was found fairly constant within certain limits, regardless of external influences.

The F_3 seedling plants were inoculated in sets of about 25 to 40 pots each. The total interval during which results were being recorded for the reaction to any one biologic form was approximately two weeks. In addition to running control plants of the parental varieties with each set of hybrid plants, one or more complete sets of "differential" varieties were inoculated during work with each form. In this way a complete control was obtained on the identity of the form being used. The differential varieties used were as follows: Little Club (C. I. 4066); Marquis; Kanred; Kota (C. I. 5878); Arnautka (C. I. 4072); Kubanka (C. I. 2094); Mindum (Minn. No. 470); Acme; Einkorn (C. I. 2433); Vernal emmer (Minn. No. 1165); and Khapli emmer (C. I. 4013).

The percentage of infected plants obtained, on the average, was very satisfactory. Of a total of more than 10,321 plants which were inoculated 796 showed no infection.

In recording the types of infection the symbols prepared by Stakman and Levine (*25a*) in their work on biologic specialization were used. These classes of host reaction and of corresponding types of infection resulting from inoculation of seedling wheat plants with spores of *Puccinia graminis tritici* are as follows:

Classes of host reaction	Types of infection [6]
Immune	0. No uredinia developed but definite hypersensitive areas present.
Very resistant	1. Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive areas; hypersensitive areas lacking uredinia also may be present.
Moderately resistant	2. Uredinia isolated and small to medium in size; hypersensitiveness present in the form of necrotic areas or circles; uredinia often surrounded by green islands.
Moderately susceptible (apparently resistant in the field)	3. Uredinia midsize; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.
Very susceptible	4. Uredinia large or varying from midsized to large, numerous and confluent; true hypersensitiveness entirely absent; chlorosis seldom present.
Resistant to moderately susceptible (apparently resistant in the field)[7]	x − Uredinia very variable; apparently includes two or more of the 0, 1, 2, and 3 types of infection on the same leaf blade; no mechanical separation possible; on reinoculation one type of uredinia may produce another; infection ill-defined.
Moderately susceptible to very susceptible (probably susceptible in the field)	x + Uredinia very variable; apparently including combinations of one or more of the types 0, 1, and 2, with type 4 on the same leaf blade; no mechanical separation is possible; on reinoculation one type of uredinia may produce a different type; infection ill-defined.

SELECTION OF BIOLOGIC FORMS

In October, 1921, about 15 of the 37 known biologic forms were available. The reaction of Mindum to these forms was known but Kubanka No. 8 and Pentad had not been tested. It was hoped that among the 15 forms some could be found that would react reciprocally on the parents of the crosses to be tested. Extensive studies disclosed that Forms I and XXXIV reacted reciprocally on Mindum and Pentad. However, similar success was not achieved with respect to Kubanka No. 8 and Pentad. Therefore, only one form, No. XXXIV, was used for the Kubanka 8 × Pentad cross.

The first rust form, designated as Form I, was collected at St. Paul, Minn., in September, 1915. When used for the present study it had been cultured through approximately 90 urediniospore generations and there was no indication of a change in its parasitic capabilities. Mindum is decidedly resistant to this form, whereas Pentad and Kubanka No. 8 are normally susceptible.

The second form, known as Form XXXIV, was collected by G. F. Puttick at Potchefstroom, South Africa, in April, 1921. When it was received at the Minnesota station it was evidently mixed with one other form. The separation of these forms was not completed until December, 1921. Since then more than seven generations have been produced without any indication of a change in the constancy of the form. In contrast to its reactions to Form I, Mindum is quite susceptible to Form XXXIV and Pentad is resistant. Kubanka No. 8 is susceptible to this form.

[6] These descriptions of types of infection are based on those given by Stakman and Levine (*25a*).
[7] Unpublished results of cooperative rust breeding work carried on at the Minnesota station.

ACCURACY OF THE RESULTS

In estimating the accuracy of the types of infection that appeared on the hybrids the behavior of the rust on pure varieties was taken as a criterion of the average amount of variation that occurred. In Table I the results of inoculations made with Form I on February 10 and 20 are given for a number of varieties, including Mindum and Pentad. In several cases, as those of Little Club and of Khapli, the results from the two periods are practically identical. In the majority of cases, however, the rust development from the inoculations of February 10 was weaker than from those inoculations made February 20. For example, einkorn had type 3 infections from the first inoculation but type 4 from the later inoculation. Similarly, with Kota, the February 10 inoculations produced type 3 infections whereas type 4 resulted from the February 20 inoculations.

TABLE I.—*A comparison of the types of infection produced by biologic Form 1 on several varieties of wheat at two different periods*

Group and variety.	Date inoculated.	Rust development.	Number of plants apparently not infected.	Types of infection.							Total of infected plants.
				0	1	2	X−	3	X+	4	
Common wheats:		*Days*									
Little Club	Feb. 10	17	.....	....	....	....	....	....	....	24	24
	20	16	.....	....	....	....	....	....	....	13	13
Marquis	10	17	.....	....	....	....	....	....	....	12	12
	20	16	2	....	....	....	....	....	....	12	14
Kanred	10	17	14	....	....	....	....	....	....	....	14
	20	16	10	....	....	....	....	....	....	1	11
Kota	10	17	.....	....	....	....	....	14	....	....	14
	20	16	.....	....	....	....	....	....	....	15	15
Durum wheats:											
Arnautka, C. I. 1493.	10	17	.....	11	....	....	1	....	....	3	15
	20	16	.....	9	....	....	....	....	....	4	13
Kubanka, C. I. 4063.	10	17	5	....	....	....	....	6	....	2	13
	20	16	3	....	....	....	....	....	....	12	15
Acme	10	17	2	....	....	....	....	11	....	2	15
	20	16	.....	....	....	....	....	....	....	11	11
Mindum	10	17	1	19	8	....	....	....	....	....	28
	20	16	.....	30	....	....	....	....	....	1	31
Pentad	10	17	4	4	3	....	10	1	2	....	24
	20	16	.....	....	1	....	3	....	8	12	24
Einkorn	10	17	.....	....	....	....	....	14	....	....	14
	20	16	.....	....	....	....	....	....	....	14	14
Emmers	10	17	1	12	....	....	....	....	....	....	13
Vernal	20	16	3	11	....	....	....	....	....	....	14
Khapli	10	17	.....	....	13	....	....	....	....	....	13
	20	16	.....	....	14	....	....	....	....	....	14

The appearance of a type 4 infection on 1 out of 11 Kanred plants inoculated February 20 and similarly on 1 out of 31 Mindum plants, indicated accidental infection from some other form of rust, or impurity in the seed due to mechanical mixture or to natural crossing. The

Arnautka seed was known to be impure. This explains why 8 out of the 28 plants inoculated did not appear to be immune. Hayes (*14*) and Hayes and Garber (*15, p. 35–36, 77–78*) have reported from 2 to 3 per cent of natural crossing in wheat grown at St. Paul, Minn. A rather large difference is shown between the inoculations of February 10 and February 20 on Pentad. This demonstrates the value of running several pots[8] of the parental varieties with each set of hybrid families.

From the foregoing discussion it should not be concluded that the variations were so great as to obscure the results. The reaction of the F_3 plants to the different forms of rust was constant within certain limits and the fact that the plants which were tested were of the third hybrid generation, and consisted of families each arising from a single F_2 plant, makes the interpretation of the various reactions a fairly definite matter.

BASIS FOR INTERPRETING RESULTS

As the determination of the genetic nature of resistance was the chief object of this investigation, the results obtained were interpreted on the basis of the reactions of the parental varieties to the forms of rust used. The F_3 families were placed in five classes with reference to reaction to the different biologic forms, viz, resistant, near-resistant, heterozygous, near-susceptible, and susceptible. In the work with Form I the class designations "immune" and "near-immune" were used instead of "resistant" and "near-resistant" owing to the nature of the Mindum reaction.

F_3 families showing resistance similar to that of the resistant parent were termed resistant. Similarly, the F_3 families showing susceptibility equivalent to that of the susceptible parent were classed as susceptible. Those F_3 families having 9 or more apparently resistant plants and one plant showing susceptibility were classed as near-resistant. Likewise F_3 families with 9 or more plants showing susceptibility and one plant appearing resistant were placed as near-susceptible.

It is probable that some of the F_3 families placed in the near-resistant or near-susceptible class were in reality heterozygous. It is also probable that others placed in these classes were resistant or susceptible, respectively. Owing to the likelihood of the occurrence of certain errors, which have been discussed in a previous section of this paper, it was not possible to determine the exact genetic nature of the "near-resistant" and "near-susceptible" F_3 families. It was best, therefore, to leave these doubtful F_3 families in classes which by their names indicate their character.

All F_3 families not falling in one of the preceding four classes were termed heterozygous. The heterozygosity of various F_3 families varied from a preponderance of apparently susceptible plants to a preponderance of plants showing resistance. Of the five different classes the resistant class is of greatest importance, as it represents the F_3 families which appeared homozygous for resistance.

[8] The word "pot" is used throughout this article to denote the group of plants of a pure line parent variety growing in a single pot, such a group being comparable to a group of F_3 plants (an F_3 family) likewise growing in one pot.

EXPERIMENTAL RESULTS

THE SEED COLOR RATIO

The inheritance of seed color in wheat has been studied for a number of years. Ratios of 3:1, 15:1, and 63:1 of red-seeded and white-seeded plants, respectively, have been obtained in the F_2 of crosses between red-seeded and white-seeded wheats (*3*, *18*, *19*, *11*). Apparently there may be from one to three factors for red seed color present in different wheats. When one or more of the factors for red are present red seed results.

The results obtained in the present study are given in Table II. Of 364 F_2 plants of the Kubanka No. 8 × Pentad cross that were classified, 295 were red-seeded like the Pentad parent and 69 were white-seeded like the Kubanka No. 8 parent. The observed ratio was 3.21:0.79 with red seed color dominant. There were 599 F_2 plants of the Mindum × Pentad crosses classified. The red-seeded totaled 449 and the white-seeded 150, the ratio being almost exactly 3:1 with red seed color dominant. In both cases the presence of a single factor for seed color is indicated.

TABLE II.—*Classification of F_3 families on the basis of seed color*

Cross.	Family numbers of F_2.	No. of F_3 families.		
		Red-seeded.	White-seeded.	Total.
Kubanka No. 8 × Pentad	774 to 792	295	69	364
Mindum × Pentad	793 to 810	247	82	329
Mindum × Pentad	644 to 680	202	68	270

THE F_2 SEED COLOR IN RELATION TO THE RUST REACTION OF THE PROGENY

TABLE III.—*The relation of seed color to the reaction of the F_3 generation of two durum crosses to two biologic forms of P. graminis tritici*

Parents of F_3 families.		Biologic form used.	Reaction of F_3 families.					
			Classes of infection.					
Cross.	Seed color.		Resistant or immune	Near-resistant or near-immune.	Heterozygous.	Near-susceptible.	Susceptible.	Total number of families.
Kubanka No. 8 × Pentad	Red	XXXIV.	8	4	70	15	104	201
Do	White	XXXIV.	1	1	14	5	24	45
Mindum × Pentad	Red	I	23	7	39	6	6	81
Do	White	I	21	6	43	4	11	85
Do	Red	XXXIV.	9	2	48	10	11	80
Do	White	XXXIV.	5	4	20	1	0	30

Table III summarizes separately the results for the F_3 families arising from red-seeded F_2 parents and for those from white-seeded F_2 parents. The Kubanka 8 × Pentad F_3 families reacted with Form XXXIV as follows: Of those from red-seeded parents, 8 appeared to be resistant, 4 near-

resistant, 70 heterozygous, 15 near-susceptible, and 104 susceptible; of those from white-seeded parents, 1 was classed as resistant, 1 near-resistant, 14 heterozygous, 5 near-susceptible, and 24 susceptible. A brief comparison of these two sets of figures reveals their similarity, that is to say, no correlation of seed color with resistance or susceptibility to rust was indicated.

Mindum × Pentad F_3 families when inoculated with Form I gave the following results: Of those from red-seeded parents, 23 were classed as immune, 7 near-immune, 39 heterozygous, 6 near-susceptible, and 6 susceptible; of those from white-seeded parents, 21 were placed as immune, 6 near-immune, 43 heterozygous, 4 near-susceptible, and 11 susceptible. Here again the distribution of families from red seed was similar to that of the progeny of white-seeded plants. A lack of correlation of seed color with resistance or susceptibility to rust was evident.

Mindum × Pentad F_3 families when tested with Form XXXIV reacted in the following ways: Of those from red-seeded parents, 9 appeared resistant, 2 near-resistant, 48 heterozygous, 10 near-susceptible, and 11 susceptible; of those from white-seeded parents, 5 were classed as resistant, 4 near-resistant, 20 heterozygous, 1 near-susceptible, and none susceptible. A comparison of the distribution of the progeny of the red-seeded parents with that of families arising from white-seeded parents shows differences in the near-susceptible and susceptible classes. These, however, can not be given much weight owing both to the small number of families classified and to the fact that Mindum, the white-seeded parent, was susceptible, whereas only 1 of the 30 families from white-seeded F_2 was in the near-susceptible and susceptible classes. If anything, a negative correlation is indicated.

A summarization of the data concerning the mode of inheritance of rust resistance with respect to seed color shows that no correlation was found to exist between the two. It is important to note that each of the three sets of data shows everal F_3 families which were homozygous for the reaction of the Pentad parent and also homozygous for the seed color of Mindum or Kubanka No. 8 parents. Rust resistance and seed color appeared to be inherited independently.

RESULTS OF INOCULATIONS OF KUBANKA NO. 8 × PENTAD F_3 FAMILIES WITH BIOLOGIC FORM XXXIV.

The data obtained for Kubanka No. 8 × Pentad with Form XXXIV are summarized in Table IV. About 35 control pots of Kubanka No. 8 were inoculated. Of the 308 plants that were infected, 21, or about 7 per cent, had type 1 infections and 287 showed types ranging from x− to 4. In view of the fact that possible errors and variations may result in types of infection outside of the normal range, the type 1 infection on these 21 plants was regarded here as exceptional. The range of infection types on Kubanka No. 8 tested with Form XXXIV was therefore considered to be from x− to 4 (Pl. I, B). All Kubanka No. 8 pots with plants giving types of infection falling within this range were classified as susceptible. Those pots with 9 or more plants, one of which had a type 1 infection, were placed in the near-susceptible class. Pots having a greater percentage of plants with type 1 infections were classed as heterozygous. The distribution of the Kubanka No. 8 pots in the five classes of infection was 5 heterozygous, 5 near-susceptible, and 25 susceptible. There is no reason to believe that 5 of the parent control pots were actually heterozygous

but, rather, the method of classification shows the accuracy with which the determination of pure-line material could be made.

TABLE IV.—*The reaction of Kubanka No. 8×Pentad and F_3 families to biologic Form XXXIV*

Parent varieties and F_3 families.	Class of infection.	Distribution of F_3 plants according to the type of infection shown.							Total of pots of parent varieties or F_3 families.
		0	1	2	X−	3	X+	4	
Kubanka No. 8	Heterozygous	.....	15	....	10	.....	6	14	5
Do.	Near-susceptible	.....	6	....	5	6	14	15	5
Do.	Susceptible	.....	.....	....	56	1	24	136	25
Total		.....	21	....	71	7	44	165	35
Pentad	Resistant	4	270	....	30	.....	.....	.....	30
Do.	Near-resistant	.....	36	....	4	.....	4	.....	4
Do.	Heterozygous	.....	12	....	2	.....	7	.....	2
Total		4	318	....	36	.....	11	.....	36
F_3	Resistant	.....	70	....	.....	.....	.....	.....	9
Do.	Near-resistant	.....	52	....	10	.....	.....	.....	5
Do.	Heterozygous	.....	297	....	183	.....	91	256	84
Do.	Near-susceptible	.....	5	....	30	3	31	112	20
Do.	Susceptible	.....	1	....	108	13	114	1,025	128
Total		.....	425	....	331	16	236	1,393	246

The legitimacy of the foregoing method of analysis will become evident if a brief study is made of the type of data obtained and the object of the work. To make possible a classification which would form an adequate basis for the comparison of the reactions of the F_3 hybrids with those of the parental varieties it was necessary to establish a range for the types of infection normal for each parent. It might appear that the total distribution of types of infection of a parental variety should be considered its normal range, and the hybrid reactions judged accordingly. This would throw all the pots of each parent into a single class, viz, with Kubanka No. 8 there would be 35 pots in the susceptible class, which would be the same as ignoring the possibility of any error in the results. Obviously that would lead to inaccuracies in classifying the hybrids, for it is well established that even with carefully controlled conditions a certain amount of error is unavoidable. The method used has on the contrary much in its favor, for it tends to throw all doubtful F_3 families into the near-susceptible, heterozygous, or near-resistant classes.

Thirty-six control pots of Pentad were inoculated. Of the 369 plants that were infected, all but 11 ranged from 0 to X− in type of infection. These 11 showed infection of the X+ type. Classification of the pots on the same basis as was used in the case of Kubanka No. 8 resulted in 30 being placed as resistant, 4 near-resistant, and 2 heterozygous (Pl. I, A).

The use of these terms to designate the different classes of infection is relative. It is convenient to call one parent susceptible and the other resistant regardless of whether the susceptibility or resistance is partial or complete. For the purpose of studying the genetics of the inheritance

of the rust reaction it is immaterial just what the classes are called. In the present cross, the Kubanka No. 8 parent is only partially susceptible but the Pentad parent is quite resistant. It thus happens that the ranges for the two parent varieties overlap somewhat at the x− type of infection.

A total of 246 F_3 families were tested. The number of F_3 plants that showed infection was 2,401. The distribution of F_3 plants according to their types of infection and to the susceptible classes of the various families to which they belonged is given in Table IV. The 246 F_3 families were classified as 9 resistant, 5 near-resistant, 84 heterozygous, 20 near-susceptible, and 128 susceptible (Pl. I, C–E). Then, one-sixth of the pots of Pentad, the resistant parent, were classed other than resistant. Considering the F_3 on this basis, there were 9 plus 1.5, or 10.5 families which were as resistant as the resistant parent. The ratio of this number to that of the remaining families is 1:22.4. The presence of 2 factors for rust reaction fairly satisfactorily explains the results. There was no evidence that either susceptibility or resistance was dominant, for the F_3 plants in the heterozygous class occurred in approximately equal numbers on either side of the x− type.

RESULTS OF INOCULATIONS OF MINDUM × PENTAD F_3 FAMILIES WITH BIOLOGIC FORM I

The data obtained with Form I are summarized in Table V. Twenty-three control pots of each of the parental varieties were inoculated. Of the 274 Mindum plants that were infected, 269 had 0 and 1 types of infection with a killing of the leaf tips in most cases, and 5 had type 4 infections. These 5 plants clearly were either not Mindum or else they had been infected by chance spores of some other form. Accidental infection is almost unavoidable where many rust forms are being worked with but fortunately it is of infrequent occurrence and usually can be detected.

TABLE V.—*The reaction of the F_3 of Mindum × Pentad to biologic Form I*

Parent varieties or F_3 families.	Class of infection.	Distribution of F_3 plants according to the type of infection shown.							Total pots of parent varieties or F_3 families.
		0	1	2	X−	3	X+	4	
Pentad	Heterozygous	13	4	....	9	1	5	.....	4
Do.	Near-susceptible	1	2	....	6	3	10	.....	3
Do.	Susceptible	.....	.....	....	10	13	14	76	16
Total		14	6	....	25	17	29	76	23
Mindum	Immune	236	12	....	.....	.....	.....	.....	20
Do.	Near-immune	15	.....	....	.....	.....	.....	2	2
Do.	Heterozygous	6	.....	....	.....	.....	.....	3	1
Total		257	12	....	.....	.....	.....	5	23
F_3	Immune	603	.....	....	.....	.....	.....	.....	44
Do.	Near-immune	158	.....	....	.....	7	1	5	13
Do.	Heterozygous	723	9	2	16	60	22	190	82
Do.	Near-susceptible	10	3	....	10	22	15	68	10
Do.	Susceptible	.....	.....	....	6	72	2	165	17
Total		1,494	12	2	32	161	40	428	166

The range of infection for Mindum was considered to include 0 and 1 types of infection. In the immune class, 236 of the 248 plants had 0 type infections, hence the immunity of Mindum to Form I is evident. The pots of Mindum were classified as 20 immune, 2 near-immune, and 1 heterozygous (Pl. 2).

In the 23 control pots of Pentad 167 plants were infected. Of these, 147 had types of infection ranging from x− to 4, and 20 showed types 0 and 1. Leaving these 20 plants out of consideration, the range of the type of infection was from x− to 4, as was the case with Kubanka No. 8 when inoculated with Form XXXIV. At first glance it would seem that 20 is a rather large number of plants to leave out of consideration in a population of 167. It is not as significant as it appears to be, however, for many of the plants with 0 type infections showed only a few vague necrotic flecks which were not clearly distinguishable from similar effects sometimes produced on seedlings as a result of environmental conditions. The classification of Pentad pots gave 16 as susceptible, 3 near-susceptible, and 4 heterozygous (Pl. 2).

A total of 166 F_3 families were tested. The number of F_3 plants inoculated was 2,340. Of these 2,169 showed infection. The distribution of F_3 plants according to their types of infection and to the classes of the various families to which they belonged is given in Table V. The 166 F_3 families were classified as 44 immune, 13 near-immune, 82 heterozygous, 10 near-susceptible, and 17 susceptible (Pl. 2).

A larger proportion of families was classified as immune and near-immune than as susceptible and near-susceptible. This was to be expected from the progeny of the parents, one of which was immune and the other only moderately susceptible. Approximately one-eighth of the pots of Mindum, the immune parent, fell outside the immune class. The F_3 progeny, considered on this basis, shows 44 plus 5.5, or a total of 49.5 families which appeared to be as immune as the immune parent. The ratio of 49.5 to 166, which is the total number of families classified, would seem to indicate the presence of a single differential factor. The variable results obtained with the Pentad parent prevent a more detailed analysis.

The distribution of plants in the heterozygous class with respect to the various types of infection shows a total of 734 with 0, 1, and 2 types of infection (723 being of the 0 type) and 288 with types ranging from x− to 4. The dominance of immunity is evident.

RESULTS OF INOCULATIONS OF MINDUM×PENTAD F_3 FAMILIES WITH BIOLOGIC FORM XXXIV

The results obtained are summarized in Table VI. Thirty-three control pots of each of the parental varieties were inoculated. The reactions of Mindum and Pentad to Form 34 were the reciprocals of their reactions to Form I. Of the 375 Mindum plants that showed infection all but 9 had x+ and 4 types of infection. The remaining 9 plants had x− and 3 types of infection. Mindum is here the susceptible parent with a range of types of infection which is narrower than that of the susceptible parents in the work previously discussed. The pots of Mindum were classified as 2 heterozygous, 4 near-susceptible, and 27 susceptible (Pl. 3).

TABLE VI.—*The reaction of the parent varieties and F_3 families of Mindum×Pentad o biologic Form XXXIV.*

Parent varieties or F_3 families.	Class of infection.	Distribution of F_3 plants according to the type of infection shown.							Total pots of parent varieties or F_3 families.
		0	1	2	X−	3	X+	4	
Mindum	Heterozygous	.....	.....	....	3	2	2	14	2
Do	Near-susceptible	.....	.....	....	1	3	7	31	4
Do	Susceptible	.....	.....	....	.....	.....	39	273	27
Total		.....	.....	....	4	5	48	318	33
Pentad	Resistant	37	115	3	131	2	.....	.....	26
Do	Near-resistant	3	18	....	24	.....	5	.....	5
Do	Heterozygous	.....	2	....	9	3	4	1	2
Total		40	135	3	164	5	9	1	33
F_3	Resistant	38	53	8	95	.....	.....	.....	14
Do	Near-resistant	12	29	1	53	.....	8	.....	6
Do	Heterozygous	38	48	8	400	144	232	139	68
Do	Near-susceptible	.....	.....	....	8	4	47	90	11
Do	Susceptible	.....	.....	....	.....	1	39	109	11
Total		88	130	17	556	149	326	338	110

Infection appeared on 357 of the inoculated Pentad plants. Of these 9 reacted with x+ type infections and 1 gave a 4 type. The other 347 Pentad plants gave a range of infection types from 0 to 3. Only 5 plants showed type 3 infections, therefore this type would appear not to belong to the range for the resistant parent. However, the type 3 infection is much more like the x− type than like the x+. In fact, experiments indicate that a variety of which the seedling plants give type 3 infection in the greenhouse is resistant in the field, whereas varieties showing x+ in the greenhouse probably are not resistant in the field. Consequently, the 3 type infection may well be included in the range of the Pentad parent. Classification of Pentad pots gave 26 resistant, 5 near-resistant and 2 heterozygous (Pl. 3).

A comparison of the results obtained for Pentad in the study with the Kubanka No. 8 × Pentad cross with those obtained here reveals considerable differences in the number of plants showing the various types of infection. The two series of inoculations were made at different periods, and consequently the conditions were not comparable. This strikingly brings out the fact that the value of the data taken on the hybrid material lays in the direct comparison made with the results from pots of parental plants grown under identical conditions. Reactions which, under one set of circumstances, would place an F_3 family in the near-resistant class might, under other circumstances, cause it to be classed as heterozygous, depending on the parental reactions at each period.

It was noticeable that both parent and hybrid plants showed an abundance of hypersensitiveness to Form XXXIV. This probably accounts in great measure for the appearance of such large numbers of x− and x+ types of infection. Sharp hypersensitive areas occurred around a large proportion of the uredinia of types 3 and 4 infections. Usually type 4 shows

very little chlorosis and no necrosis. Here, however, about 50 per cent of the type 4 uredinia showed such a decided necrotic bordering that the advisability of creating a special class for this type of infection was considered. Eventually it was concluded that the establishment of an additional type would possibly lead to some confusion without really proving of value, as it was apparent that plants infected in this manner were susceptible, on account of the development of numerous large uredinia.

A total of 110 F_3 families were tested. There were 1,711 F_3 plants inoculated, of which 1,604 showed infection. The distribution of F_3 plants is summarized in Table VI. Classification of the 110 F_3 families gave 14 as resistant, 6 near-resistant, 68 heterozygous, 11 near-susceptible, and 11 susceptible (Pl. 3). Since between a third and a fourth of the Pentad pots were placed outside of the resistant class, it may be considered that 14 plus 4 or 18 F_3 families were as resistant as the resistant parent. This number of resistant families is too small to indicate definitely the presence of a single factor, and it is much too large to make a two-factor hypothesis feasible. The single factor is the more plausible explanation.

If the near-resistant and resistant classes are grouped together, and similarly, the near-susceptible and susceptible classes, a fairly satisfactory 1:2:1 ratio is obtained. The presence of a single factor would appear probable in this case. The results do not make possible a more exact genetic analysis. Examination of the distribution of F_3 plants in the heterozygous class showed a lack of dominance for either susceptibility or resistance.

COMBINATIONS OF SUSCEPTIBILITY AND RESISTANCE OF MINDUM × PENTAD F_3 FAMILIES TO FORM I AND FORM XXXIV

Table VII correlates the results obtained with Form I and Form XXXIV for those families which were inoculated with both forms. Various combinations of susceptibility, resistance, and heterozygosity to the two biologic forms appear. Six F_3 families were definitely resistant to Form XXXIV and immune from Form I as, for example, 809-21, 805-16, and 796-4 (Pl. 4, A, B, C). Two families were quite susceptible to both forms, as 804-7 (Pl. 4, E). Other families were resistant to or immune from one form and susceptible to the other. Still other F_3 families were resistant or susceptible to one form and heterozygous to the other, as 806-24 (Pl. 4, D). Many families were heterozygous to both forms. There were also combinations of near-resistance and near-susceptibility, as 809-8 (Pl. 4, F).

TABLE VII.—*The classified reaction of Mindum × Pentad F_3 families to biologic Forms I and XXXIV*

Reaction to Form XXXIV.	Reaction to Form I.					Total.
	Immune.	Near-immune.	Heterozygous.	Near-susceptible.	Susceptible.	
Resistant	6	0	8	0	0	14
Near-resistant	1	0	2	1	1	5
Heterozygous	14	7	36	3	7	67
Near-susceptible	3	1	5	1	1	11
Susceptible	3	1	3	1	2	10
Total	27	9	54	6	11	107

The most significant feature of Table VII is that six F_3 hybrid families are shown to have been definitely resistant to both biologic Forms I and XXXIV. Considering the reaction of the parental varieties to these biologic forms, it is very likely that at least one of the two near-resistant families was likewise homozygous in its reaction. On this basis it would seem that two main differential factors may explain the results obtained. These factors, if present, are inherited independently, which makes possible the combining of resistance to both forms in a single variety.

THE REACTION OF THE MINDUM × PENTAD F_3 FAMILIES FROM WHITE-SEEDED PARENTS TO FORM I AND FORM XXXIV

Table VIII correlates the results obtained for F_3 families from white-seeded parents when inoculated with Forms I and XXXIV. Out of a total of 34 families 2 were resistant to Form XXXIV and immune from Form I. The indications of 3: 1 ratios for Forms I and XXXIV are here substantiated in a very close 15: 1 ratio.

TABLE VIII.—*The classified reaction of the progeny of Mindum × Pentad white-seeded F_2 plants to biologic Forms I and XXXIV*

Reaction to Form XXXIV.	Reaction to Form I.					Total.
	Immune.	Near-immune.	Heterozygous.	Near-susceptible.	Susceptible.	
Resistant	2	0	3	0	0	5
Near-resistant	1	0	2	0	2	5
Heterozygous	3	2	11	1	2	19
Near-susceptible	0	0	0	0	1	1
Susceptible	0	0	0	0	0	0
Total	6	2	16	1	5	30

The object of giving special treatment to the F_3 progeny of white-seeded F_2 plants is a recognition of the fact that the economic aspects of a problem warrant careful consideration. Pentad, although high in rust resistance, is a poor milling wheat. Owing to the difficulty of distinguishing the thrashed grain of one red durum from another, millers have come to discriminate against all red durums. There is consequently no immediate future for a new rust-resistant red durum, even though it be of good milling quality.

DISCUSSION

The study of the reaction of the F_3 generation of the durum crosses Mindum × Pentad and Kubanka No. 8 × Pentad to two biologic forms of *P. graminis tritici* has shown that all combinations of susceptibility and resistance to these forms may appear. The reaction of Kubanka No. 8 × Pentad F_3 families to Form XXXIV indicated the presence of two factors with neither susceptibility nor resistance dominant. The results obtained from Mindum × Pentad F_3 families inoculated with Form I can be explained on the basis of a single main factor differenc with immunity dominant. The data for the reaction of Mindum × Penta

F_3 families to Form XXXIV gave some indication that a single factor was present, but dominance was not apparent. No evidence of a correlation between seed color and rust reaction was found.

As the action of each biologic form in general was fairly uniform on individual plants of the differential varieties and also on the plants of Kubanka No. 8, Mindum and Pentad and the F_3 families, barring the x− and x+ types of infection, it may be concluded that the urediniospores of each form probably were of the same genetic constitution. Therefore, the factors concerned with susceptibility and resistance must have been located in the plants themselves. Furthermore, as different biologic forms differ in their parasitizing capabilities on the same varieties of wheat, their genetic constitution can not be the same. It is probable, then, that factors governing susceptibility and resistance to different biologic forms used in this experiment are different in nature and are located in different chromosome pairs. The results of the present investigation show that when two varieties of wheat react reciprocally to two biologic forms of *P. graminis tritici*, one variety being resistant to one form and the other to the other form, it is possible by means of crossing these varieties to obtain progeny resistant to both forms of rust. These results furnish further evidence for the belief that eventually a variety of wheat can be produced which will be resistant to all biologic forms of this rust.

SUMMARY

(1) A study was made of the parasitic capabilities of two biologic forms of *Puccinia graminis tritici* on the F_3 progeny from crosses between three varieties of *Triticum durum*.

(2) Two of the parental varieties, Mindum and Pentad, reacted reciprocally to two of the biologic forms used.

(3) The constancy of the parasitic capabilities of the biologic forms had been determined previously and, as additional evidence, complete sets of differential varieties were inoculated at the commencement and at the completion of the work with each form. Form XXXIV attacked Kubanka No. 8 but had little effect on Pentad. Form I produced no uredinia on Mindum but developed vigorously on Pentad. On the other hand, Form XXXIV infected Mindum severely but developed weakly on Pentad.

(4) A separate set of 8 to 20 plants from each F_3 family was grown for use with each form of rust. Only first leaves were inoculated.

(5) With respect to the reaction of the F_3 families to each of the two biologic forms of rust there were some families that were as resistant as the resistant parent, some as susceptible as the susceptible parent, and others which were heterozygous.

(6) The results of using Form XXXIV on Kubanka 8 × Pentad F_3 families indicated the presence of two differential factors.

(7) A single main factor difference explained fairly satisfactorily the results obtained for Mindum × Pentad F_3 families inoculated with Form I.

(8) The results obtained when Mindum × Pentad F_3 families were infected with Form XXXIV, gave some indication of the presence of a single factor. The data would not permit, however, a definite genetic analysis.

(9) No relation was found between seed color and rust resistance.

(10) All combinations of susceptibility and resistance of individual Mindum × Pentad F_3 families to Form I and Form XXXIV appeared. Out of a total of 110 F_3 families 6 were highly resistant to both forms of rust and 2 of these families were from white-seeded parents.

(11) With the varieties of wheat used, it was found possible to combine, in a single variety, resistance to two biologic forms of stem rust of wheat, when crosses were made between two varieties which reacted reciprocally to these rust forms.

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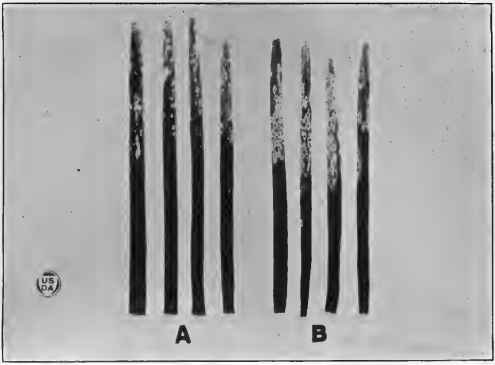
PLATE 1

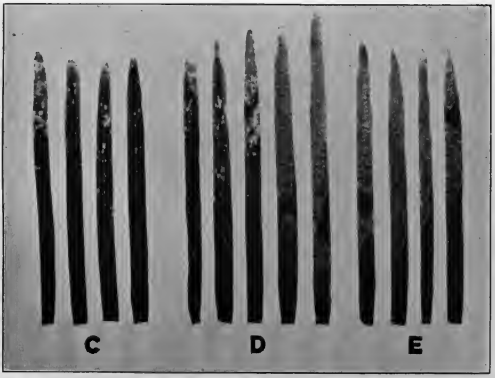
The reaction of Pentad, Kubanka No. 8, and F_3 hybrids to biologic Form XXXIV.

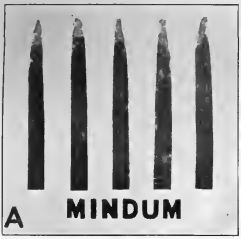
A.—Pentad, the resistant parent, normally giving the o-type of infection with an occasional uredinium.

B.—Kubanka No. 8, the susceptible parent, giving numerous uredinia more or less surrounded by chlorotic areas.

C, D, E.—Typical F_3 families that appeared resistant, heterozygous, and susceptible, respectively.



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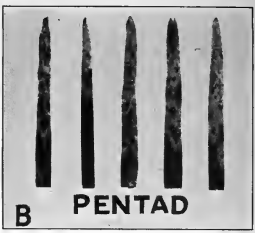
MINDUM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PENTAD


C
SEGREGATING F_3

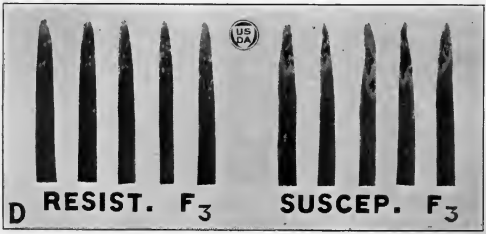

D
RESIST. F_3
SUSCEP. F_3

PLATE 2

The reaction of Mindum, Pentad, and F_3 hybrids to biologic Form I.

A.—Mindum is immune, normally giving a few hypersensitive flecks.

B.—Pentad is susceptible with types of infection varying from x− to 4.

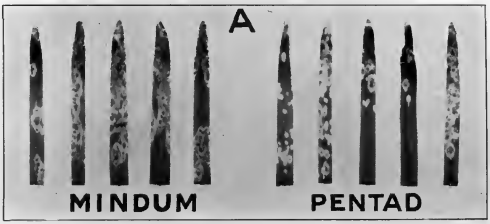
C, D.—The F_3 families shown are, respectively: 802–6, which appeared heterozygous; 809–5, which was immune like Mindum; 804–17, which showed greater susceptibility than Pentad.

PLATE 3

The reaction of Mindum, Pentad, and F_3 hybrids to biologic Form XXXIV.

A.—Mindum, the susceptible parent, normally giving x+ and 4 types of infection, principally the latter; Pentad, the resistant parent, with a range of infection types from 0 to x−.

B, C.—The F_3 families shown are, respectively: 807–18, heterozygous; 805–16, as resistant as the resistant parent; 804–17, susceptible like Mindum.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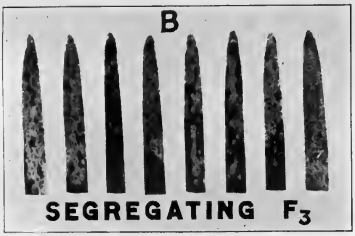
MINDUM
PENTAD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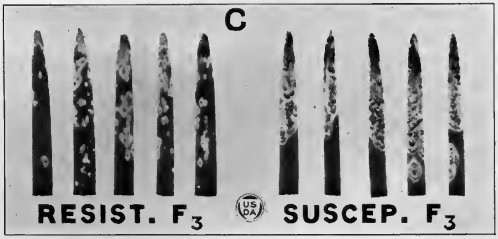
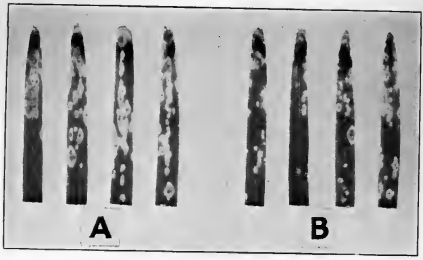
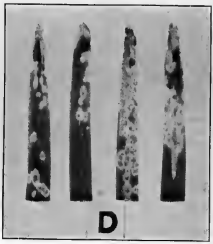
SEGREGATING F_3


C
RESIST. F_3
SUSCEP. F_3


A
B


C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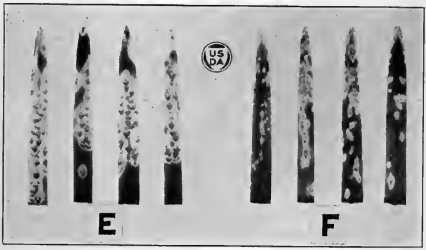
E
F

PLATE 4.

The reaction of certain Mindum × Pentad F_3 families to biologic Form XXXIV.

A, B.—Two F_3 families (809–21 and 805–16, respectively) from white-seeded parents, immune from Form I and resistant to Form XXXIV.

C.—Family 796–4, from red-seeded parents and similar in reaction to A and B.

D.—Family 806–24, which was heterozygous to Form I and resistant to Form XXXIV.

E.—Family 804–7, which was highly susceptible to both forms of rust.

F.—Family 809–8, which fell into the near-susceptible class with Form I and in the near-resistant class with Form XXXIV.

A STUDY OF RUST RESISTANCE IN A CROSS BETWEEN MARQUIS AND KOTA WHEATS [1]

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INTRODUCTION

A knowledge of the mode of inheritance of certain plant characters is essential if the breeder is to outline his particular problem. Accordingly one of the first steps in the investigations carried on for the purpose of producing rust-resistant spring wheats has been to determine the genetic possibilities of attaining the desired end.

Several years ago it was a common belief that the rust organism changed its infection capabilities so rapidly as to seriously interfere with the production of rust-resistant wheat. The investigations of Stakman and his coworkers (*6*, *7*, and *8*)[2] at the Minnesota station have proved that the variable results which have been obtained under field conditions are due to numerous biologic forms of rust which can only be differentiated by their manner of infecting particular pure lines of wheat. This has led naturally to the survey to determine the prevalence of particular rust forms and to the use of these forms in breeding experiments. The method is to determine those wheat varieties which are resistant to particular rust forms and then by means of crosses to build up desirable rust-resistant varieties.

Kota (*9*) is the only rust-resistant spring common wheat which so far has been discovered, except for certain recently produced hybrids. Under humid conditions, however, Kota does not yield as well as Marquis and is seriously lacking in strength of straw.

The purpose of this paper is to present certain studies of a cross between Kota and Marquis which were carried on with the hope of solving the following questions:

1. Is the resistance of Kota to different biologic forms due to a single genetic factor, as in the case of Kanred?

2. Given Kota and Marquis, which react reciprocally to two biologic forms of rust, is it possible to produce from a cross of Kota and Marquis a variety resistant to both biologic forms?

3. To determine the possibility of using the reaction of F_3 seedlings in the greenhouse to particular biologic forms of stem rust as a means of isolating F_3 families which have the resistance of the Kota parent under field conditions.

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[2] Reference is made by number (italic) to "Literature cited," p. 1012.

THE PRESENT STATUS OF THE PROBLEM

For reviews of earlier studies which have led to the present mode of attack, the reader is referred to previous papers (*3*, *4*). In the present paper it seems sufficient to sum up the facts now at hand without making an extended review of literature.

Thirty-seven biologic forms of stem rust have been discovered (*6*), and of these 21 have been found in the upper Mississippi Valley. If the resistance of a wheat variety to each of these biologic forms was due to a separate set of independently inherited factors the problem would be a very difficult one. If the resistance to one biologic form was due to a genetic factor which was allelomorphic to a factor for resistance to a second form the problem of obtaining a wheat resistant to all biologic forms would be an impossibility. No variety of common wheat has been found which is resistant to all biologic forms of rust; but Khapli, an early maturing emmer, is resistant to all 37 forms.

Crosses of Khapli with common varieties have given self-sterile F_1 progeny. This has led to the attempt to transfer the resistance of Khapli to a durum variety, with the hope of then transferring this resistance to common wheats by a subsequent cross.

That the resistance of durum wheats can be transferred to common wheats has been shown. Several years ago a cross was made between Iumillo and Marquis. There was found to be a strong linkage between the durum characters and resistance although this was overcome by growing large numbers. Several plants were obtained from an F_3 population of from 20,000 to 30,000 plants which appeared to have the spike characters of common wheat and which also appeared rust resistant. Selection has been practiced further and the wheats thus obtained appear of desirable field type and have shown high resistance in field experiments.

Kanred, a rust-resistant winter wheat, produced at the Kansas station, proved immune from 11 of the 21 biologic forms of rust which are found in the North-Central States. This immunity was transferred to spring wheats by crosses between Kanred and Marquis. The immunity from all 11 biologic forms was apparently due to a single genetic factor (*1*, *2*).

Puttick (*5*), in a study of the F_2 progeny of a cross between Mindum and Marquis, obtained seedling wheats which combined the resistance of both parents. In this study, two biologic forms of rust were used to which Marquis and Mindum reacted reciprocally, one parent being resistant and the other susceptible to the respective rust forms. Puttick, however, did not grow the seedlings to maturity so it was impossible to say whether they were of common or durum types.

In the paper preceding this, Harrington and Aamodt (*2a*) have presented the results of a cross between Pentad, which is resistant to biologic Form XXXIV and susceptible to biologic Form I, and Mindum, which reacts in a reciprocal way to these rust forms. The resistance of Pentad to Form XXXIV and of Mindum to Form I appears to be due to a single main genetic factor in each case. These two factors appear to be independently inherited and in the production, from the cross Pentad × Mindum, of a wheat which is resistant to both biologic forms, it is only necessary to grow enough plants to obtain the necessary recombination of the two factors for resistance.

The brief outline given shows the present status of the problem, the mode of attack being to build up synthetically, by certain crosses, wheats which are resistant to as many biologic forms as possible. For this mode of attack a knowledge of the number and nature of the inherited factors is essential.

MATERIAL AND METHODS

Certain general methods of work have been used as in previous studies. For this reason only brief descriptions will be made.

Pure lines of the parental varieties were used in making the crosses. The F_1 generation plants were grown in the greenhouses[3] at Washington, D. C., during the winter of 1920–21 and the F_2 generation was grown during the summer of 1921. The season was a very unfavorable one and the seed produced was rather badly shrivelled in certain cases. Individual plant data on spike characters, average seed length, seed plumpness, and seed texture were taken and the F_3 seedlings obtained from sowing seed of individual F_2 plants were inoculated in the greenhouse with the rust forms chosen.

In deciding which rust forms[4] to use, a study was made of the degrees of infection of seedlings of Marquis and Kota when inoculated at the same time and handled in a similar manner.

Eleven biologic forms were available in the greenhouse and all were used in inoculations for the purpose of discovering two to which the two wheat varieties reacted reciprocally. The reactions obtained on Marquis and Kota are given in Table I. Although Kota is rather highly resistant as tested under field conditions, the difference in seedling infection of Marquis and Kota in the greenhouse is not very great. In nearly all cases, however, where there is a difference, Kota shows a lower degree of infection than Marquis. The meaning of the symbols used to designate the types or degrees of infection is given after Table I.

TABLE I.—*Reaction of Kota and Marquis to 11 forms of stem rust, expressed by type of infection produced*

Variety.	Type of infection produced by biologic form of rust No.—										
	I.	III.	IX.	XVII.	XVIII.	XIX.	XXI.	XXVII.	XXIX.	XXXII.	XXXIV.
Marquis.....	4	4−	3	4	4	1 and 2	4	2+	4	3+	3+
Kota.....	3+	3+	3	4−	3	3	3+	o	3+	3	3

[3] The writers are indebted to Mr. J. Allen Clark, of the Office of Cereal Investigations, for growing the F_1 generation and returning seed of the same.

[4] The biologic forms used were obtained from M. N. Levine, who kindly made them available for our use

Explanation of classes of host reaction and of corresponding types of infection resulting from inoculation of seedling wheat plants with spores of Puccinia graminis tritici [5]

Classes of host reaction.	Types of infection.
Immune (immune in the field)	0. No uredinia developed but definite hypersensitive areas present.
Very resistant (highly resistant under field conditions)	1. Uredinia minute and isolated; surrounded by sharply-defined, continuous, hypersensitive areas; hypersensitive areas lacking uredinia also may be present.
Moderately resistant (highly resistant under field conditions)	2. Uredinia isolated and small to midsized; hypersensitiveness present in the form of necrotic areas in circles; uredinia often surrounded by green islands.
Moderately susceptible (somewhat resistant in the field)	3. Uredinia midsized; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.
Very susceptible (entirely susceptible under field conditions)	4. Uredinia large or varying from midsized to large, numerous and confluent; true hypersensitiveness entirely absent, chlorosis seldom present.

The studies which have been made show that a pure-line wheat variety inoculated with a single biologic form frequently shows a variation in infection from type 3 to type 4 and occasionally from type 2 to type 3, due to varying environmental conditions to which the seedlings are exposed, even when the conditions of the greenhouse test are kept as uniform as possible. These variations apparently are not due to changes in the genotype.

The most striking differences in the infection of Kota and Marquis were obtained with Form XIX and Form XXVII and accordingly these two forms were chosen for the study. Form XIX was collected in Pocatello, Idaho, in November, 1918, and has been cultured through approximately 51 urediniospore generations. The Form XIX used to inoculate the F_4 material was collected in North Dakota in July, 1921, and has been cultured through approximately 17 generations. Form XXVII was collected in Barges, France, in August, 1919, and has been cultured through approximately 45 urediniospore generations.

Between 15 and 20 seeds of each individual F_2 plant were sown in a single pot, but, owing to the unfavorable conditions under which the F_2 plants were grown, the germination of these seeds was low. No pots were used in the study in which the number of F_3 plants per pot fell below 8. While 8 plants is much too small a number to prove absolutely the genetic nature of the individual F_2 parent, it gives a fair indication of the genetic class in question. Larger numbers of seeds could not have been used without depleting the quantity of seed reserved for sowing to obtain F_3 families under field conditions. The results obtained with the small numbers of seed used are reliable enough, however, to furnish a good idea of the approximate number of factors involved, and, from the plant-breeding standpoint, the information obtained is sufficient to answer the main questions for which the investigation was undertaken. It is recognized that from the genetic standpoint larger numbers of seedlings in each F_3 family should be inoculated.

[5] These descriptions of types of infection in the greenhouse are based on those given by Stakman and Levine (6).

One of the problems which it was hoped to solve was the discovery of a greenhouse test of F_3 seedlings which would give an accurate estimate of the behavior of Marquis × Kota F_3 families under field conditions. As no such test was available, the larger part of the Marquis × Kota material was grown in the rust nursery and an artificial epidemic of stem rust induced by the use of the available biologic forms of stem rust which were present in the greenhouse and which were known to have been found in the spring-wheat region. The forms of rust used for the field epidemic in 1922 are those listed in Table 1, with the exception of Form XXVII, which was originally collected in Barges, France, and Form XXXIV which was collected in South Africa. These two forms were not used for the nursery epidemic.

Between 25 and 50 plants were grown in the field nursery from each selected F_3 family. The rust infection was heavy on susceptible types, as shown by the infection on Marquis, and consequently the determination of those F_3 families which had the Kota type of resistance to the nine biologic forms in question could be made with accuracy.

EXPERIMENTAL RESULTS

DWARF PLANTS OBTAINED IN THE F_2 PROGENY

The F_2 material of the Marquis × Kota cross grown in the plant-breeding nursery in 1921 was not severely infected with stem rust and, therefore, was used for the greenhouse studies. Considerable material also was grown in the rust nursery. With most hybrid material in which Kota had been used as one parent, dwarf plants appeared in the F_2 generation. The frequency of the appearance of such plants was determined for the F_2 progeny which grew in the rust nursery in 1921. Of a total of 787 F_2 plants, 666 were of normal type and 121 were dwarfs. Seed from some of these dwarfs was sown in the greenhouse and only dwarf plants resulted. The results may be explained genetically by the assumption that one of the parents contains two factors, one for dwarf habit and the other which inhibits the expression of the dwarf factor. On this basis a 13:3 ratio of normals and dwarfs is expected in F_3. The number of dwarfs expected in a population of 787 would be 147. The number of dwarf plants obtained was somewhat less than the expectation for a 13:3 ratio.

THE AWNLESS OR BEARDED CHARACTER IN RELATION TO SEED CHARACTERS

Kota is a bearded wheat, while Marquis has short apical awns and is commonly classified as awnless. The F_1 hybrids have somewhat longer apical awns than Marquis. At harvest time some of these tip awns were broken off and it was impossible to separate the awnless from the hybrid types with certainty. For this reason awnless and apically-awned (hybrid) forms were placed together. In an earlier study of a cross between Marquis and Preston, experimental data gave reason for the belief that the awn was an important physiological organ under the conditions of the experiment. Accordingly a somewhat similar study was made of the Kota × Marquis plants (Tables II, III, IV).

TABLE II.—*Distribution of F_2 plants of the cross between Marquis and Kota into classes according to average length of seed, in relation to plant and spike characters*

Plant type.	Classes for seed length (in millimeters).						Total plants.	Mean.
	4.8	5.1	5.4	5.7	6.0	6.3		
Normal:								
Awnless	.....	12	72	174	114	12	384	5.73±0.01
Bearded	.....	2	11	51	64	9	137	5.85±0.01
Dwarf:								
Awnless	5	9	5	3	1	1	24	5.26±0.07
Bearded	3	3	6	.....	.....	.....	12	5.18±0.05

TABLE III.—*Distribution of F_2 plants of the cross between Marquis and Kota into classes according to the percentage of hardness of seed, in relation to plant and spike characters*

Plant type.	Classes for percentage of hardness (100=corneous).								Total plants.	Mean.
	40	45	50	55	60	65	70	75		
Normal:										
Awnless	2	....	53	119	162	24	23	1	384	58.92±0.18
Bearded	....	1	24	51	46	10	5	....	137	57.01±0.29
Dwarf:										
Awnless	....	....	13	11	7	2	....	....	33	54.70±0.54
Bearded	....	....	3	8	3	....	....	....	14	55.00±0.04

TABLE IV.—*Distribution of F_2 plants of the cross between Marquis and Kota into classes according to the percentage of plumpness of seed, in relation to plant and spike characters*

Plant type.	Classes for percentage of plumpness.											Total plants.	Mean.
	30	40	45	50	55	60	65	70	75	80	85	90	
Normal:													
Awnless	2	2	...	13	...	30	14	82	115	36	86	4	384	74.02±0.33
Bearded	...	...	...	1	...	9	6	31	33	33	18	6	137	75.44±0.44
Dwarf:														
Awnless	...	5	1	11	1	4	2	4	5	...	...	...	33	56.82±1.38
Bearded	...	...	1	3	1	1	2	5	1	...	...	...	14	61.79±1.73

Apparently the presence of the awn results in the production of a somewhat longer seed on the normal plants, the average difference being about 0.1 mm. The texture of the seeds of the awnless normal plants was somewhat harder than that of those on the bearded plants. The percentage of plumpness of the seed of the bearded normal plants averaged slightly higher than in the case of the awnless plants although, in the light of the probable error, the difference obtained is not significant. In the determinations of texture, 100 is the standard for completely corneous seed, the border line between subcorneous and soft being around 50. The percentage of plumpness of seed is taken in a somewhat similar manner.

REACTION OF F_3 SEEDLINGS AND THEIR PARENT VARIETIES TO BIOLOGIC FORMS XIX AND XXVII

Pots of the parent varieties, Marquis and Kota, inoculated and handled in the same manner as the hybrid F_3 families, were used as controls. In order to assist in the study the parents and the various F_3 families were classified into groups on the basis of their resistance or susceptibility to infection by these two forms of rust as follows:

Classes of resistance and susceptibility to infection in the Marquis-Kota cross when F_3 seedling families and the parent varieties were inoculated with biologic Forms XIX and XXVII in the greenhouse in the winter of 1921–22

BIOLOGIC FORM XIX

IR=Resistant in classes of infection types 1 to 2, or that of the Marquis parent.
HR=Heterozygous with the Marquis or types 1 and 2 reaction dominant.
H=Heterozygous with the Kota or type 3 reaction dominant.
HS=Reaction mainly of the Kota type, type 3, with an occasional plant of the Marquis type of reaction.
IS=Reaction of type 3, like the Kota parent.

BIOLOGIC FORM XXVII

R=Infection of type 0, or the immunity of the Kota parent.
IR=Reaction of type 2, the resistance of the Marquis parent. The uredinia were somewhat larger than for the normal type 2 infection.
H_1=Heterozygous with reactions of types 0 and 2.
H_2=Heterozygous with reactions of types 0, 2, and 4.
H_3=Heterozygous with reactions of types 2 and 4.
H_4=Heterozygous with reactions of types 0 and 4.
S=Susceptible with reaction in type 4 only.

The results obtained from inoculating the parents and F_3 hybrids with biologic Form XIX are given in Table V.

TABLE V.—*Distribution of F_3 seedlings of the cross between Kota and Marquis according to types of infection caused by biologic Form XIX*

Classes of parents and F_3 crosses.	Distribution by types of infection.										Number of plants.	Number of families.
	0	−1	1	1+	−2	2	2+	−3	3	3+		
F_3 families IR	4	17	170	18	14	150	39	.....	.....	....	412	40
F_3 families HR	9	1	147	15	13	163	15	13	101	1	478	44
F_3 families H	23	....	118	8	15	187	27	123	768	....	1,269	114
F_3 families HS	7	1	20	3	....	17	3	45	381	....	477	45
F_3 families IS	35	....	.....	....	....	.....	....	131	2,117	25	2,308	212
Marquis IR	5	....	215	1	....	40	3	.....	.....	....	264	23
Marquis HR	16	....	326	39	12	107	9	33	83	2	627	50
Kota HS	....	....	1	....	....	.....	....	.....	15	....	16	1
Kota IS	13	....	.....	....	....	.....	....	3	962	....	978	81

The infection of the Kota parent fell within type 3 with the exception of one plant which had a type 1 infection. With the conditions under which the studies were made, occasional infection with a different biologic form could not be avoided. The infection of the Marquis parent fell mainly within types 1 and 2, although there were 118 plants out of 891 which showed infection of type 3.

The Marquis seed used was from a carefully selected sample and was typical of the Marquis variety. There was the possibility that these Marquis seedlings which in the greenhouse gave type 3 infections were of a different genetic nature than the greater part of the Marquis seedlings, due to mixtures or some other causes. Accordingly, seedlings which showed the type 3 and the type 1 or 2 infection in the greenhouse were planted in the field and grown to maturity. All plants appeared to be of the Marquis variety. Seeds from several plants in each group were saved separately and progeny of these plants tested in the greenhouse by inoculating with spores of Form XIX. Five out of six of the progeny of the seedling plants which had type 3 infections again gave seedlings which were placed in the 3 group, while the infection of one family was mainly in types 1 and 2. Six out of seven progeny lines which descended from seedlings having type 2 infections again had similar degrees of infection, while one family bore infection mainly of type 3. The reason, then, for the greater part of the type 3 infections obtained in the Marquis seedlings appears to be genetic differences in the Marquis material.

To determine their reaction to biologic Form XIX, 455 F_3 families were tested. Some proved homozygous in the moderately resistant and the moderately susceptible types. Other F_3 families proved to be heterozygous. The number of susceptible types is too large to be explained on the basis of a single factor difference. Forty resistant families were obtained.

Certain of the F_2 parent plants of these various F_3 families were selected and their progeny grown in the rust nursery in 1922. Some lines appeared desirable and individual plants within these lines were selected. Seedlings were grown in the greenhouse from the individually selected plants and were inoculated with Form XIX. Progenies of 3, 4, and 5 plants of three different F_3 families, which were classified as IS, or of the Kota type of infection, were grown in the greenhouse. All 12 plants bred true to the IS type of infection. One family from the IR group was tested in a similar manner. Progeny of 5 plants of this family were tested, and all showed type 2 infections, as was expected.

The reactions to Form XXVII were of a more definite nature than those to Form XIX, as will be seen in Table VI. Of the Kota plants all except 33 out of a total of 1,040 were in the immune class, while Marquis had 9 plants in type 4 and 725 in type 2 infections. The deviations obtained could all be explained on the basis of natural causes, for the frequency of such natural crossing is in the neighborhood of 2 to 4 per cent in Minnesota under nursery conditions.

A total of 462 F_3 families were tested. Of these, 76 showed either no infection or infection of the 0 or immune type, 14 were resistant, and 39 were susceptible, and there were four separate classes of heterozygous types. Immunity is clearly dominant over resistance or susceptibility as shown by the results for the heterozygous classes H1 and H4.

The segregating classes obtained when F_3 families were inoculated with Form XXVII could result from two independently inherited genetic factors for resistance and immunity each allelomorphic to a factor for susceptibility. Furthermore, if the factor for immunity was epistatic to the factor for resistance, three times as many homozygous immune families would be obtained as homozygous for either resistance or susceptibility. It is possible that a much more complex genetic condition may be the cause of the classes of segregation obtained. The clear proof that

some susceptible types may be obtained from a cross of resistant parents is of much interest.

TABLE VI.—*Distribution of the F_3 seedlings of the cross between Kota and Marquis according to types of infection caused by biologic Form XXVII*

Classes of parents and F_3 crosses.	Types of infection.						Number of plants.	Number of families.
	0	2−	2	2+	4−	4		
F_3 families R	1,159	.....	.....	.....	.....	.....	1,159	76
F_3 families IR	12	.....	55	88	.....	.....	155	14
F_3 families H1	588	2	66	113	.....	.....	769	62
F_3 families H2	707	.....	52	113	164	29	1,065	82
F_3 families H3	23	23	67	225	264	29	631	50
F_3 families H4	1,312	.....	.....	.....	305	98	1,715	139
F_3 families S	22	.....	.....	.....	387	87	496	39
Marquis H3	.....	.....	28	67	8	1	104	8
Marquis IR	9	.....	172	458	.....	.....	639	55
Kota H1	16	.....	1	.....	.....	.....	17	1
Kota H4	179	.....	.....	.....	32	1	212	15
Kota R	811	.....	.....	.....	.....	.....	811	59

Several hybrid F_3 families, whose seedling reaction to Form XXVII was known, were grown in the rust nursery in 1922, and individual plants from various progeny lines were selected. Three such lines, which in a previous greenhouse test gave reactions which placed them in the R or immune group, were again tested for their reaction to Form XXVII for the purpose of checking up the accuracy of the preceding year's study. A total of 16 plants belonging to these three families had type 0 infection and thus were in the immune class. Two lines of the susceptible group were similarly tested. One gave evidence of breeding true for susceptibility while another segregated, giving both susceptible and resistant types. If similar results were obtained, the number of families in the susceptible group should be decreased from that presented in Table VI.

The Marquis and Kota pots which were inoculated at the same time were used as controls on the purity of the biologic forms. In the hand-inoculated pots, the killed areas around the uredinia on Marquis were much less sharply defined than in the studies made the previous winter. In two pots, Marquis seedlings which were inoculated by brushing with seedlings infected with biologic Form XXVII were heavily infected with rust over the greater part of their leaf surface. The uredinia on these heavily infected seedlings were surrounded by sharply defined hypersensitive areas. Apparently environmental conditions may influence, to a rather marked degree, the reaction of seedlings to a particular biologic form.

INFECTION UNDER FIELD CONDITIONS

One of the purposes of the investigation was to determine the possibility of using the greenhouse test as a means of determining those F_3 families having the type of resistance which Kota shows under field conditions. While Kota in the field frequently is heavily infected with stem rust, the uredinia do not break out in the same manner as on susceptible varieties and are much smaller and often surrounded by a

hypersensitive area. For this reason the seed of Kota is generally well filled even under rust-epidemic conditions.

The F_3 families studied in their reaction to Form XIX and Form XXVII in the greenhouse were the progeny of F_2 plants taken at random from an F_2 population. The seed characters of these plants were examined and the plants which appeared to have desirable seed from an agronomic standpoint were selected and their progeny grown in the rust nursery in 1922. The various F_3 lines were exposed, as has been previously noted, to at least 9 biologic forms of stem rust. The epidemic was a satisfactory one. Individual plants within the different F_3 families were carefully examined and on the basis of the infection obtained the F_3 families were placed in the following classes:

R=Resistant; while there was considerable infection the plants all showed the Kota type of resistance.
NR=Semiresistant, all plants infected in a similar way. Much more resistant than Marquis, but more heavily infected than Kota.
H=Heterozygous, containing both resistant and susceptible plants.
S=Susceptible, all plants heavily infected, with large uredinia, similar to the infection shown by Marquis.

The results of the field experiment are presented in Table VII. The 9 control plots of Kota were all clearly resistant while the 20 control plots of Marquis were fully susceptible.

TABLE VII.—*Distribution of 206 F_3 families of the Marquis-Kota cross, which had not been tested previously under rust-epidemic conditions, when inoculated in the rust nursery with a mixture of 9 different rust forms, in 1922*

Parents or hybrids.	Number of families.			
	Homozygous.		Heterozygous.	Susceptible.
	Resistant.	Semi-resistant.		
F_3 awnless homozygous	3	18	3	22
F_3 bearded homozygous	15	35	16	22
F_3 heterozygous	10	33	12	17
Kota, bearded	9	.....	.....	.....
Marquis, awnless	.....	.....	.....	20

Of a total of 206 F_3 families 28 were homozygous resistant, 61 were susceptible, 86 appeared semiresistant, and 31 were clearly heterozygous. These results can not be explained on a single factor basis, because only 28 out of 206, which is a ratio of 1 to 7.36, were as resistant as Kota. It should be remembered that 9 biologic forms of rust were used to produce the field epidemic. Apparently the Kota type of resistance to all 9 forms depends on more than a single genetic factor.

In 1921 an F_2 generation of the Kota-Marquis cross was planted in the rust nursery. Several of the 9 biologic forms used in 1922 to produce the epidemic were available, although only three or four biologic forms were used to produce the 1921 epidemic. A total of 666 normal plants, which were carefully examined, consisted of 80 bearded susceptible, 233 awnless susceptible, 89 bearded apparently resistant, and 264 awnless apparently resistant. The 353 plants which appeared resistant were harvested and on examination 47 appeared to have desirable seed char-

acters. Progenies of these were grown in 1922 in the field and the rust reaction determined. Eleven of the 47 families were resistant, 14 were semiresistant, 4 were susceptible, and 12 were heterozygous. If the same relative proportion of the entire 353 resistant families had bred true to resistance there would have been 82 out of 666 F_2 plants that were homozygous in their resistance to all eight biologic forms. This is a ratio of 1 homozygous resistant to 8.1 of other types, which is very similar to the previously discussed result of the inoculation of 206 F_3 families of which 28 bred true for the Kota type of resistance.

THE GREENHOUSE TEST AS A MEANS OF ISOLATING LINES RESISTANT IN THE FIELD

One of the purposes of a study of the greenhouse reactions to Forms XIX and XXVII was to determine the possibility of using this test as a means of isolating lines which would exhibit the Kota type of resistance in the field. Fifty-two families whose reactions to Form XIX were known in the greenhouse and 48 families whose reactions to Form XXVII were known were grown in the field nursery in 1922 and classified under field conditions as resistant, near-resistant, heterozygous, and susceptible, as shown in Table VIII.

TABLE VIII.—*Distribution into classes according to reaction in the greenhouse of F_3 seedlings from F_2 plants in relation to behavior of F_3 families in the field rust nursery under artificial epidemic conditions*

Reaction under field conditions.	Greenhouse reaction—											
	To Form XIX.					To Form XXVII.						
	IR.	HR.	H.	HS.	IS.	R.	IR.	H_1.	H_2.	H_3.	H_4.	S.
R	0	1	1	0	3	1	1	0	0	0	3	0
NR	1	3	10	2	11	5	0	2	6	3	5	3
H	3	0	2	1	4	2	2	0	1	0	3	1
S	1	0	4	1	4	0	0	4	2	0	2	0

A study of the results shows that the greenhouse reactions to the biologic Forms XIX or XXVII can not be used to determine which families will exhibit the Kota type of resistance to several biologic forms in the field. Thus of the five families which gave the Marquis type of reaction to Form XIX in the greenhouse, namely, the IR group, none bred true for resistance under field conditions. Similarly, of the 22 families which gave the Kota type of reaction in the greenhouse and which were placed in the IS group, only 3 proved resistant in the field. The reaction of Kota to Form XXVII was of the immune or R type. Eight hybrid families of this type were grown in the field and only one proved as resistant as Kota. These results are further evidence which proves that the resistance of Kota to several biologic forms of stem rust is due to more than a single genetic factor.

QUESTION AS TO WHETHER THE RESISTANCE TO FORM XIX AND FORM XXVII CAN BE COMBINED IN A SINGLE VARIETY

In order to build up wheats resistant to all biologic forms, it is necessary to combine in a single variety the resistance exhibited by different wheat varieties to particular biologic forms. The reactions of F_3 families to Forms XIX and XXVII are correlated in Table IX.

TABLE IX.—*Distribution of F_3 families classified according to reaction to form XIX in relation to reaction to Form XXVII*

Reaction to Form XXVII.	Reaction to Form XIX.					
	IR.	HR.	H.	HS.	IS.	Total.
R	3	9	13	5	44	74
IR	4	2	1	0	1	8
H1	6	6	12	4	14	42
H2	7	11	13	7	29	67
H3	1	5	15	3	13	37
H4	3	9	27	15	59	113
S	1	2	8	1	19	31
Total	25	44	89	35	179	372

Three out of a total of 372 families proved immune from Form XXVII and were resistant to Form XIX, and 4 families were resistant to both biologic Forms XIX and XXVII. It is possible, therefore, to combine in a single variety the resistance of the Marquis parent to Form XIX, and the immunity of the Kota parent from Form XXVII. These facts give further hope for the belief that resistance to different biologic forms exhibited by different wheat varieties is due to independently inherited Mendelian factors or that the linkage of these factors is so slight that crossovers frequently occur. From the genetic standpoint the facts so far learned give reason for the belief that a wheat variety can be produced which will be resistant to all biologic forms of stem rust.

CORRELATION BETWEEN MORPHOLOGIC AND SEED CHARACTERS AND RUST RESISTANCE

The F_2 plants which were the parents of the F_3 families tested under field conditions were classified as normal awnless or bearded and dwarf awnless or dwarf bearded. For the purpose of determining whether there was any marked correlation between these characters and reaction to rust, the reactions of these separate morphologic groups to Form XIX and Form XXVII have been arranged in correlation, as shown in Tables X and XI.

TABLE X.—*Distribution of F_2 normal awnless (A) and bearded (B) plants and dwarf awnless (DA) and bearded (DB) plants, classified according to their reaction to Form XIX in relation to spike characteristics of F_2 plants*

F_2 plant type.	F_3 reaction to Form XIX.				
	IR.	HR.	H.	HS.	IS.
A	28	35	78	28	142
B	10	12	23	11	46
DA	1	0	12	1	19
DB	0	2	3	1	5
Total	39	49	116	41	212

TABLE XI.—*Distribution of F_3 normal awnless (A) and bearded (B) plants and dwarf awnless (DA) and bearded (DB) plants, classified according to their reaction to Form XXVII in relation to spike characteristics of F_2 plants*

F_2 plant type.	F_3 reaction to Form XXVII.						
	R.	IR.	H_1.	H_2.	H_3.	H_4.	S.
A	62	11	47	60	32	101	27
B	28	3	11	20	11	30	8
DA	2	0	1	1	2	5	2
DB	1	0	1	2	2	2	2
Total	93	14	60	83	47	138	39

Three times as many awnless families as bearded were expected. The fact that there were about three times as many awnless as bearded families in each of the groups classified according to reaction to both biologic forms seems very convincing proof that the factors which govern susceptibility or resistance to either Form XIX or Form XXVII are inherited separately from the factors which govern the presence or absence of awn development.

To Form XXVII the reaction of the F_3 families from the dwarf types seems very similar to that obtained from normal plants. For Form XIX, however, the percentage of dwarf plants which were as resistant as Marquis and which were placed in the IR group is very small and certainly much less than the percentage of similar families from the normal plants.

In a somewhat similar manner the relation between average length of seed of the F_2 plants and the reaction of these plants to Forms XIX and XXVII was determined, as shown in Tables XII and XIII.

TABLE XII.—*Distribution of F_2 plants classified according to their reaction to Form XIX in relation to length of seed*

Classes by reaction to Form XIX.	Classes for length of seed (in millimeters).						Total.	Mean.
	4.8	5.1	5.4	5.7	6.0	6.3		
IR	.....	.....	5	17	9	3	34	5.79±0.03
HR	1	3	5	21	18	1	49	5.74±0.03
H	3	7	18	50	30	2	110	5.69±0.02
HS	1	.....	6	18	15	.....	40	5.75±0.03
IS	2	10	33	81	67	9	202	5.74±0.01

TABLE XIII.—*Distribution of F_2 plants classified according to their reaction to Form XXVII in relation to length of seed*

Classes by reaction to Form XXVII.	Classes for length of seed (in millimeters).						Total.	Mean.
	4.8	5.1	5.4	5.7	6.0	6.3		
R	.....	2	15	46	24	6	93	5.75±0.02
IR	.....	.....	4	5	3	2	14	5.77±0.06
H_1	2	3	8	25	20	2	60	5.72±0.03
H_2	.....	2	17	32	28	3	82	5.75±0.02
H_3	1	4	11	19	10	1	46	5.63±0.03
H_4	1	3	17	60	52	2	135	5.77±0.01
S	.....	4	5	15	13	1	38	5.72±0.03

The ranges of variability for seed length are somewhat greater, as would be expected, for the reaction groups which contain the largest number of families. The differences obtained in the means are not sufficiently great to indicate any marked linkage between the factors of inheritance which govern seed length and those which govern the manner of reaction to the forms of stem rust used in the study.

SUMMARY

(1) Kota, which is resistant to stem rust in the field but which has weak straw when grown under humid conditions, was crossed with Marquis, which is susceptible to stem rust but which possesses other desirable economic characters.

(2) A survey of available biologic forms led to the selection of Form XIX, to which Marquis was resistant and Kota moderately susceptible, and Form XXVII, from which Kota was immune and to which Marquis was resistant. These two forms of stem rust were chosen for the study with the hope of finding a greenhouse test which could be used as a means of isolating F_3 families which would prove as resistant as Kota under field conditions.

(3) Out of a total of 787 F_2 plants of the Kota-Marquis cross which were grown under field conditions, 666 were of normal type and 121 were dwarfs. The results were explained on the basis that one of the parents contained a factor for dwarf habit and another factor which prevented the production of dwarfs. The other parent apparently lacked both factors.

(4) The seeds of the F_2 bearded plants were 0.1 mm. longer on an average than the seeds from the awnless plants. The seeds of the awnless plants had slightly harder texture but were slightly inferior in plumpness to those from the bearded plants. Apparently the beard of wheat, under the conditions of the experiments, leads to the production of somewhat better developed seed.

(5) Marquis, in the greenhouse, was more resistant than Kota to Form XIX. Varying environmental conditions, however, resulted in the production of a type of infection on Marquis which approached that obtained normally with Kota. The reactions of Kota to Form XIX under the same conditions did not show similar variations in the types of infection. F_3 hybrid families were obtained which reacted in a manner similar to Marquis and Kota, while other families were obtained which were clearly heterozygous. The results could not be explained on the basis of a single genetic factor.

(6) Kota proved immune from Form XXVII in the greenhouse, while Marquis proved resistant. F_3 families were obtained which were as homozygous for immunity and resistance as Kota and Marquis, respectively, while others were obtained which were entirely homozygous for susceptibility. Four types of heterozygous families were obtained. Immunity appeared to be dominant to both resistance and susceptibility. The number of seedlings inoculated in each family available for the study was too small to make the experiment absolutely conclusive. The results could be quite satisfactorily explained on the basis of two independently inherited factors for immunity and resistance contained in the Kota and Marquis parents, respectively, each factor being allelomorphic and dominant to a factor for susceptibility.

(7) Nine biologic forms of stem rust which were collected in the North-Central and Northwestern States were used for producing a field epidemic. Kota proved rather highly resistant under field conditions while Marquis was very susceptible. In one group of 206 F_3 families there were 28 which were as resistant as Kota. This is a ratio of 1 to 7.36. In another test there was a ratio of 1 resistant F_3 family to 8.1 susceptible and heterozygous.

(8) The reaction of these F_3 families to Forms XIX and XXVII in the greenhouse was correlated with the behavior of such F_3 families under field conditions when an epidemic of stem rust was induced by infection with nine biologic forms of stem rust to which Kota proved resistant in the field. The study of F_3 seedlings in the greenhouse in their reaction to Forms XIX and XXVII was not satisfactory as a means of isolating F_3 lines which would exhibit the Kota type of resistance under field conditions.

(9) The resistance of Marquis to Form XIX and the immunity of Kota from Form XXVII were combined in 3 F_3 families out of a total of 372 families studied. This is further evidence in support of the belief that there are several genetic factors which determine the differential reactions of Marquis and Kota to the biologic forms in question. That the resistance of one parent to Form XIX and the immunity of the other parent from Form XXVII can be combined in a single hybrid family is added reason for the hope that resistance to all biologic forms can be obtained eventually.

(10) There was no close linkage in inheritance of the factors which govern the presence or absence of the awns or of seed length with those which determine the resistance or susceptibility to either biologic form used in the greenhouse study.

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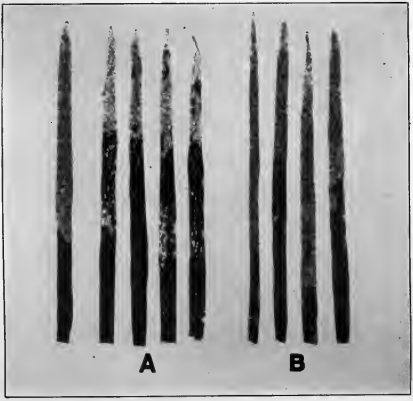
PLATE 1

Comparative lodging of Kota and Marquis. At left, Kota, badly lodged; at right, Marquis, fully erect. Photograph taken at University Farm, St. Paul, Minn., 1922.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B

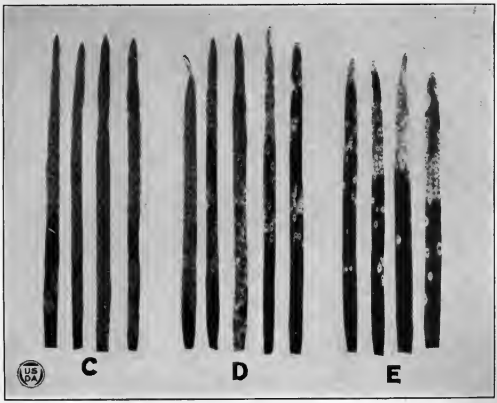

C
D
E

PLATE 2

Reaction of Marquis and Kota and of F_3 families of the Marquis × Kota cross to biologic Form XIX.

A.—Reaction of Marquis of types 1 and 2 except the leaf at the extreme left, which proved to have a mixture of infection types.

B.—Reaction of Kota of type 3.

C.—F_3 family of the immune-susceptible group.

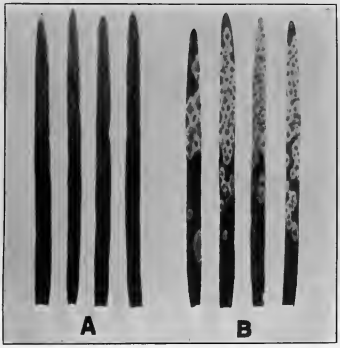
D.—F_3 heterozygous family.

E.—F_3 family of the immune-resistant group.

PLATE 3

Reaction of Marquis and Kota and of F_3 families of the Marquis×Kota cross to biologic Form XXVII.

A.—Kota, showing immunity represented by infection of type o.
B.—Marquis, showing resistance which classifies it in the immune-resistant group.
C.—Immune family.
D.—Resistant family.
E.—Susceptible family.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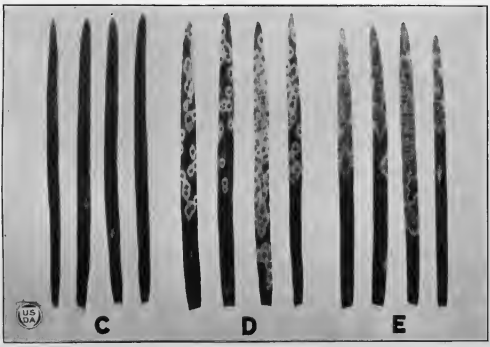
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BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON VARIETIES OF AVENA SPP.[1]

By E. C. STAKMAN, *Plant Pathologist, Department of Agriculture, University of Minnesota, and Pathologist and Agent of the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture;* M. N. LEVINE, *Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture; and* D. L. BAILEY, *Assistant in Plant Pathology, Department of Agriculture, University of Minnesota, and Agent, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*[2]

Puccinia graminis tritici Erikss. and Henn., which originally was considered as a single biologic form, has been shown by Stakman and Piemeisel (*11*),[3] Levine and Stakman (*5*), Melchers and Parker (*7*), Stakman, Levine, and Leach (*10*), and Stakman and Levine (*9*) actually to consist of many forms which differ in their parasitic action on certain varieties of *Triticum* spp. Hoerner (*4*) showed that there is a similar specialization of *Puccinia coronata* Cda. on varieties of *Avena* spp. and Mains and Jackson (*6*) found two biologic forms of *P. triticina* Erikss. on varieties of wheat. It seemed quite likely, therefore, that *P. graminis avenae* Erikss. and Henn. might also consist of several biologic forms. Experiments, therefore, were begun in the fall of 1918 to ascertain whether this was true.

It is obvious that one of the most direct methods for ascertaining whether there are biologic forms of rust, with different parasitic capabilities on varieties of cereals, is to find forms of rust capable of infecting varieties ordinarily practically immune from described rust forms, or vice versa. As a result of preliminary studies on *Puccinia graminis avenae* it was found that Victory (both C. I. No. 1145[4] and Minn. 514), Improved Ligowa (Minn. 281), Minota (Minn. 512), and many other varieties of oats were extremely susceptible to the rust strains[5] used, while White Tartar (White Russian) (Minn. 339 and two pure-line selections made by the section of plant pathology at the Iowa Experiment Station and designated as Rust Nursery Row No. 101½ and 102½), was quite resistant. The writers, therefore, began collecting uredinial material of *P. graminis* Pers. on oats from as many different localities as possible, in this and other countries.

Since the fall of 1918, more than 100 collections of *P. graminis* on oats have been obtained from 21 States of the United States, from two Provinces in Canada, and from one State in Mexico. Seventy-six of the collections have been cultured in the greenhouse of the section of plant pathology at University Farm, St. Paul, Minn. Many of these were used for inoculating seedlings of about 70 different varieties and selections

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[2] The writers are indebted for rust material to Prof. W. P. Fraser, pathologist in charge of Cereal Disease Investigations, Dominion Laboratory of Plant Pathology, in cooperation with the University of Saskatchewan, Saskatoon, Sask, Canada; to Prof. Wallace Butler, of San Antonio, Tex.; to Mr. G. F. Puttick, of the Department of Agriculture of the Union of South Africa; and to Mr. Fred Griffee, assistant plant breeder, Department of Agriculture, University of Minnesota. They also are indebted to other Federal and State pathologists for collections of rust.

Dr. H. K. Hayes, plant breeder, Department of Agriculture, University of Minnesota and collaborator of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, made valuable suggestions for which the authors wish to express their appreciation.

[3] Reference is made by number (italic) to "Literature cited," p. 1017–1018.

[4] C. I.=Cereal investigations accession number.

[5] The term "strain" is used only to designate a culture of rust from a single collection.

of oats, belonging to 6 different species of the genus Avena, namely: *A. brevis* Roth., *A. nuda* L., *A. sativa* L., *A. orientalis* L., *A. sterilis* L., and *A. strigosa* Schreb.

It was found that definite preliminary results were likely to be obtained by using only three varieties of oats, viz: Victory, White Tartar (White Russian) and Monarch Selection (of Etheridge). All collections were run to Victory and White Tartar (White Russian). Victory was completely susceptible to all of the strains tried, White Tartar was very highly resistant to most of them, while Monarch Selection, which was inoculated only with the collections made in 1921 and 1922, was extremely susceptible to some of these collections and practically immune from others. Attempts then were made to find forms of stem rust which would infect the White Tartar variety heavily. This was achieved with rust material procured from Europe and Africa.

Victory was completely susceptible to, White Tartar very resistant to, and Monarch Selection (of Etheridge) practically immune from, a form collected by Mr. Fred Griffee at St. Paul, Minn. This is designated hereafter as Form I.

A form of rust collected by Prof. Wallace Butler at San Marcos, Tex., infected Victory normally, developed only lightly on White Tartar, but produced large, vigorous uredinia on Monarch Selection. These results were confirmed by repeated inoculations, and clearly indicated the existence of a second biologic form of *Puccinia graminis avenae*, as it was capable of infecting Monarch Selection normally, which the form collected by Mr. Griffee could not infect. This is designated as Form II.

A rust strain sent by Mr. G. F. Puttick from Potchefstroom, Union of South Africa, infected Victory normally, White Tartar moderately, and Monarch Selection normally. This undoubtedly was a third form and is hereafter called Form III.

Finally, Stakman found a form at Upsala, Sweden, which infected White Tartar just as heavily as it did Victory and Monarch Selection. This, then, was a fourth form,[6] designated as Form IV.

Table I summarizes the action of these four forms on the varieties of oats which served as the differential hosts.

It is quite evident from Table I that Form I (Pl. 2, A) infects Victory normally, but develops only lightly on White Tartar (White Russian) and exceedingly lightly on Monarch Selection. Form II (Pl. 2, B) infects Victory and Monarch Selection heavily, but attacks White Tartar only lightly. Form III (Pl. 3, A), like Form II, attacks Victory and Monarch Selection very heavily and in addition attacks White Tartar moderately. Form IV (Pl. 3, B) infects all three varieties very heavily. Repeated inoculations have been made and the same results have been obtained consistently.

Two different forms were isolated from a single collection several times. In some cases the X type of infection described for wheat rust (*9*, *p.* 5) also developed on oats. Several strains produced this X type of infection on Monarch Selection, thus indicating the probable existence of a fifth biologic form which infects Victory very heavily, White Tartar only lightly and Monarch Selection heterogeneously (Pl. 4). There are now known, therefore, at least four, and probably five, biologic forms of *Puccinia graminis avenae* which produce different reactions on certain varieties of *Avena* spp.

[6] Proper disposition is being made of the spore material of the South African and Swedish forms, as they have not yet been found in the United States.

TABLE I.—*Results of inoculating three differential varieties of Avena spp., with four biologic forms of Puccinia graminis avenae*

Biologic Form No.	Place of collection.	Summary of inoculation results.					
		Victory (C. I. No. 1145).		White Tartar (Minn. 339).		Monarch Selection (of Etheridge).	
		Number of trials[a]	Character of infection.	Number of trials.	Character of infection.	Number of trials.	Character of infection.
I	St. Paul, Minn.	19	Normal, heavy infection. Uredinia large, numerous and coalescing.	8	Infection light. Uredinia usually small and scattered. Hypersensitiveness (sharp chlorosis to definite necrosis) ordinarily present.	6	Infection exceedingly light. Uredinia, when present, minute, scattered and surrounded by very sharply defined necrotic areas. Distinct hypersensitive flecks also occur quite frequently.
II	San Marcos, Tex.	16	.....do.....	8	.....do.....	6	Normal, heavy infection. Uredinia large, coalescent and numerous.
III	Potchefstroom, South Africa.	7	.....do.....	5	Infection moderate. Uredinia medium in size and only slightly confluent. True hypersentitiveness absent, but light chlorotic areas usually present.	3	Do.
IV	Upsala, Sweden.	2	.....do.....	2	Normal, heavy infection. Uredinia large, many, and confluent.	2	Do.

[a] From 10 to 15 plants were inoculated in each trial.

These newly discovered biologic forms are quite as distinct as are those of *P. graminis* on wheat varieties (*12*). The inoculations were made on seedlings in the greenhouse, but, as a result of previous work, it is safe to conclude that the reaction of seedlings to rust forms is a fairly accurate index of the reaction of older plants. It usually is more difficult to obtain infection on older plants than it is on seedlings, but this apparently is due very largely to the fact that the film of water necessary for spore germination and entrance of germ tubes through the stomata, is not easily maintained on old plants. However, when this difficulty is overcome, by atomizing the plants several times a day, infection occurs normally.

The new forms apparently are constant. Repeated inoculations and cross inoculations have been made with most of them and the results have been consistent. There are variations, of course, in the intensity of rust development when the environmental conditions are unfavorable for the development of host or parasite or both. When light intensity is low, and when the temperature is either too high or too low, the rust develops subnormally. If the host plants are weakened by poor growing conditions, by mildew, root rots, or other factors, the rust usually does not develop well. This variation, however, does not indicate any change in the genotypic constitution of the rust forms, but is only the temporary result of environment. In order to draw accurate conclusions regarding the parasitic behavior of biologic forms it is necessary to grow the host plants and the rust fungus within the range of environmental conditions in

which they will develop normally. Optimum conditions are not essential but extremely unfavorable conditions should be avoided.

It is important to determine whether the urediniospores of these new forms are really of the avenae type, as *P. graminis phleipratensis* (Erikss. and Henn.) Stak. and Piem. develops moderately well from artificial inoculation on some varieties of oats and may possibly occur on some in the field. *P. graminis agrostis* Erikss. also can attack some oat varieties weakly. These forms, however, can be distinguished easily from *P. graminis avenae* by the morphological characters of the urediniospores. The urediniospore characters of the new forms clearly are those of *P. graminis avenae* (*8*). However, it is not yet known whether these individual forms differ appreciably from each other morphologically.

The geographical distribution of these biologic forms of *P. graminis avenae* is not yet known definitely. The sources of 23 collections, made during 1921 and 1922, are given in Table II. Form I has been collected from Saskatchewan to Mexico, in the central part of the continent, and probably is widely distributed.

TABLE II.—*Distribution of biologic forms of stem rust of oats collected during 1921 and 1922*

Biologic Form.	Place of collection.	Original host.
II	Saskatoon, Sask., Canada	Avena sativa L.
[a] V	Lloydminster, Sask., Canada	Do.
[a] V	Weyburn, Sask., Canada	Do.
I, II	Zelma, Sask., Canada	Do.
II	Presque Isle, Me.	Do.
I, II	Litchfield, Minn.	Do.
I, II	Rosetown, Minn.	Do.
I	St. Paul, Minn.	Do.
II	Redfield, S. Dak.	Do.
II	Huron, S. Dak.	Do.
[a] V	Colmar, Iowa	Do.
I, II	Lafayette, Ind.	Poa trivialis L.
I	Huntley, Ill.	Avena sativa.
II	Chillicothe, Mo.	Do.
II	Norman, Okla.	Dactylis glomerata L.
II	Lawton, Okla.	Avena sativa.
II	San Marcos, Tex.	Do.
I, II	Boerne, Tex.	Do.
I, II	San Antonio, Tex.	Do.
I, II	Robards, Tex.	Do.
I, II	Saltillo, Mexico	Do.
IV	Upsala, Sweden	Do.
II, III	Potchefstroom, South Africa	Do.

[a] If not a mechanical mixture of I and II.

Form II seems to be even more abundantly distributed than Form I in the central part of North America, from Saskatchewan to Mexico, and has been found also in Maine, as well as in South Africa.

Forms III and IV have not yet been found in North America. It seems improbable that they are abundant in the northern Mississippi Valley, because the White Tartar (White Russian) oat is very resistant in the field. Furthermore, Garber (*3*) produced Victory × White Russian hybrids which were resistant to stem rust, at least in Minnesota. As these

hybrid strains combine the desirable characters of Victory with the rust resistance of White Tartar, it is to be hoped that Forms III and IV of *P. graminis avenae* do not exist in the United States. If they do not, the problem of developing good varieties of oats resistant to stem rust seems to be well on the way to solution.

The provisional Form V was collected in two localities in Saskatchewan, Canada, and in one in Iowa. It may be a mechanical mixture of Forms I and II. Inoculation experiments to determine this fact are in progress.

It is especially interesting to note that the Swedish form of rust is more virulent than any of those so far found in the United States, because Eriksson (2) long ago called attention to the fact that *P. graminis avenae* was exceptionally virulent in Sweden. The senior author was also impressed with this fact during a recent visit to Sweden. Several grasses, which are moderately susceptible to the avenae form of rust in the United States, but which seldom rust heavily in the field, were very generally and severely infected in Sweden. The fact that there are tremendous numbers of barberry bushes in some oat-growing regions of Sweden, and the additional fact that oats are so commonly grown might account for the abundance of the rust. In addition, however, Eriksson evidently was correct in assuming that the stem rust of oats was especially virulent in Sweden. This emphasizes still more the fact that the specialization of *P. graminis avenae* may be different in different countries. The present results indicate that the forms may also differ even in various regions of the same country. On the other hand more than one biologic form may occur in the same locality or even on the same plant.

While the virulent Swedish form of stem rust of oats may possibly exist in the United States, fortunately it does not seem to be either abundant or widespread in those Northern States in which White Tartar is commonly grown. The work of Durrell and Parker (1) indicates also that White Tartar is quite generally resistant to stem rust.

The practical significance of the existence of several biologic forms of *Puccinia graminis* on oats in the United States will depend on the number and the virulence of forms which may be found in the future. This problem is now being thoroughly investigated.

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PLATE 1

Classes of host reaction: Resistant (R), susceptible (S), and intermediate (X), as indicated by different types of infection (0, 1, 2, 3, 4, and X) produced by biologic forms of stem rust on different varieties of oats.

Class R (resistant), including 3 subclasses corresponding to infection types 0, 1, and 2.

A.—Practically immune (0). No uredinia are developed, but sharply defined hypersensitive flecks are usually present.

B.—Extremely resistant (1). Infection very light; uredinia minute and scattered and surrounded by very sharply defined necrotic areas.

C.—Moderately resistant (2). Infection light; uredinia usually small and scattered; hypersensitive areas varying from sharply defined necrosis to pronounced chlorosis.

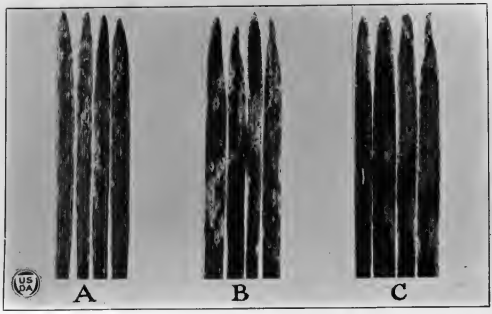
Class S (susceptible), including two subclasses corresponding to infection types 3 and 4.

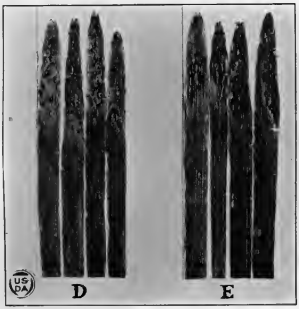
D.—Comparatively susceptible (3). Infection moderate; uredinia midsized with a tendency to coalesce; true hypersensitiveness absent, but light chlorotic areas usually present.

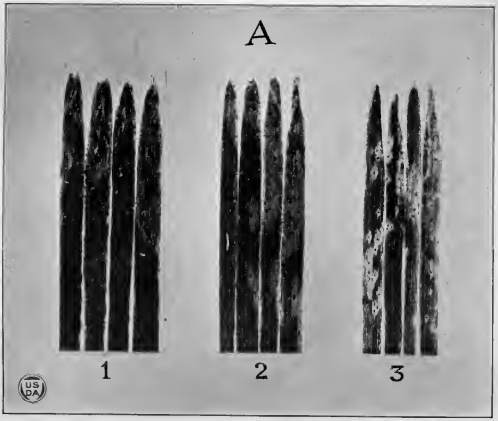
E.—Completely susceptible (4). Infection normal and heavy; uredinia large, numerous and confluent; hypersensitiveness entirely absent, but chlorosis may be present when cultural conditions are unfavorable.

Class X (intermediate), representing the heterogeneous (X) type of infection. (No subdivisions of this class are recognized.)

F.—Uredinia very variable, apparently including all types and quantities of infection, often of the same blade; no mechanical separation seems to be possible, as, on reinoculation, spores from small uredinia may produce large ones, and vice versa. In general, the infection is ill-defined.



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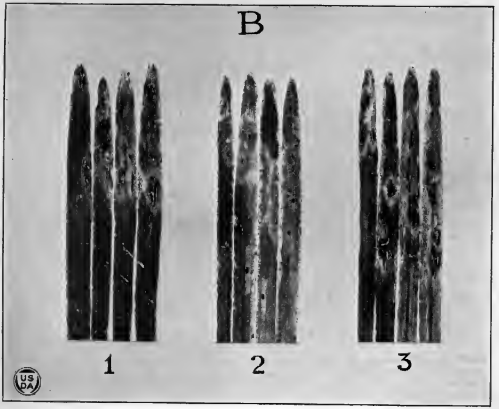
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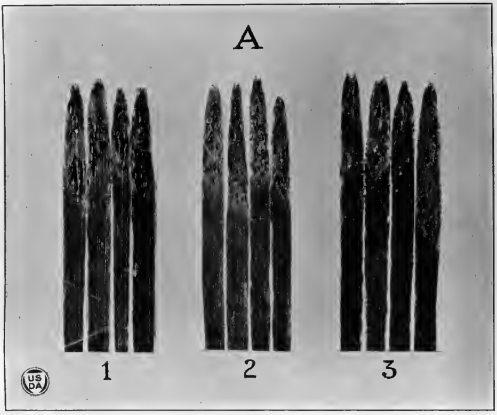
PLATE 2

A.—*Puccinia graminis avenae* (Form I): (1) Victory, very susceptible; (2) White Tartar, quite resistant; (3) Monarch Selection, practically immune.

B.—*Puccinia graminis avenae* (Form II): (1) Victory, very susceptible; (2) White Tartar, quite resistant; (3) Monarch Selection, highly susceptible.

PLATE 3

A.—*Puccinia graminis avenae* (Form III): (1) Victory, completely susceptible; (2) White Tartar, moderately susceptible; (3) Monarch Selection, very susceptible.
B.—*Puccinia graminis avenae* (Form IV): (1) Victory, completely susceptible; (2) White Tartar, highly susceptible; (3) Monarch Selection, very susceptible.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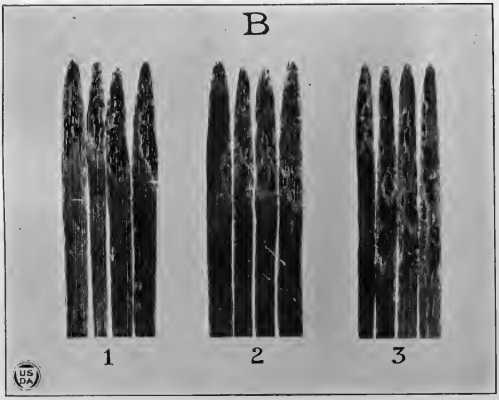
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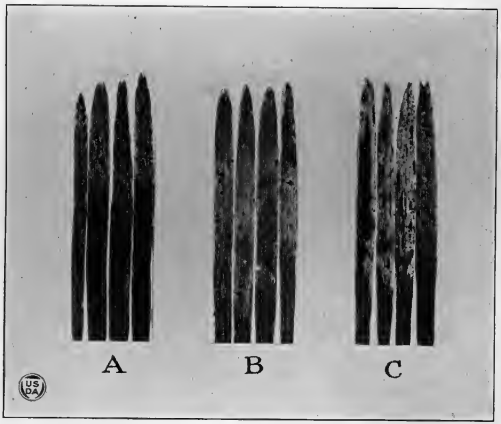
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PLATE 4

Puccinia graminis avenae (Form V)

A.—Victory, susceptible.
B.—White Tartar, resistant.
C.—Monarch selection, intermediate.

DISEASE RESISTANCE TO ONION SMUDGE[1]

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INTRODUCTION

In an earlier paper on onion smudge (*24*) [3] a description of the symptoms and seasonal development of the disease, and of the morphology and life history of the causal organism is given. In connection with the above studies it was found in confirmation of the earlier observations of Berkeley (*1*), Thaxter (*18, p. 163–165*), Halsted (*8*), and Selby (*16, p. 364–366, 414*), that all white varieties of onion observed are highly susceptible to smudge while varieties with colored bulbs—yellow and red—show a high degree of resistance. Further study as to the nature of this resistant quality has shown that there are at least two substances or groups of substances within onion tissue which have a marked inhibitive effect upon the growth of the causal fungus, *Colletotrichum circinans* (Berk.) Voglino. One of these is the volatile oil,[4] and the other is a group of one or more compounds closely associated or identical with the flavone and anthocyan pigments of the scales. The present paper contains the results of investigations upon this subject.

OCCURRENCE OF VARIETAL RESISTANCE AND SUSCEPTIBILITY TO ONION SMUDGE

Observations on the relative susceptibility or resistance of onion varieties to smudge in field and storage have been continued since 1914, chiefly in Wisconsin and Illinois but incidentally in a number of other onion-growing sections in the United States. In 1922, the opportunity was given to secure field data from certain onion centers in Europe and the Canary Islands. In all cases there, as in this country, where colored and white varieties were grown simultaneously under conditions favorable for the development of smudge, white bulbs were uniformly infected while colored bulbs were completely free or infected to only a slight degree.

It should be pointed out in this connection that the pigment in colored varieties of onion appears in the outer bulb leaves or scales when the plants are about half grown, or earlier. Several of the first leaves of the plant acquire no basal thickness and the first of these slough off, without the production of much pigment, during the plant's early growth. With age the coloring becomes more intense until a short time before maturity,

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[2] The writer is indebted to Prof. L. R. Jones of the University of Wisconsin and to others in both the university and the Department of Agriculture for valuable suggestions and criticisms during the progress of the investigation.

[3] Reference is made by number (italic) to "Literature cited," p. 1038–1039.

[4] Throughout this paper the term "volatile oil" is used to designate the volatile substances which ordinarily arise from onion tissue, some of which, at least, are responsible for the characteristic onion odor.

when the yellow or red pigment, depending on the variety, is fully developed. At this time the plants have one to three thin, papery outer scales which are intensely colored. These cover the succulent scales and extend up over the neck of the bulb, where they frequently connect with their respective green portions of the tubular leaves or "top," although the latter may have disappeared by this time. Following these outer scales upward from the base, we find that the pigment ceases very abruptly above the bulb, leaving the neck sheathed largely by uncolored tissue (Pl. 1). Toward the end of the growing season there is among colored varieties a tendency toward some pigment reduction in the outer scales, especially in the portions above the soil line. This becomes more noticeable when bulbs are allowed to remain in the ground for a prolonged period after they are mature and ready for harvest.

When the disease appears early, several weeks before harvest, a few fruiting bodies of the fungus are occasionally found on the dead outer scales of colored varieties. These scales, usually only slightly pigmented, slough off before harvest. Practically all of the subsequent infection on colored varieties is confined to the outer sheath at or just above the neck of the bulb. This tissue, like the lower portions of the outer scale, is practically dead previous to harvest, but little or no pigment is present. This condition is illustrated in Plate 1, which shows one set each of red, yellow, and white varieties, all taken from the same field a few days before harvest. It is to be noted that the disease has become general on the bulb of the white variety, while in the yellow and red varieties it is confined to the uncolored sheath of the neck. In onion-set fields, this condition of infection at the neck very often becomes general on colored varieties if there is sufficient rainfall and high relative humidity to bring about dissemination of the fungus and infection. The intensely colored portions of the outer scales rarely show signs of the disease. The infection at the neck is largely sloughed off at harvest, leaving little or no sign of the disease in storage. On white varieties, as stated above, the fungus usually covers the entire bulb and continues to invade the fleshy scales, causing a gradual shrinkage.

INOCULATION EXPERIMENTS WITH BULBS

The field observations reported above have been confirmed by plot experiments in which the plants were grown on well-infested soil or were inoculated three to four weeks before harvest with spore suspensions of the causal organism. In these experiments a number of varieties of each color were included. Table I gives a list of the varieties used and the dates of the trials. These trials confirm the general field data in that the white varieties all appeared to be highly susceptible, while the colored varieties were highly resistant to the disease. In the case of the latter group, where infection did occur it was present in small amounts and usually at the neck of the bulbs where the pigment was less intense. Occasionally the outer pigmented scales bore some fungus stromata, but usually the latter were associated with a lessening of pigment due to one cause or another. In other instances, invasion of the fleshy scales was noted where they were exposed to the soil, due either to splitting or to partial disintegration of outer scales when the bulbs were not harvested promptly. This observation is in accord with the following experimental evidence which shows that when the fungus is applied directly to the fleshy colored scale, invasion readily takes place.

TABLE I.—*List of onion varieties tested as to their susceptibility or resistance to smudge*

Color.	1916	1917	1920	1921
White.	White Globe. White Portugal. Queen. Mammoth Silver King. Extra Early Barletta. Giant White Italian Tripoli.	White Globe. White Portugal. Queen. Giant White Italian Tripoli.	White Globe.	Crystal Wax Bermuda. White Australian. White Portugal. Early White Queen.
Yellow or brown.	Southport Yellow Globe. Michigan Yellow Globe. Yellow Globe Danvers. Yellow Danvers. Yellow Strassburg. Prizetaker. Early Neapolitan Marzola. Australian Brown.	Southport Yellow Globe. Michigan Yellow Globe. Yellow Globe Danvers. Yellow Strassburg.	Yellow Globe.	Ailsa Craig. Gigantic Gibraltar. Australian Brown.
Red.	Extra Early Red. Large Red Wethersfield. Southport Red Globe.	Extra Early Red. Large Red Wethersfield.	Red Globe.	Extra Early Red. Red Bermuda.

The inoculation studies were continued in moist chambers, where drops of spore suspension were placed directly upon marked areas of the surface of the scales. From certain of the bulbs the dry, outer scales were removed and the inoculum placed directly upon the succulent scale, while in others the natural conditions were simulated by placing the drops upon the dry scale. In no case did any infection whatever occur upon the dry outer scales of colored bulbs; and where the spores were examined in the drops of inoculum no normal germination had occurred. In nearly all instances, however, inoculations in the same or similar moist chambers upon the succulent scales of bulbs from the same original source did take effect. The infected area was characterized first by a very rapid disappearance of the pigment, often within two days after the inoculations were made, followed by a gradual collapse of the epidermal and underlying cells (Pl. 2, A). From all appearances the fungus invaded the so-called resistant varieties as aggressively as it did the susceptible ones when the dry scales were removed. The outer scales, therefore, appear to serve as the barrier and to be responsible for the resistant property of the colored varieties.

The rapid destruction of the coloring matter in succulent scales at first was very surprising in view of the marked toxicity of water extract of pigmented scales, which will be described later. In fact, it necessitated a further study of fungus invasion of the colored succulent scales. This was done by cutting tangential razor sections from the surface of the scale directly beneath the drop of inoculum so as to contain the undisturbed epidermis with a few layers of the underlying cells. These sections were then examined in water mounts under the microscope. By this method the progress of the fungus could be watched from the instant of spore germination throughout the disappearance of the pigment. Scales of a red variety were used because it was much easier to follow the latter phenomenon in them than in those of the yellow varieties. It was found that the process of invasion was exactly similar to that already described for white onion bulbs (*24*). After the formation of the appressorium and of the penetration tube, the mycelium developed first between the cuticle and sub-epidermal wall. Many cells were to be

found in which penetration of the cuticle had occurred with no apparent reduction of pigment. However, before the hyphae had developed to any great extent there was evidence of pigment reduction, which proceeded quite rapidly. From the evidence so far obtained it appears that the pigment was destroyed while the mycelium was developing in the outer wall and before actual penetration of the cell occurred. If this be true, the hyphae do not actually come into contact with the pigment as such; hence even if the latter were toxic, it might have no effect upon the fungus. The destruction of the pigment is due either to a substance secreted by the hyphae and capable of diffusing in advance of them or to independent chemical changes in the host cell brought about by the disturbances resulting from fungus invasion of the outer wall.

Of interest in this connection is the common observation made during these experiments—namely, as the lesion developed on the succulent red scales, pigment of normal intensity appeared in the lumina of several layers of the uninvaded parenchyma cells below and around the lesion. In normal, healthy scales these cells never contain pigment, the latter being confined to the cells of the outer epidermis. The significance of the appearance of pigment under these adverse conditions can not be entirely explained, but it seems to show very conclusively that the fungus infection at a given point on the scale not only affects the invaded cells but also influences either directly or indirectly the metabolism of uninvaded cells for some distance around the lesion. The stimulation of anthocyan production by fungus invasion is a matter of common observation in nature (*25*).

VARIETAL SUSCEPTIBILITY OF ONION SEEDLINGS

The writer has reported (*24*) experiments in which *Colletotrichum circinans* caused a damping off of White Globe seedlings under greenhouse conditions. It was of interest to note whether young seedlings of colored varieties would show any signs of resistance, especially since no pigment would have developed at this early stage. Sterilized greenhouse soil was inoculated with a pure culture of the organism, and seeds of a few of the varieties listed above were planted. The percentage of diseased plants in the white varieties were: White Portugal, 85 per cent; Giant White Italian Tripoli, 98 per cent; and Queen, 93 per cent; in the yellow varieties: Michigan Yellow Globe, 100 per cent; and Southport Yellow Globe, 93 per cent; and in the red varieties: Extra Early Red, 81 per cent; and Southport Red Globe, 95 per cent. The essentially equal susceptibility at this stage of all varieties tried, colored and white alike, is self-evident.[5]

The experimental and observational evidence conclusively shows that we have in colored onions a high degree of resistance to smudge but that this character is not present or at least not effective in the young seedlings. As will be shown later, the active resistant principle is a water soluble substance in the dry outer colored scales. This resistance is not to be construed as a case of absolute immunity nor is it unaffected by environmental conditions. This idea is substantiated by the fact that

[5] Under field conditions in our Northern States, the young onion seedlings, being spring-sown, are ordinarily growing in a soil too cool for the development of smudge. This probably accounts for the total lack of any report in America of damping off in the field due to the smudge fungus. In England (1922), however, this organism was found causing a damping off of young seedlings sown in August, at a time when soil temperatures were more favorable. This observation was on White Lisbon variety; no seedlings of colored varieties were available for comparison.

slight infection may occur on colored bulbs and even in rare occasions to a considerable degree if they are unduly exposed after maturity to the bleaching effects of direct sunlight as well as meteoric and soil water.

RELATION OF ONION OIL TO THE PARASITISM OF COLLETOTRICHUM CIRCINANS

The amount of research upon the chemical composition of the onion is limited. According to Remington and Wood (*15, p. 1525–1526*):

> Fourcroy and Vanquihn obtained from the ordinary onion a white acrid volatile substance containing sulphur, albumen, much uncrystallizable sugar and mucilage, phosphoric acid both free and combined with lime, acetic acid, calcium citrate, and lignin. The expressed juice is susceptible of vinous fermentation. The oil is essentially the same in chemical composition as the oil of *Allium Sativum* and consists largely of allyl sulphide $(C_3H_5)_2S$.

According to Gildemeister and Hoffman (*7, p. 546*), Semmler has found that onion oil, contrary to the reports given in most textbooks, consists not of allyl sulphid but of a series of sulphids, chiefly $C_6H_{12}S_2$. Kooper (*11*) found thiocyanic acid in the onion as well and secured positive tests for thiocyanic acid allyl ether, but no trace of acetic, formic, or allyl aldehyde. The germicidal effect of onion juice was noted by Pasteur (*12*).

RELATION OF EXPRESSED ONION JUICE TO COLLETOTRICHUM CIRCINANS

The relation of the constituents of the onion tissue to the fungus was first investigated experimentally by studying the effect of the expressed sap from onion scale upon spore germination. White onion scale tissue was macerated and the sap expressed by forcing through cheesecloth. Portions of the sap were diluted 10, 100, and 1,000 times with distilled water. Spores from a pure culture of the fungus were placed in drops of the expressed sap and in its various dilutions on glass slides in moist Petri dishes. No germination occurred in the sap as expressed from the tissue, nor in the 1 to 10 dilution. In the 1 to 100 dilution fair germination took place, and in the 1 to 1,000 dilution normal germination occurred. This experiment was repeated many times with practically the same results. When bits of fleshy onion scale tissue were added to drops of water containing spores, germination was also inhibited.

In order to determine the effect of heating on this inhibitive substance, a small amount of onion extract was secured in the usual way and divided into two equal parts, one of which was placed in live steam for 20 minutes. No germination occurred in the fresh extract as usual, while in the heated extract a high percentage of the spores germinated normally. From the results of these experiments it seemed quite certain that there was present in the extract a toxic substance which was readily removed by heat.

Since germination of the spore normally takes place in the soil or in water outside the plant, and not in contact with the host cell sap, and since the fungus would thus not come in contact with the inhibitive substance until its mycelium had penetrated the host, the question arose whether this toxic substance within the cell is capable of inhibiting the growth of the germ tube. Spores were germinated in distilled water until the germ tubes were several times the length of the spore, then a number of them were transferred to onion sap extract. The growth of the latter group was checked at once, while those left in water continued to grow at the normal rate. This showed that the mycelium could be checked by the

inhibitive substance of the cell sap. It seemed highly probable that this substance present in the living cells of the host was responsible, in part at least, for the checking of the advance of the fungus after it had penetrated the fleshy scales. It seems logical, therefore, to conclude that the restricted parasitism of the fungus is not due entirely to substances lacking in the fungus, but perhaps in some measure to the toxicity of the cell sap of the host.

EFFECT OF THE VOLATILE ONION OIL ON THE FUNGUS

Experiments were next undertaken to determine the effect of the volatile oil of the onion upon the germination and growth of the fungus. For the germination studies clean glass slides were laid in Petri dishes, two slides in each dish. The expressed juice of white onions, diluted to various degrees as in the above experiments, was used. Two drops of this medium containing the spores were placed on one slide in each Petri dish, while on the second slide in each dish were placed two drops of distilled water containing spores in suspension. The data secured from this experiment are tabulated in Table II.

TABLE II.—*Effect of volatile oil of onion extract on germination of spores of Colletotrichum circinans*

Petri dish No.	Slide No.	Medium.	Dilution.	Germination.	
				Experiment 1.	Experiment 2.
				Per cent.	*Per cent.*
1	1	Onion extract	Undiluted	0	0
	2	Distilled water		0	0
2	3	Onion extract	1 to 10	0	0
	4	Distilled water		75±	25±
3	5	Onion extract	1 to 100	75±	25±
	6	Distilled water		75±	95±
4	7	Onion extract	1 to 1,000	75±	95±
	8	Distilled water		75±	95±

The unusual and striking feature of these experiments is that in the Petri dishes containing the undiluted extract, no germination whatever occurred, either in the extract or in the drops of distilled water. This could be attributed only to the fact that a volatile substance from the expressed onion juice had been absorbed by the distilled water and had prevented the spores from germinating. The toxicity of the onion oil was thus clearly demonstrated. The toxic effect of the onion oil upon the spores in distilled water decreased as the onion extract was diluted. These experiments were repeated many times with essentially the same results.

The effect of the volatile oil was demonstrated in another way. A suspension of spores was made in two tubes of melted potato agar, which were then poured into sterile Petri dishes. After the agar had hardened the plates were inverted. In the center of the inside of the cover of one plate a small amount of onion extract was placed. The agar was thus exposed to the volatile oil, but was not in contact with the extract. The other dish served as a control (Pl. 2, B). In it the spores

germinated normally and growth soon covered the plate. In the other plate the volatile oil prevented germination of all the spores except those around the edge of the plate. In a few days the extract was decomposed by bacteria, since no attempt was made to keep it sterile; then the growth of the fungus proceeded from the margin toward the center of the plate. The fact that the spores in the center of the plate did not germinate showed that they had been killed by the volatile oil.

Another experiment was performed to determine the effect of the volatile oil on the growth of the mycelium. Three plates of potato agar were poured, and to the center of each was transferred a bit of mycelium from a pure culture of the fungus. The colonies were allowed to develop for four days, when they measured 18, 19, and 15.5 mm. in diameter, respectively. One plate was then designated as a control. Within the inverted covers of the other two were placed small amounts of onion extract as described in the previous experiment. In Table III are given the measurements of the colonies for the next five days. The colony in the control plate continued to grow at a normal rate, but those colonies exposed to the volatile oil practically ceased growth. At the end of the third day the extract was removed from one plate; the fungus colony in that plate resumed growth, showing that the mycelium had not been killed, but had merely been checked by the volatile oil. In the third plate a slight amount of growth had taken place by the fifth day, after which, as the onion extract decomposed, the fungus resumed the normal growth rate. This experiment shows that the growth of mycelium is markedly checked in the presence of the volatile oil, but that, unlike the spores, its growth processes are resumed when the inhibitive substance is removed. This fact is of significance in considering the effect of the onion oil upon the fungus hypha invading the scale tissue. One might expect in this case a slowing up or cessation of growth of the hypha, temporarily at least, but not necessarily a killing of the fungus filament.

TABLE III.—*Effect of volatile oil from onion extract on growth of Colletotrichum circinans*

Time of measurement.	Diameter of thalli.		
	Control.	Exposed.	Exposed.
	Mm.	*Mm.*	*Mm.*
Beginning of experiment	18	19	15.5
Second day	27	19	15.5
Third day	33	19	15.5
Fourth day	39	[a] 20	15.5
Fifth day	41	23	16.0

[a] Extract removed at end of third day.

ONION OIL FROM RESISTANT AND FROM SUSCEPTIBLE VARIETIES

It should be kept in mind that the foregoing experiments were conducted entirely with extracts from white onions, which have been shown to be susceptible to the disease. It was of interest to know what degree of toxicity was to be found in the extract from the yellow and the red varieties, the so-called resistant types. Accordingly, extracts were made from Red Globe and Yellow Globe bulbs, as well as from the White Globe, a single bulb being used in each case. It should be pointed out

here that there is a certain amount of pigment present in the epidermal cells of the colored fleshy scales which was necessarily included in the extract. Moreover, it will be shown later that the water extract of dry pigmented scales is toxic to the fungus. It is believed, however, that in these fleshy scale extracts the pigments were so dilute as to have had little or no effect. Various dilutions of the fleshy scale extracts in distilled water were secured, as before, and their effect upon germination of spores was determined. As described in the previous experiment, a Petri dish containing one slide for drops of spores suspended in distilled water and one slide for spores in the onion extract, was used for each dilution. Results of this experiment are included in Table IV.

TABLE IV.—*Toxicity of succulent scale extract from White, Yellow, and Red Globe varieties of onion to the spores of Colletotrichum circinans*

Dilution.	Petri dish No.	Medium.	Spore germination.[1]					
			Experiment 1.			Experiment 2.		
			White.	Yellow.	Red.	White.	Yellow.	Red.
Undiluted...	1	Extract	o	o	o	.....	.....	.....
		Distilled water	o	o	o	.....	.....	.....
	2	Extract	o	o	o	.....	.....	.....
		Distilled water	o	o	o	.....	.....	.....
1 to 10.....	3	Extract	o	o	o	o	o	o
		Distilled water	++	++	++	⊖	⊖	o
	4	Extract	o	o	o	o	o	o
		Distilled water	++	⊕	++	⊖	⊖	o
1 to 20.....	5	Extract	.....	.....	.....	o	o	o
		Distilled water	.....	.....	.....	⊕	++	⊖
	6	Extract	.....	.....	.....	o	o	o
		Distilled water	.....	.....	.....	⊕	++	++
1 to 40.....	7	Extract	.....	.....	.....	o	o	o
		Distilled water	.....	.....	.....	++	++	++
	8	Extract	.....	.....	.....	o	o	o
		Distilled water	.....	.....	.....	++	++	+
1 to 60.....	9	Extract	.....	.....	.....	o	o	o
		Distilled water	.....	.....	.....	++	++	++
	10	Extract	.....	.....	.....	o	⊖	o
		Distilled water	.....	.....	.....	++	++	++
1 to 80.....	11	Extract	.....	.....	.....	o	+	⊖
		Distilled water	.....	.....	.....	++	++	++
	12	Extract	.....	.....	.....	−	+	⊖
		Distilled water	.....	.....	.....	++	++	++
1 to 100.....	13	Extract	++	++	++	−	++	⊕
		Distilled water	++	++	++	++	++	++
	14	Extract	++	+	++	⊕	++	⊖
		Distilled water	++	++	++	++	++	++
1 to 1,000....	15	Extract	++	++	++	.....	.....	.....
		Distilled water	++	++	++	.....	.....	.....
	16	Extract	++	++	++	.....	.....	.....
		Distilled water	++	+	++	.....	.....	.....

[1] Symbols used: o=no germination; ⊖=0 to 25 per cent spores germinating; −=25 to 50 per cent; ⊕=50 to 75 per cent; +=75 to 90 per cent; ++=90 to 100 per cent.

It is quite evident that but little difference exists between the colored and white Globe varieties used as to the toxicity of their cell sap. In fact, in this respect the differences between individual bulbs of the same

variety are as great as those between varieties. It is to be expected, therefore, that the volatile oil content of these varieties of onions is a factor not necessarily responsible for differences in susceptibility or resistance to smudge.

From the foregoing experiments it may be concluded that the expressed sap from white onion scale inhibits germination and growth of *Colletotrichum circinans* and that this toxicity appears to be due, in large measure at least, to the volatile onion oil which is liberated upon crushing of the tissue. This toxic substance is thermolabile inasmuch as it is largely removed by heating the extract for 20 minutes in live steam. The fact that no appreciable difference in toxicity between sap from colored and that from white Globe scales was found indicates that the volatile oil has little to do with the difference in varietal susceptibility. It is believed, however, that this substance is not to be overlooked as a factor in limiting the parasitism of *C. circinans*.

It is of interest in this connection to note that Bernard (*2*) found the bulb tissue of Loroglossum to have a fungicidal effect upon the mycorrhizal fungi isolated from a number of closely related species of orchid. This was demonstrated by placing a piece of the bulb on agar in proximity to the thallus of the fungus. Growth of the latter did not extend within a certain zone surrounding the bit of orchid tissue.

RELATION OF SCALE PIGMENTS TO DISEASE RESISTANCE

It will not be necessary to review the literature upon plant pigments since it has already been brought together by others, including Wheldale (*25*) and Wakeman (*22*). Although a few cases of correlation between resistance and the red or yellow pigments in plants have been noted (*5*, *6*, *17*, *21*), in no previous case, so far as the writer is aware, has the relation of plant pigments to plant parasites been carefully studied. The fact that a water extract from pigmented onion scales has been proved to be highly toxic (*23*) to the smudge organism, has prompted a further inquiry into this subject. It is needless to point out that the widespread occurrence of closely related pigment compounds in the plant kingdom justifies further investigation of their relation to parasitism.

NATURE OF THE RED AND YELLOW PIGMENTS IN ONION SCALES

The red and yellow pigments of onion scales are solutes in the cell sap of the outer epidermal layer. They first appear when the plants are about half grown and continue to develop during the remainder of the growing season. At maturity the color is most intense in the outer scales which have dried down to a thin papery consistency, and it becomes progressively less marked in the fleshy scales as the center of the bulb is approached. Intensity and color of pigment vary with varieties. Colored varieties may be roughly grouped into the large classes, the yellow and the red. There are numerous varieties, however, such as the Australian Brown, which lie on the border line between the two groups. Pigmented cells of the yellow varieties when treated with alkalies turn deep brownish yellow in color, a reaction typical of the flavones (*25*). The pigmented cells of the red varieties turn pink in acid and green in alkaline solutions, which reactions are characteristic of the anthocyans. Perkin and Hummel (*14*) isolated quercetin, a flavonol,

from a hot water extract of colored onion scales, but they did not state the variety or color of onion used. Perkin and Everest (*13, p. 201*) state that attempts to isolate a quercetin glucoside from onion skins have hitherto failed. Our work shows that quercetin exists to a considerable extent in both the yellow and the red scales, and in the last case it evidently occurs along with an anthocyan. Beyond the work of Perkin, just cited, there is no information available as to the exact nature in which the red and yellow pigments exist in the onion tissue. In connection with the present problem this question is now being investigated in cooperation with the Department of Pharmacy, University of Wisconsin, and results with their bearing upon the relation between parasite and host will be published later. It is a matter of common observation that upon death of the cell, the cell sap pigments readily diffuse out. Likewise in the onion, there is considerable diffusion of soluble pigment from dead outer scales but little or none from the succulent scales.

RELATION OF ONION PIGMENTS TO THE FUNGUS

The close correlation between presence of pigment in the outer scale of the onion and resistance to smudge has already been pointed out. In further search for the substances in the plant responsible for resistance, a study was made of the effect of water extracts of the dry outer scales upon the germination and growth of the fungus.

It has been shown that when bits of the fleshy scale of the white or colored onion were placed in drops of water containing spores of *Colletotrichum circinans*, germination was entirely inhibited. Similar trials made with outer dead scales of white onion yielded no inhibition, however. Thus, it was evident, that a large part of the volatile oil present in the fleshy tissue is lost as the outer scale dries down. It is assumed, therefore, where dry outer scales were used that toxicity of the volatile oil did not enter in to any appreciable degree in the following experiments.

METHODS

The methods used were as follows: Drops of distilled water containing spores of the fungus were placed on clean glass slides, two drops on each slide. Petri dishes lined with moistened filter paper were used as moist chambers; two slides were placed in each Petri dish, one serving as a control, the other as a means of testing the effect of scale extract on spore germination. In the drops on the latter slide bits of onion scale about 2 mm. square were placed, usually two such pieces per drop. Observations were made and recorded after 18 to 24 hours.

EFFECT UPON SPORE GERMINATION

Further experiments, conducted with bits of tissue from the dead outer scales, resulted in quite a different reaction on the part of the fungus than had hitherto been noted. No indication of the injury to control drops so evident in the last experiment was noted, showing that the fungicidal effect of the volatile oil is largely, if not entirely, absent in the dry outer scales. In the case of drops of spore suspension containing bits of the dry white scale, germination occurred and appressoria formed as usual. The behavior of the organism was thus very

similar to that commonly observed in distilled water or on nutrient media. However, where red or yellow scale tissue was added to the drops distinctly abnormal germination occurred. In a large majority of cases, the spore sent out a germ tube, which usually acquired a length of only about one micron, when the wall at its tip either dissolved or ruptured and a portion of the cell contents exuded and collected in a naked mass at the end of the very short and usually indistinguishable tube. Thus at the end of 12 to 18 hours most of the spores were to be found each with a naked mass of the cell contents adjacent to them. This feature is illustrated in figure 1 and Plate 3. For the purpose of the present paper this condition is referred to as "rupturing" of the germ tube, although the exact details of the process have not been worked out and it is realized that further study may necessitate another descrip-

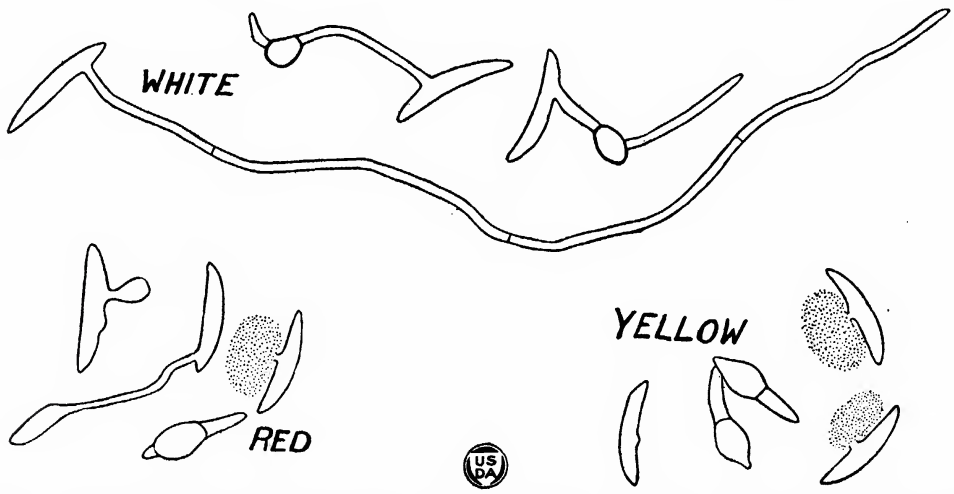

FIG. 1.—Effect of water extract of outer onion scales upon spore germination of the smudge fungus. Bits of dry scales of white, yellow, and red onions were added to drops of spore suspension in distilled waters. Note typical germ tubes and appressoria in the case of the white scale as compared with abnormal germination in the case of the colored scales. The abnormalities are of three types: (1) Ruptured germ tubes, (2) short, thick germ tubes, (3) swelling of spores without production of germ tubes.

tive term. This phenomenon usually prevented further functioning of the spore.

In a smaller percentage of cases there occurred other abnormal types of reaction, which may be divided into two general classes. The first class includes those which will be referred to as "swollen spores." In this group there is a marked swelling of the spores, usually at one end, with septation occurring at the limit of this enlargement (fig. 1); no germ tubes are formed. In the second class, designated as having "abnormal germ tubes," the tube grows for a short distance, is usually swollen at the tip or abnormally large in diameter, and never produces an appressorium (fig. 1).

The percentages of various types of germination obtained in the first experiment are recorded in Table V. The results were determined by counting several microscopic fields from each slide. It is to be noted that ruptured germ tubes occurred with a large majority of the spores where red or yellow scale tissue was added to the drops. Moreover, the spores reacted essentially alike to the red and yellow tissue. Numerous repetitions of this experiment yielded essentially similar results. The data from one of these experiments are recorded in Table VI.

TABLE V.—*Effect of dry outer scale tissue of red, yellow, and white varieties of onion upon the spore germination of Colletotrichum circinans (experiment 1)*

Variety.	Germination.				
	No germination.	Normal germination.	Ruptured germ tubes.	Abnormal germ tubes.	Swollen spores.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Red	0	21	78	0	1
Yellow	0	35	61	2	2
White	10	90	0	0	0

TABLE VI.—*Effect of dry outer scale tissue of red, yellow, and white varieties of onion upon the germination of Colletotrichum circinans (experiment 2)*

Petri dish No.	Color of tissue used.	Germination.				
		No germination.	Normal germination.	Ruptured germ tubes.	Abnormal germ tubes.	Swollen spores.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	Red	7	2	45	42	4
	Control	9	91	0	0	0
2	Red	12	0	88	0	0
	Control	12	88	0	0	0
3	Yellow	34	0	66	0	0
	Control	7	93	0	0	0
4	Yellow	24	0	76	0	0
	Control	11	89	0	0	0
5	White	8	92	0	0	0
	Control	9	91	0	0	0
6	White	13	87	0	0	0
	Control	47	53	0	0	0

EFFECT UPON GROWTH

The experiments just described show conclusively that the water extract of pigmented dry scales is highly toxic to spores of *Colletotrichum circinans*, while a similar extract from white scales has no such inhibitory effects. The fact that the toxic substance (or substances) is so readily diffusible leads one to surmise that the plant may be protected by its gradual solution into the soil water immediately surrounding the bulb. The smudge organism develops normally in the soil; hence it is conceivable that in some cases at least the spore may germinate at some distance from the bulb; thus the mycelial hyphae may be the first to come into close contact with the bulb or with the soil solution immediately surrounding it. Accordingly an experiment was carried out to determine the effect of the pigmented extract upon the growth of the fungus mycelium.

Spores were germinated in water until the germ tubes were several times the length of the spores. Small pieces of dry outer red scales were then added to certain of the drops, while others were left as controls. After 24 hours, examination showed that whereas the hyphae in the control drops had continued to grow normally, those to which the bits of red scale had been added had grown little if at all from the time these pieces

were added. This is best shown in figure 2, which contains camera-lucida sketches of representative spores and thalli, made at the time when the scale tissue was added to the drops of spore suspension and at the conclusion of the experiment. It is quite evident that the toxic substance (or substances) here concerned inhibits the growth of hyphae as well as the normal germination of spores of *Colletotrichum circinans*.

EFFECT OF COLORED AND OF UNCOLORED PORTIONS OF THE SAME SCALE

It has already been pointed out that ordinarily little or no pigment is formed in the outer scales at the neck of the bulb. Moreover, under suitable conditions this particular portion of an otherwise resistant plant is readily attacked. It was important, therefore, to know whether the inhibitive substance already demonstrated in the pigmented tissue could be found in this closely adjacent uncolored area. The outer scale of a red onion set (Red Wethersfield variety) which had become naturally infected in the uncolored portion at the neck was carefully removed.

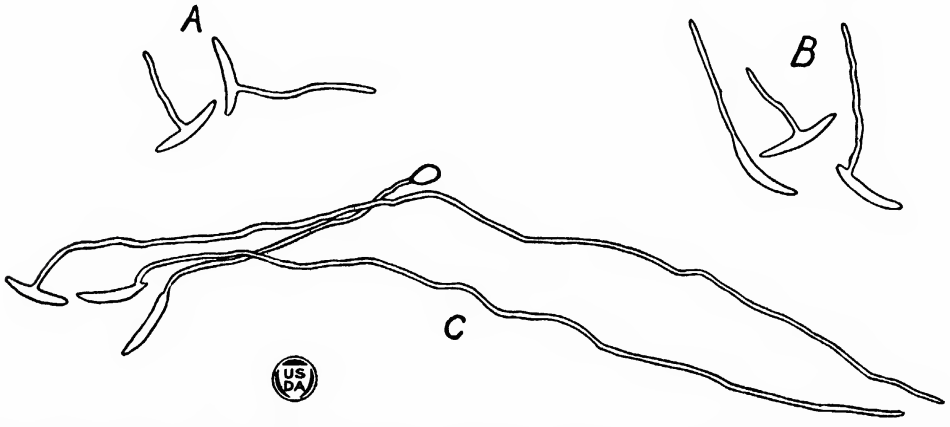

FIG. 2.—Effect of colored outer scale extract upon growth of hyphae of the smudge fungus. Spores were germinated in drops of distilled water to the point illustrated by camera lucida sketches in A. To some of the drops bits of red outer scale were then added. Growth was recorded by further sketches 24 hours later. In B are shown typical spores from drops to which red scale tissue has been added, while in C are shown typical spores from control drops. Growth was almost, if not entirely, checked when the scale tissue was added.

Bits of tissue were then cut from the colored part of the scale just below the area of infection, and from the uncolored part about 1 inch above this point. The scale after the removal of these bits of tissue is shown in Plate 4. The bits of tissue from the two points were placed in drops of spore suspension according to the previously described method. In the drops containing uncolored tissue normal germination occurred, while in those containing colored tissue practically all of the spores developed ruptured germ tubes. Representative spores from each lot are illustrated in Plate 4. The experiment has been repeated several times with similar results, showing conclusively the close correlation existing between the pigment and the toxic entity.

EFFECT OF DILUTING THE PIGMENT EXTRACT

In order to secure a more nearly uniform basis of comparison between white, yellow, and red bulbs, an equivalent amount of dry scale tissue from each variety was weighed. The three lots were then allowed to steep for 24 hours at room temperature in distilled water added at the

rate of 20 cc. per gram of tissue. The extract was filtered and various dilutions of the filtrate with distilled water were used as media for spore

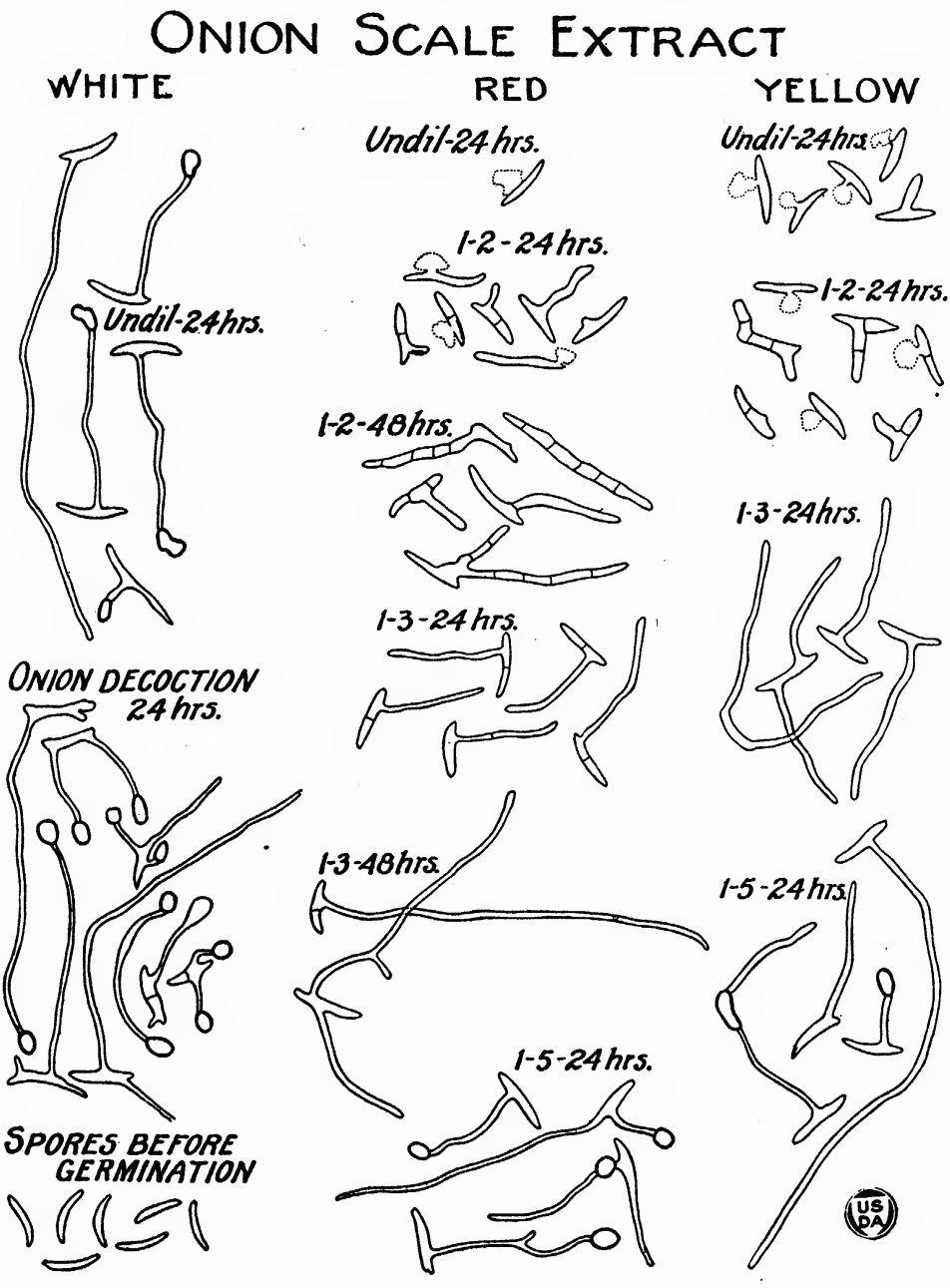

FIG. 3.—Spore germination of *Colletotrichum circinans* in extracts made from dry outer scales of white, red, and yellow varieties of onion, and in onion decoction. One gm. of dry scale was extracted over night in 20 cc. of distilled water at room temperature. Dilutions were then made up to 1 to 5 with distilled water. Note that germination and growth were normal in onion decoction and undiluted white scale extract, while in the undiluted colored scale extract typical abnormal germination occurred as shown in fig. 1. With dilution the toxicity of the extract was reduced, however, quite normal germination occurring at 1 to 5. Normal ungerminated spores are shown in the lower left-hand corner. See further explanation in the text.

germination. The results are recorded below; representative spores from each dilution are illustrated by camera lucida sketches in figure 3.

White scale tissue:

Undiluted—Good germination, growth, and appressorium formation.

Yellow scale tissue:
- Undiluted.....At 24 hours, a large percentage of ruptured germ tubes; at 48 hours, no change.
- 1 to 2.....At 24 hours, many ruptured germ tubes and many short, thick, germ tubes; at 48 hours, practically no change.
- 1 to 3.....At 24 hours, short, thick germ tubes, no appressoria; at 48 hours, good growth and appressorium formation.
- 1 to 5.....At 24 hours, good growth and appressorium formation.

Red scale tissue:
- Undiluted.....At 24 hours, very little germination; when it occurred it resulted in ruptured germ tubes; at 48 hours, no change.
- 1 to 2.....At 24 hours, ruptured and short, thick, abnormal germ tubes; at 48 hours, some additional growth of latter.
- 1 to 3.....At 24 hours, germ tubes slightly longer than in 1 to 2, no appressoria, an occasional ruptured germ tube; at 48 hours, considerable additional growth, but no appressoria.
- 1 to 5.....At 24 hours, good germination and appressorium formation.

It will be seen that in the undiluted extract of white scales, germination was quite normal and compared favorably with that in onion decoction. In the undiluted red and yellow scale extract "ruptured" germ tubes were common. As the colored extracts were diluted, however, there was a gradual diminution of the toxic effect with seemingly normal germination occurring in the 1 to 5 dilution This shows that the inhibition in the concentrated solutions was not due to a lack of nutrients. Moreover, the toxic entity was apparently about equally concentrated in the extract from the red and that from the yellow scales, if we may judge from the extent to which germination was reduced in equal dilutions of the two extracts.

RELATION OF CELL SAP ACIDITY TO THE FUNGUS

The reaction of the cell sap of the host plant as a factor in resistance to parasites has been emphasized by Comes (*3*, *4*), but Jones, Giddings, and Lutman (*10*), Hawkins and Harvey (*9*), Vavilov (*20*), and others found no positive evidence of its importance as the cause of resistance. Since this point is not one to be overlooked, a study of the acid toleration of *Colletotrichum circinans* was made.

Onion decoction, a medium which had proved very favorable for germination and growth, was used in this study. Two hundred gm. of fresh onion bulb tissue and 1,000 cc. of distilled water were cooked in the steamer for one hour. This decoction was then filtered through filter paper and the hydrogen-ion concentration of the filtrate determined. The medium was then divided among ten 200-cc. flasks and the acidity of each adjusted with standard solutions of hydrochloric acid or with sodium hydroxid so as to cover the following range of P_H values: 1.8, 2.0, 3.0, 3.6, 4.4, 5.2, 6.4, 7.4, 8.8, and 9.4. A portion from each flask was used for spore germination tests and the remainder sterilized for 30 minutes at 7 pounds pressure. The acidity of each flask was again tested after sterilization and the following values noted: 1.6, 1.8, 2.2, 3.4, 4.6, 5.2, 6.2, 6.8, 7.4, and 8.2. Spore germination tests were also made with portions from these lots after sterilization. Good germination occurred in the alkaline media and in the acid up to P_H 3.4. When the acidity increased beyond that point, germination was reduced and abnormal. In the unsterilized extract at P_H 1.8 and 2.2, 10 to 15 per cent of the spores began to germinate, but the germ tubes appeared to rupture and exude cytoplasm in a manner strikingly similar to that observed in the water extract from dry

colored onion scales. Occasionally there was a slight swelling of the spores (fig. 4). At P_H 3, about 43 per cent of the spores germinated, but the hyphae were abnormal in size and stunted, and in many cases the germ tubes ruptured. In the sterilized extract no germination occurred at P_H 1.6 to 2.2, except for an occasional ruptured germ tube at P_H 2.2.

The growth of the fungus on onion decoction of various P_H values was tested. The medium was made as described above, except that 2 per cent dextrose was added. Duplicate portions of 75 cc. each in 300-cc. Jena glass flasks were used. The medium was inoculated by transferring a piece of stroma and mycelium from a potato agar culture of the organism to each flask. The flasks were kept in diffused light at room temperature (20° to 25° C.) for 54 days. The mycelial growth was then removed from the remaining culture medium by filtration and the reaction of the filtrate determined. The P_H value of the medium before and after filtra-

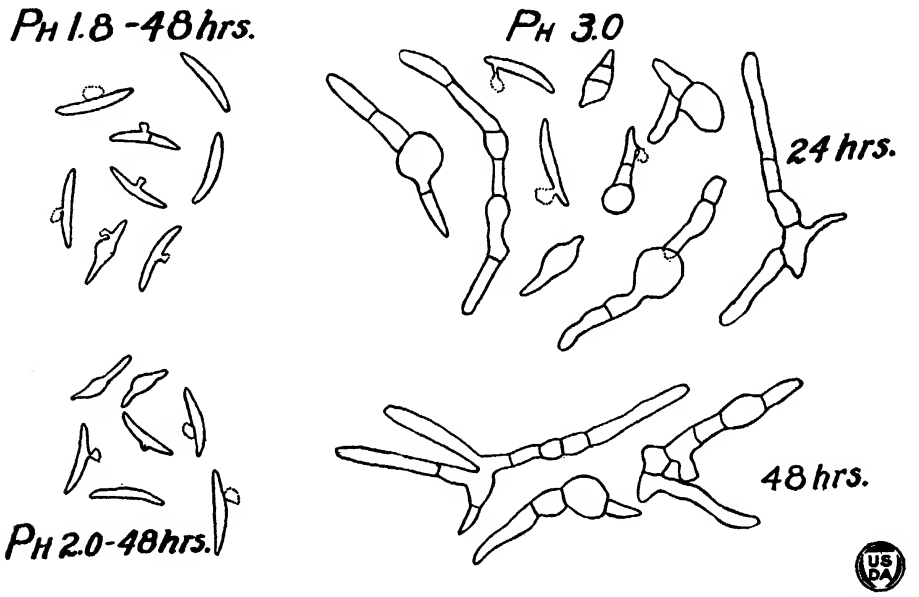

FIG. 4.—Spore germination of *Colletotrichum circinans* in onion scale decoction adjusted to the various degrees of acidity indicated. Note the ruptured germ tubes at P_H 1.8 and 2.0, and the same together with swollen spores and short thick germ tubes at P_H 3. See further explanation in the text.

tion, and at the end of the experiment, as well as the amount of fungus growth occurring in the flasks, are recorded in Table VII.

A repetition of this experiment with Pyrex flasks and the use of spores instead of mycelium as inoculum yielded essentially the same results.

It may be concluded from the above experiments that both the spores and the mycelium of *Colletotrichum circinans* germinate and grow well in onion decoction ranging in P_H value from about 3.5 to 8. As the actual acidity increases beyond 3.5 there is a sharp reduction in germination and growth, with a tendency toward rupturing of the germ tubes. It is important also to note that in the flask cultures, where good growth occurred, the reaction of the medium changed decidedly toward the alkaline during the period of the experiment. The rapidity of this change and the actual variations in the reaction from day to day were not determined.

The acid toleration of the fungus having been studied, the reaction of the onion tissue was next considered. Plants of the Red, Yellow, and White Globe varieties growing out-of-doors were pulled at about 8.30

a. m., July 31, 1920, and determinations made directly by the electrolytic method.[6] The tops and the scales were chopped up separately and the expressed juice used for the determinations. The results are given in Table VIII. It is to be remembered, of course, that this method does not necessarily give the actual reaction which the fungus hyphae encounter as they invade the plant. It is conceivable that there may be natural variations in reaction in different parts of the bulb and at different hours of the day, while secretions of the fungus itself might change its immediate surroundings. Valleau (*19*) suggests in the case of brown-rot of plums (*Sclerotinia cinerea*) that, "it is very probable that in the slow development of the fungus in the resistant fruits enough oxalic acid is produced by the hyphae to actually become toxic to them." However, there was no evidence of wide variations in acidity between resistant and susceptible onion bulbs, and the acidity of the juice expressed from either was much lower than that of the onion decoction in which germination and growth of the fungus were inhibited. Moreover, the fungus tended to change very acid solutions to a more alkaline, and thus more favorable, reaction.

TABLE VII.—*Growth of Colletotrichum circinans in onion decoction adjusted to various P_H values (duration of experiment, 54 days)*

Flask No.	P_H value.			Amount of growth.
	Before sterilization.	After sterilization.	At end of experiment.	
1	2.0	1.6	1.8	None.
2	2.0	1.6	1.8	Do.
3	3.0	3.0	2.0	Do.
4	3.0	3.0	2.0	Do.
5	3.2	3.0	2.6	Very slight.
6	3.2	3.0	2.6	Do.
7	3.8	3.6	5.2	Good growth and sporulation.
8	3.8	3.6	4.8	Do.
9	4.6	4.6	6.2	Do.
10	4.6	4.6	6.2	Do.
11	5.2	5.4	7.2	Good growth.
12	5.2	5.4	7.0	Do.
13	6.4	6.4	7.4	Do.
14	6.4	6.4	7.4	Do.

TABLE VIII.—*Acidity of expressed juice of scales and tops of Red, Yellow, and White Globe onions*

Variety.	P_H values.	
	Tops.	Succulent scales.
Red Globe	5.2	5.3
Yellow Globe	4.8	5.4
White Globe	5.2	5.4

[6] The writer is indebted to Mr. O. C. Bryan of the Department of Soils, University of Wisconsin, for making the hydrogen-ion determinations.

The possibility of a connection between acidity and toxicity in cold water extracts of dry outer scales was considered. Outer scales of red, yellow, and white bulbs were extracted for 24 hours in redistilled water having an approximately neutral reaction. For each gram of tissue 15 cc. of water were used. The hydrogen-ion concentration of each extract was then determined and portions of each were used for spore germination tests. The results are given in Table IX. Although the acidity of the outer scale extracts is somewhat higher than that of the succulent tissue, it was still in all cases within the range tolerated by the fungus, and the toxicity, therefore, must be due to something beside acidity. In none of these determinations was there any evidence of wide variation in acidity between resistant and susceptible bulbs.

TABLE IX.—*Acidity of cold water extracts of dry outer scales of white and colored onions*

Variety.	P_H value of extract.	Reaction of spores.				
		Ungerminated.	Normal germination.	Ruptured germ tubes.	Swollen spores.	Short, abnormal germ tubes.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Yellow Globe	3.70	52	0	47	1.0	0.0
Yellow Globe	4.67	24	0	74	1.0	1.0
Red Globe	3.55	91	0	8	0.5	0.5
White Globe	4.03	Percentage germination fairly high; good growth; numerous appressoria; new conidia being formed at 48 hours, making spore counts impossible.				

DISCUSSION OF RESULTS

The resistance of the common colored varieties of onions to smudge is well established. The water extracts from the dry outer scales of red and of yellow onions have shown a marked toxicity to spores and mycelium of *Colletotrichum circinans*, and the reaction of the fungus is quite different from that brought about by the onion oil. In the scales of white varieties the toxic entity ordinarily associated with flavone or anthocyan pigments in the colored varieties, is apparently absent or too slight to be detected. Moreover, in the colored varieties, it is confined to that tissue in which there has been production of pigment. Its absence in the unpigmented portions of the colored scales about the neck of the bulb is correlated with a marked susceptibility to the disease in this limited region. The fact that this toxic substance is readily diffusible in water indicates that meteoric or soil water immediately adjacent to colored bulbs may become sufficiently toxic to ward off invasion by the fungus. In any case, we are led strongly to suspect that by means of this toxic substance the dry outer scales of colored bulbs serve as a barrier between the parasite in the soil and the underlying fleshy scales, which have been shown to be susceptible to infection.

The exact identity of the toxic substance (or substances) remains to be determined. Its very close association with the scale pigments is suggestive. The toxicity may be due to the color compounds or to compounds closely associated with them. Attempts are now being made to isolate the substance in pure form, but it must be recognized

that such an analysis breaks down to some extent the complex balance of compounds as they exist in the tissue.

The marked toxicity of the expressed juice of fleshy scales of both the susceptible and the resistant varieties is also of interest. It seems to be due largely to the volatile onion oil, but apparently it does not contribute to the noted differences in varietal susceptibility of the northern Globe types. The juice from colored scales and that from white scales are essentially equal in toxicity. Moreover, as stated above, infection of the fleshy scales seems to progress as readily in resistant as in susceptible varieties. The relation of the volatile oil to the aggressiveness of the parasite may well be considered, however. The smudge organism causes an exceedingly slow decay of the bulb compared with certain other bulb parasites, such as *Botrytis allii*, for instance, although penetration by the former is accomplished very readily and easily and in fact much more generally than in the case of the latter fungus, which usually requires a wound in order that invasion may take place. When we consider the marked toxicity of the onion oil to the smudge fungus, we are led to wonder that invasion occurs at all. However, several possible explanations at once present themselves. In the first place, the oil as it is released from the expressed sap may be in a different state than that in which it actually exists in the living cell. In the allied case of the mustard oil of the crucifers, allyl isothiocyanate, for instance, the oil exists in certain cells as the glucoside, sinigrin, while in adjoining cells is contained a glucoside-splitting enzym, myrosin. When the tissue is crushed the enzym comes into contact with the glucoside and the volatile oil is released. Does a similar condition prevail in the onion? In the second place, under ordinary natural conditions the mycelium of the smudge fungus apparently does not invade the living host cells, but weakens and kills them, usually slightly in advance of the hyphae, by means of some diffusible substance. Moreover, the change in color and texture of the cell contents and, in the case of red scales, the tendency toward formation of coloring matter in the parenchymatous cells both indicate certain effects on host metabolism ahead of the parasite. It may be that through these changes in the cells the volatile oil is either broken down or released and hence that its toxic effects are reduced, at least to the point where invasion is merely retarded and not entirely checked.

The study of penetration indicates that the fungus is capable of penetrating the cuticle and dissolving the cellulose of the walls equally well in all varieties. The acid range tolerated in culture media is wider than the range occurring in the host. It is assumed, therefore, that differences in cell membranes and in acidity of cell sap do not materially affect resistance to the smudge organism.

SUMMARY

(1) Onion smudge is a common disease of the bulb scales. In general, white varieties are susceptible to the disease while colored varieties show a high degree of resistance.

(2) When the dry outer scales of colored varieties were removed the fleshy scales were found to be very susceptible, invasion taking place in the same manner as in the white varieties. The highly colored dry outer scales apparently serve as a barrier to the entrance of the parasite.

(3) In the seedling stage all varieties tried were equally susceptible.

(4) The expressed juice of succulent onion scales is highly toxic to the fungus; this effect is seemingly due in large measure to the volatile onion oil. The extracts from resistant and from susceptible varieties have essentially equal degrees of toxicity. The onion oil apparently is not a factor contributing to varietal resistance to smudge in the northern Globe types, but its importance in limiting the parasitic action of the fungus is suggested.

(5) The red and yellow colors in onion bulbs are due to pigments which are present in the cell sap of the outer epidermal layer. This color is most intense in the dry outer scales, and at least a portion of the pigment in these is readily soluble in water.

(6) Water extract of dry outer pigmented scales causes abnormal germination and retards growth of the fungus, while that from dry outer white scales does not. Essentially the same reaction was recorded with the yellow as with the red scales.

(7) A substance (or substances) present in the dry outer scales of resistant bulbs and closely associated or identical with the red and yellow pigments is apparently the chief factor causing resistance to smudge. Studies are now being directed toward isolation and identification of the toxic entity.

(8) Within the range determined for host tissue, variation in the hydrogen-ion concentration of onion decoction as a medium affects neither germination nor growth of the fungus.

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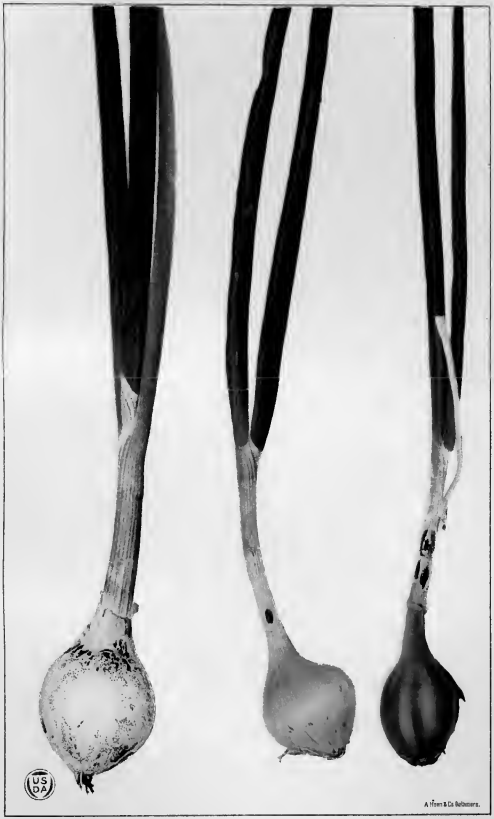
PLATE 1

Varietal resistance to onion smudge.

White, yellow, and red onions grown in the same field of smudge-infested soil and gathered shortly before harvest. In the case of the white (susceptible) variety the fungus has already developed abundantly on the outer scale of bulb and neck. In the red and the yellow (resistant) varieties, the colored outer scales have remained free from the disease; infection has occurred only at the neck, where little or no pigment developed.



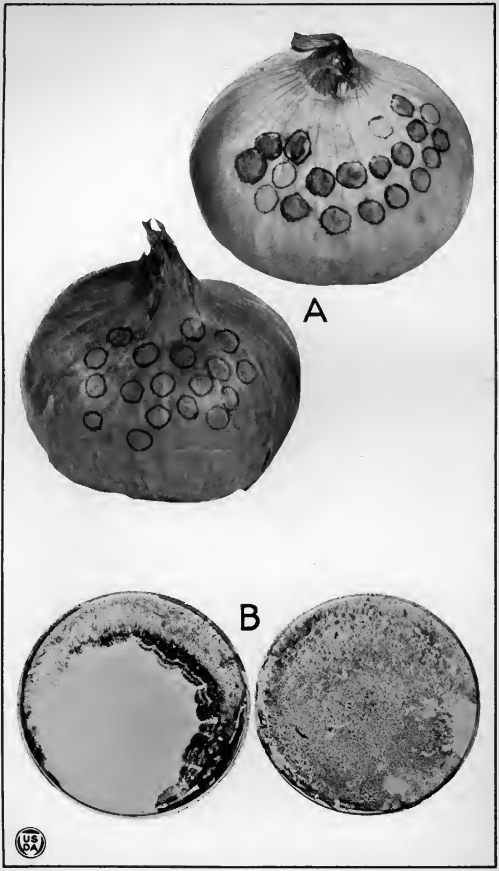

A
B

PLATE 3

Effect of cold water extract from dry outer onion scales upon spore germination of *Colletotrichum circinans*.

A.—Photomicrograph of spores about 17 hours after being placed in an extract from the yellow scales. Note the masses of naked cytoplasm which have exuded from the spores after the tip of the young germ tubes have ruptured. Compare with B.

B.—Photomicrograph of spores about 17 hours after being placed in an extract from white scales. Note the normal germ tubes and appressoria. Compare with A. See also figure 1 and further explanation in the text.

PLATE 2

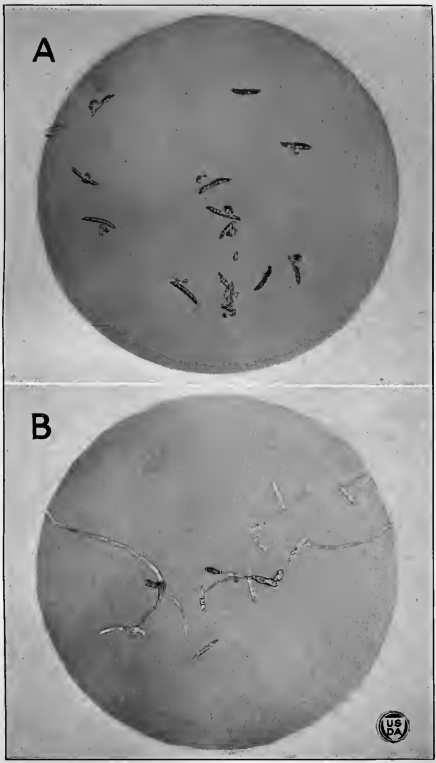
Relation of volatile oil and scale pigment to the onion smudge organism.

A.—Relation of outer colored scale to infection: Two bulbs of Yellow Globe (resistant) variety inoculated by placing drops of spore suspension, in onion decoction, at points indicated by black circles.

Left: Inoculations made on dry, highly pigmented outer scale; no infection resulted.

Right: The thin, dry, outer pigmented scales were removed from this bulb and the inoculations were made on the first fleshy scale, which contained relatively little pigment; scale infection resulted in a majority of cases.

B.—Effect of volatile oil upon spore germination: Petri dish cultures prepared by pouring melted potato agar in which spores of the smudge organism were suspended. Plate at left exposed to volatile oil of onion by being inverted over a small amount of expressed onion juice for several days. The spores were all killed except those around the outer edge of the plate. As the juice deteriorated due to bacterial contamination, growth progressed inward from the unaffected area. Plate at right untreated; normal germination and growth occurred thoroughout.


A
B

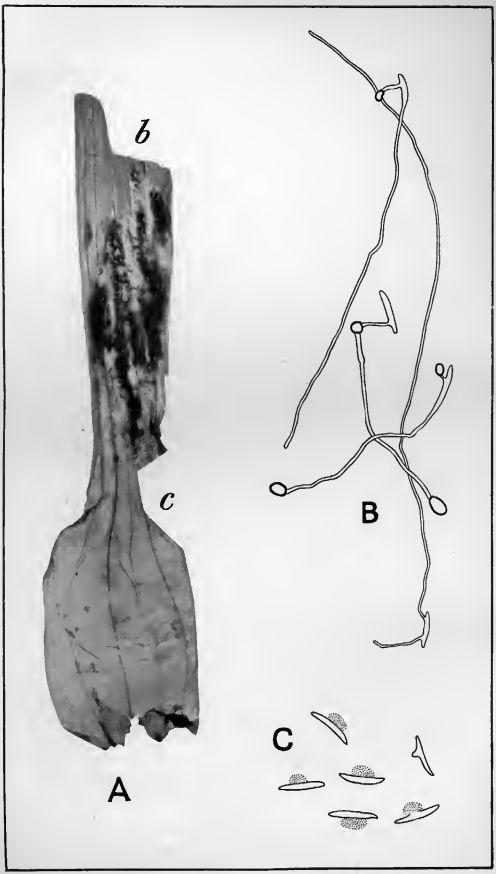

b
c
A
B
C

PLATE 4

Correlation of pigment with onion smudge infection and with the occurrence of the toxic entity.

A.—Portion of outer scale removed at harvest time from a red onion set similar to that shown in Plate 2. (Enlarged × 3.) Note that heavy smudge infection occurred at the neck above the point where pigment developed, while below that point the scale remained free from the disease. The scale tissue in the respective portions was tested for the presence of the toxic entity. Results of spore germination tests are shown in B and C.

B.—Normal germination resulting when bits of uncolored portion of scale from *b* in A were added to a drop of distilled water in which spores were suspended.

C.—Abnormal spore germination similar to that shown in figure 1, resulting when bits of the colored portion (*c*) of the same scale were placed in another drop of spore suspension. Note "rupturing" of germ tubes or production of short tubes of abnormally large diameter.

THE EFFECT OF RESPIRATION UPON THE PROTEIN PERCENTAGE OF WHEAT, OATS, AND BARLEY[1]

By F. W. McGINNIS, *Assistant Professor of Farm Crops, Division of Agronomy and Farm Management,* and G. S. TAYLOR, *Analyst, Division of Chemistry, Department of Agriculture, University of Minnesota*

INTRODUCTION

Scientific literature contains considerable information dealing with the protein content of small grains. In a review of the available information bearing upon this subject, which has accumulated during the past century, one chief point of interest seems to be the variation in protein percentage found in the different grains when produced under different conditions of soil and climate.

Much study and investigational work has been conducted to ascertain the cause of the variation found in this regard, especially with wheat. Wheat has been the subject of greatest study, because of the interest of those engaged in the manufacture of wheat products, where the chemistry of the grain is of paramount importance in determining the quality of the output.

The reason why grain produced under a given environment will yield a berry entirely different in chemical structure from that produced under a condition of a different nature, has been a subject of much study with the grain chemist as well as with those engaged in physiological investigations.

It seems to be the generally accepted idea at the present time that the percentage of protein in wheat is due to a certain environmental condition which prevails during the growth and maturity of the grain. Upon analysis of the grain, grown under widely different environmental conditions, this is generally found to be true. It is common knowledge that wheats grown in the north central section of the United States are of a higher protein percentage and milling value than those grown in the eastern or far western sections. There is no doubt that the varieties being grown for the highest total yield in these respective areas, account, to a certain degree, for the difference in protein percentage in the grain. But when consideration is confined within a variety, the variation is found to be very wide.

Some investigators have attributed the difference to the available nitrogen in the soil, others to varietal differences, while most students of the subject have recognized it to be a physicochemical phenomenon correlated more directly with the climatic conditions which prevail during the life processes. In general, the protein percentage has been found to be dependent somewhat upon the length of the growing season and to a greater degree upon the length of the ripening period. As these two factors are dependent largely upon the amount of available moisture in the soil, the strongest correlation has been found between the amount of rainfall, its distribution, and the protein percentage.[2]

[1] Accepted for publication Feb. 20, 1923. Published with the approval of the Director as paper 397 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] McGINNIS, F. W. THE RELATIVE EFFECT OF ENVIRONMENT UPON THE CHEMICAL COMPOSITION OF WHEAT, OATS, BARLEY, AND CORN. Unpublished thesis. Copies on file in Cornell University Library, Ithaca, N. Y. 1918.

That the external environmental conditions are responsible largely for the protein percentage found in the grain has been noted from the earliest observations. A realization, however, of the manner in which the contents of the grain are influenced by the capricious nature of external conditions and the physiological complexities which may arise during the life of the plant, is of more recent discovery. In order to understand fully how an environmental condition may affect a variation in the chemistry of the grain, it is necessary to follow out, from the earliest stages, the development of a kernel and the processes through which it reaches maturity.

Early investigational work along this line consisted of chemical analyses of the grain being made shortly after the time of flowering or when the mold of the grain was first formed, and continuing progressively at short intervals until the grain was mature. The nitrogen percentage was found to be highest as the kernel mold was formed and became decreasingly less as the grain filled and approached maturity. These observations led investigators to assume that most, or practically all, of the nitrogenous material was taken from the soil and moved into the grain during the earlier stages of growth, and that the filling of the endosperm with starch or carbohydrate material was carried out in the later stages of growth. Thus, it was concluded that the percentage of protein would be influenced directly by the amount of carbohydrate produced and translocated during the filling stage. As the environmental conditions in most of the grain sections are favorable to maximum growth during the early stage of development, and very often too severe to permit later growth to proceed or the grain to be filled, this was the natural conclusion to be drawn.

More recent work by Brenchley (*2*)[3] of the Rothamsted Experiment Station, Thatcher (*5, 6*), of the Minnesota Agricultural Experiment Station, and others, has brought out some slightly different ideas on the subject. These investigators have shown that the greater amount of nitrogenous material of the wheat or barley kernel is not taken up during the early stage of growth, but that the nitrogen inflow is continuous until the grain has reached maturity. According to Brenchley the nitro-carbohydrate movement into the grain is in constant ratio during the entire period of kernel development. It has been suggested that the variation in protein content is not due to the failure of the plant to produce and translocate carbohydrate material at the later stages of growth, but that the percentage of nitrogen found in the kernel is determined by the amount of carbohydrate lost by respiration during the desiccation process throughout the ripening period.

The difference of opinion as to which process is most effective in determining the character of the grain contents suggested the necessity of obtaining definite data to verify the later idea, which appears from available information to be an unwarranted conclusion. The authors have conducted a project through the years 1920 and 1921 to determine the variation in protein in wheat, oats, and barley, as affected by respiration, and to show the relative variation in protein when the same grains are grown under widely different conditions.

[3] Reference is made by number (italic) to "Literature cited," p. 1048.

PLAN OF EXPERIMENT

Marquis wheat, Improved Ligowa oats, and Manchuria barley were grown in 17 localities in the United States during the years 1920 and 1921. The grains were grown at experiment stations and substations in eight States which represent extremes, so far as the quality of grain produced is concerned. The seed was sent to each station from University Farm, St. Paul, Minn., each year, so that the seed planted would be identical. Plantings were made at the regular planting time in the spring and harvested when the grain was mature. Samples of the crop in each instance were sent to University Farm, where nitrogen determinations were made for total protein.

During the summer of 1921, respiration studies were made upon these grains during the ripening or desiccation period at University Farm, to ascertain the amount of material lost during this time. The studies were started when the grains reached the dough stage, before the desiccation process of maturity had begun, and continued until complete maturity.

Each morning at 8 a. m. samples of head material of each of the grains were collected, placed in an air-tight container, and taken to the laboratory. Twenty-five grain samples were picked from the spike by hand and immediately placed in a respirometer. The grains were subjected for 2 hours to a temperature of 30° C., after which the carbon dioxid respired was swept through the modified Truog absorption tower and collected in saturated barium hydroxid solution. The carbon dioxid evolved in this time was calculated in milligrams by titration with *N/10* hydrochloric acid and computed into carbon dioxid lost per 100 gm. of dry matter in 24 hours.

Table I shows the results of this work. It should represent the maximum loss by respiration which could be expected under field conditions. A constant temperature of 30° C. would average the high rate of respiration during the heat of the day and the lower rate during the cool night time.

Table I shows the amount of carbon dioxid produced and the actual amount of carbohydrate material lost from the grain, per 100 gm. of dry matter, in the process of respiration throughout the 16-day ripening period. The amount of carbohydrate material lost was calculated from the data showing the carbon dioxid evolved. The evolution and estimation of carbon dioxid is probably the most accurate method of determining the loss in weight due to oxidation, and is intended to account for the entire collective activities of respiration. While the process of respiration is a complex one, and it is impossible to account for the exact changes which take place, the carbohydrate material lost can be very closely estimated by assuming the process in general to be oxidation of sugars and starch.

The oxidation process results in the formation of carbon dioxid and water according to the empirical formula, $C_6H_{10}O_5 + O_{12} \rightarrow 6CO_2 + 5H_2O$. One part of carbohydrate with a molecular weight of 162 when oxidized by free oxygen, results in the formation of 6 molecules of carbon dioxid with an equivalent weight of 264. From these figures it will be seen that the formation of 100 gm. of carbon dioxid will require 61.36 gm. of carbohydrate material. Thus, the amount of carbohydrate lost is calculated as 61.36 per cent of the carbon dioxid evolved. For wheat this gives a loss of 10.038 gm.; oats, 11.092 gm.; and barley, 15.12 gm. of carbohydrate per 100 gm. of dry matter in 16 days, as indicated in Table I.

TABLE I.—*Carbon dioxid evolved, and carbohydrate material lost by respiration, from wheat, oats, and barley, through a 16-day ripening period*

Crop.	Date.	Weight taken.	Dry matter.	Moisture.	Weight of dry matter.	CO_2 respired in 2 hours.	CO_2 respired per hour, per 100 gm. dry matter.	CO_2 respired in 24 hours, per 100 gm. dry matter.	Carbohydrate lost in 24 hours, per 100 gm. dry matter.
	July.	*Gm.*	*Per cent.*	*Per cent.*	*Gm.*	*Mgm.*	*Mgm.*	*Gm.*	*Gm.*
Wheat	5	23.70	56.13	43.87	13.30	28.39	106.73	2.561	1.571
	6	24.14	54.27	45.73	13.10	31.30	119.47	2.867	1.759
	7	25.00	60.25	39.75	15.06	26.13	86.85	2.084	1,278
	8	25.00	60.00	40.00	15.00	21.48	71.60	1.718	1.054
	9	25.00	59.10	40.90	14.77	20.28	68.66	1.647	1.010
	11	25.00	66.80	33.20	16.70	18.41	55.12	1.322	.811
	12	25.00	76.66	23.34	19.16	16.26	44.26	1,062	.651
	13	25.00	81.78	18.22	20.44	6.46	15.81	.379	.232
	14	25.00	77.17	22.83	19.29	6.72	17.42	.418	.256
	15	25.00	78.74	21.26	19.68	7.85	19.94	.478	.293
	16	25.00	84.87	15.13	21.22	3.33	7.85	.188	.115
	18	25.00	80.27	19.73	20.07	3.88	9.67	.232	.142
	19	25.00	85.21	14.79	21.30	3.49	8.19	.196	.120
	20	25.00	85.56	14.44	21.39	4.94	11.55	.277	.170
	21	25.00	87.50	12.50	21.87	10.01	22.89	.549	.336
	22	25.00	87.29	12.71	21.82	7.11	16.29	.391	.340
								16.369	10.039
Oats	11	25.00	58.62	41.38	14.65	29.49	100.65	2.415	1.481
	12	25.00	51.75	48.25	12.94	35.63	137.67	3.304	2.027
	13	25.00	52.40	47.60	13.10	24.87	94.92	2.278	1.397
	14	25.00	64.91	35.09	16.23	21.01	64.73	1.553	.953
	15	25.00	65.36	34.64	16.34	19.54	59.79	1.434	.880
	16	25.00	71.06	28.94	17.76	17.12	48.20	1.156	.709
	18	25.00	77.25	22.75	19.31	12.11	31.36	.752	.461
	19	25.00	79.79	20.21	19.95	11.30	28.35	.680	.417
	20	25.00	83.38	17.62	20.59	6.46	15.69	.376	.231
	21	25.00	79.25	20.75	19.81	9.69	24.58	.590	.362
	22	25.00	85.41	14.59	21.35	8.40	19.68	.472	.289
	23	25.00	83.88	16.12	20.97	13.24	31.57	.757	.464
	25	25.00	86.09	13.91	21.52	17.45	40.54	.973	.597
	26	25.00	89.60	10.40	22.40	7.75	17.30	.415	.254
	27	25.00	90.09	19.91	22.52	7.92	17.58	.422	.258
	28	25.00	85.42	14.58	21.35	5.98	14.00	.336	.206
	29	25.00	84.99	15.01	21.22	3.07	7.23	.173	.106
								18.086	11.092
Barley	5	16.983	47.99	53.01	8.13	48.61	298.95	7.175	4.402
	6	16.619	47.79	52.21	7.94	40.37	254.22	6.100	3.742
	7	25.00	53.98	46.02	13.49	23.26	86.21	2.070	1.270
	8	25.00	58.24	41.76	14.56	25.19	86.50	2.080	1.276
	9	25.00	56.13	43.87	14.03	19.35	69.07	1.660	1.018
	11	25.00	68.65	31.35	17.16	16.63	48.75	1.170	.717
	12	25.00	68.62	31.38	17.16	16.67	48.57	1.165	.714
	13	25.00	71.57	28.43	17.89	9.21	25.74	.617	.378
	14	25.00	75.53	24.47	18.88	6.80	18.01	.432	.265
	15	25.00	81.82	18.18	20.45	3.07	7.51	.180	.110
	16	25.00	86.22	13.78	21.55	2.68	6.22	.149	.091
	18	25.00	85.29	14.71	21.32	2.91	6.59	.158	.097
	19	25.00	86.52	13.48	21.63	7.75	17.91	.429	.263
	20	25.00	86.04	13.96	21.51	4.94	11.48	.275	.168
	21	25.00	87.85	12.15	21.96	9.37	21.32	.512	.314
	22	25.00	86.71	13.29	21.68	8.40	19.38	.465	.285
								24.637	15.120

It was the intention to show the relative variation between wheat, a naked grain, and oats and barley, to which the glumes adhere, in the amount of material lost. As oats and barley are apparently less variable in their protein percentage than wheat,[4] it was thought that the adhering glumes might be influential in inhibiting respiration and consequently account for this lesser fluctuation. This demonstration was made impossible through inability to obtain samples of uniform moisture content, which, no doubt, is largely responsible for the wide difference in the results.

EFFECT OF RESPIRATION UPON PROTEIN PERCENTAGES

The result of the loss of carbohydrate material as affecting a variation in the protein percentage can be ascertained by estimating the percentage of the grain which is subjected to respiration activities. The composition of the average grain is approximately 12.50 per cent protein, 2.50 per cent ash, and 85 per cent carbohydrate. Respiration being confined to the carbohydrate portion of the grain, it would require approximately 7 per cent variation in this material to effect a 1 per cent difference in the protein percentage. As the number of grams of carbohydrate loss, in this case, is based upon the 100 gm. dry basis, these same figures represent the loss in percentage. The protein will vary 1 per cent for each 7 per cent loss or gain in carbohydrate.

Calculating from the amount of carbohydrate material lost as given in Table I, it becomes apparent that there is a possible variation in wheat of 1.46 per cent, oats 1.62 per cent, and barley 2.2 per cent protein, due to respiration.

While these figures seem upon first thought to be definitely significant, it is not probable that respiration creates a variation in the protein content as wide as the above possibilities suggest. As indicated, considerable carbohydrate is lost during the ripening period, but fresh material is no doubt being moved into the grain until complete maturity, which is sufficient at least to counterbalance that which is lost. It must be recognized, however, that the amount of material being moved into the grain will be a variable factor and dependent largely upon the environmental conditions which prevail at that time. Brenchley (*1*) has shown that in the development of the barley grain the maximum dry weight of 1,000 gm. is reached when the moisture content is approximately 40 per cent, and that this dry weight remains practically constant and does not fall off during the desiccation process, while the moisture content is being lowered to 21 per cent. The fact that the weight of dry matter is not lowered indicates that up to the time of maturity considerable material is being moved into the grain to compensate for that lost by respiration.

More confirmation of this supposition is presented by Harlan (*3*) in his study of the development of the barley grain, where he has shown that deposits of dry matter in the kernel continue until very near the point of absolute ripeness. He concludes (*4*) from irrigation studies that plants are able to utilize water to the date of full maturity, with much increase in dry matter content.

[4] McGINNESS, F. W. OP. CIT.

The fact that the movement into the grain is continuous while respiration activities are in operation makes it impossible to determine the exact effect of respiration upon the variation in protein percentage. Assuming that the possible variation in protein is as great as mentioned above, namely, 1.46 per cent in wheat, 1.62 per cent in oats, and 2.20 per cent in barley, this would not be sufficient to account for the wide variation in the percentage as found in grain when produced in different localities, as given in Table II.

Upon analysis of Table II, a very wide variation in protein percentage is found for the grains grown in different localities and also for different years when grown in the same locality. In the year 1921 wheat grown in the hard spring wheat section at Fargo, N. Dak., yielded 16.10 per cent protein, while the same variety at Puyallup, Wash., shows 9.98 per cent. Another extreme variation may be seen by examination of the results at Havre, Mont., showing a protein content of 18.24 per cent, and Waterville, Wash., 9.19 per cent in the year 1920. In the former case there is a variation of 6.12 per cent in this regard, and 9.05 per cent in the latter. It would not be possible to attribute these differences to the respiratory process alone, as the above results show a 1.46 per cent variation in the protein which can be due to this phenomenon.

The data for oats indicate a much greater uniformity in this respect than is found in wheat. While this uniformity is especially pronounced throughout the year 1920 for the different areas, there are several cases in 1921 which vary considerably. The oats produced at Moccasin, Mont., Moro, Oreg., and Umatilla, Oreg., have a protein content which will average 14.12 per cent. Those grown at Waterville and Puyallup, Wash., and Corvallis, Oreg., average approximately 11.32 per cent. The spread in oats is not so great as in wheat, but this fluctuation of 2.80 per cent is much greater than that caused by the effect of respiration.

While the loss of carbohydrate material from the barley grain is much greater than that from wheat or oats, owing probably to the higher moisture content, it is not sufficient to account for the protein variation where the grain is grown under different conditions. Barley grown at Fargo, N. Dak., in 1920, yields 16.06 per cent protein, while that produced at Corvallis, Oreg., yields only 11.11 per cent. This is a difference of 4.95 per cent, which is more than twice the difference which could be due to respiration. In 1920, 14.50 per cent barley was produced at Havre, Mont., and Logan, Utah, yields 11.11 per cent. Here, again, is a spread of 3.39 per cent in the protein content, or 54 per cent greater than that attributed to respiration.

In a number of the analyses of each of the grains, the differences in protein percentage falls within the respiration possibility. These smaller differences may or may not be due to respiration, depending largely upon the environmental conditions under which the grain is matured. It is quite possible that respiration is effective to a degree in shaping the percentage composition of the grains when grown in the areas as indicated in Table II, but it is also possible that respiration plays a rather unimportant rôle in this regard when the synthetic process which goes forward throughout the period of desiccation is taken into account.

The exact influence of respiration upon the protein percentage composition of the grain is yet to be determined, but there is no foundation for the general sweeping statement that the variation in composition is dependent upon the respiratory process.

TABLE II.—*Physical and chemical composition of wheat, oats, and barley grown in 16 different localities in 1920–21*

Crop and locality.	Weight 1,000 grains.		Corneous.		Protein.	
	1920	1921	1920	1921	1920	1921
Wheat:	*Gm.*	*Gm.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Original seed	34.20	34.90	38	80	12.80	12.57
Havre, Mont	19.60	29.75	100.	100	18.24	18.91
Moccasin, Mont	25.85	25.00	100	95	15.85	16.00
Fargo, N. Dak	28.90	22.50	100	100	12.34	16.10
Morris, Minn	18.25	24.05	100	100	14.68	15.01
St. Paul, Minn	24.00	25.80	92	91	13.42	16.46
Duluth, Minn	28.75	21.50	67	100	13.80	11.76
Grand Rapids, Minn	27.80	17.80	100	100	12.43	17.79
North Platte, Nebr	20.00	22.70	100	100	14.55	15.01
Lincoln, Nebr	25.00	20.60	100	94	12.81	14.52
Garden City, Kans	22.10	19.30	98	100	15.24	15.10
Moro, Oreg	26.10	23.95	100	100	16.82	15.88
Umatilla, Oreg	24.25	18.60	100	100	15.57	18.52
Waterville, Wash	30.35	33.85	0	98	17.10	15.65
Puyallup, Wash	38.80	38.55	22.5	23.0	9.19	9.98
Corvallis, Oreg	33.08	34.17	75.0	81.0	12.40	10.47
Logan, Utah	30.24	34.60	100	100	14.22	14.97
Barley:						
Original seed	34.45	36.70	.....	.....	12.46	12.48
Havre, Mont	19.00	31.35	.....	.....	14.50	11.21
Moccasin, Mont	24.65	33.10	.....	.....	14.01	13.81
Fargo, N. Dak	36.05	22.22	.....	.....	12.66	16.06
Morris, Minn	25.10	33.70	.....	.....	11.08	13.13
St. Paul, Minn	28.22	24.70	.....	.....	11.06	13.01
Duluth, Minn	32.16	40.15	.....	.....	14.00	11.08
Grand Rapids, Minn	33.75	27.15	.....	.....	11.92	12.42
North Platte, Nebr	25.75	28.15	.....	.....	12.44	13.03
Lincoln, Nebr	24.15	31.05	.....	.....	11.83	12.33
Garden City, Kans	22.12	23.52	.....	.....	12.12	12.41
Moro, Oreg	17.50	28.40	.....	.....	13.05	10.88
Umatilla, Oreg	27.79	16.75	.....	.....	12.62	13.71
Waterville, Wash	36.11	36.00	.....	.....	14.34	13.89
Puyallup, Wash	33.75	37.45	.....	.....	10.70	11.88
Corvallis, Oreg	33.25	38.75	.....	.....	11.14	11.11
Logan, Utah	37.60	33.45	.....	.....	11.11	12.11
			Hull.			
Oats:						
Original seed	26.20	29.90	29.70	27.32	11.90	11.77
Havre, Mont	24.60	28.55	25.22	28.63	13.82	13.29
Moccasin, Mont	26.45	23.25	25.00	25.76	13.67	14.20
Fargo, N. Dak	30.36	30.00	23.12	26.12	11.11	12.40
Morris, Minn	27.75	28.55	26.12	27.30	13.47	11.39
St. Paul, Minn	25.40	21.45	26.70	32.29	11.96	12.23
Duluth, Minn	30.30	22.70	28.74	28.20	11.35	12.22
Grand Rapids, Minn	30.13	28.70	24.87	30.01	12.52	12.10
North Platte, Nebr	27.05	19.00	28.03	22.00	12.25	12.26
Lincoln, Nebr	27.70	21.00	25.56	21.00	11.78	13.94
Garden City, Kans	28.80	21.40	28.40	24.60	11.36	12.15
Moro, Oreg	24.65	27.30	29.36	30.02	13.69	14.05
Umatilla, Oreg	26.65	28.10	34.21	25.90	12.21	14.23
Waterville, Wash	27.60	31.80	27.64	30.66	12.25	11.31
Puyallup, Wash	32.10	33.20	25.78	26.21	11.10	11.20
Corvallis, Oreg	29.80	33.50	27.04	30.12	11.22	11.45
Logan, Utah	28.95	33.05	27.48	23.82	11.39	12.04

SUMMARY

(1) The loss of carbohydrate material during the ripening of the wheat, oats, and barley grains is considerable. The greater percentage of the loss occuring before the process of desiccation begins while the grain contains above 40 per cent moisture.

(2) The protein composition of wheat, oats, and barley is influenced to a marked degree by the loss of carbohydrate material during the ripening period. Factors other than respiration or in connection with the process contribute largely to the formation of high protein grains.

(3) Wheat is more variable in the protein percentage than barley or oats, and barley appears to be more variable than oats.

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