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Experiments on Spore Germination and Infection in Certain Species of Oomycetes[1]

I. E. MELHUS

INTRODUCTION

Some two years ago special work was undertaken upon Cystopus and other Oomycetes aiming to learn more as to the methods of spore germination, zoospore formation, methods of infection, and as to the possible existence of so-called "physiological species" in the genus Cystopus. The parasite *Cystopus candidus* was common on the garden crop of radish, *Raphanus sativus*, and attempts were made at the outset to produce infection by transferring spores from this to radishes growing in the greenhouse. Although repeated trials were made in connection with these early studies, only meager and irregular infections resulted. This suggested that some variable factors of unknown nature were present in the greenhouse trials. Difficulty was also encountered at the outset in securing uniform results in spore germination by the methods described by earlier workers. Thus it soon became evident that some more specialized methods were necessary in order to secure the germination of the conidia and host infection in abundance and with a satisfactory degree of certainty.

We were thus led to attempt to determine the relations of various conditions to spore germination and infection with this fungus, not only host relations, but also relations of the age

[1] The author wishes to express to Dr. R. A. Harper and Dr. L. R. Jones his sincere appreciation for the kind criticism and keen interest shown during the progress of this work and preparation of the manuscript.

and maturity of the spores, moisture, food, chemical stimuli, light and temperature. Other species were subsequently tested. The results secured are of such definiteness and breadth of applicability as to justify their publication, although much remains to be done upon the problems as originally defined. Before discussing my own work and conclusions, a brief review will be made of the results of previous studies upon these and closely related matters.

REVIEW OF EARLIER WORK

The germination of the asexual spores of Cystopus was first observed over a century ago by Prevost (1807:29). He studied the species commonly parasitic on crucifiers and purslane respectively, then known as *Uredo candida* and *Uredo portulacae*. His description is clear and interesting. He states that the spores germinated one or two hours after immersion, sometimes within 40 or 45 minutes, owing in all probability to differences in temperature, which, during the observation, fluctuated between 12° and 16° R. (equivalent to 15° and 20° C.). In the process of germination the spores absorb water and become bottle shaped; soon a globule (zoospore) is seen on the outside and this is immediately followed by several others, sometimes as many as six more. He states that these globules instantly reunite into a mass which moves as a unit by rolling about in the water. The globules, as a rule, separate from one another in a very short time; sometimes, however, two or even three globules may remain attached together, either immediately touching or as if joined by a string. Those globules which separate from one another, and they are by far the greater number, are sometimes a little angular and possibly a little hollowed or pushed in at one side. They swim about in the same way as when united in mass. Soon the movement of the globules ceases and they become fixed at the surface of the water or at the edge of the drop. He observed that the swarmspores developed germ tubes, regarding which however he gives little detail. Prevost likewise studied the Cystopus on salsify and Amaranthus and found the last two forms much more difficult to germinate.

Tulasne (1854:77) states that he germinated the spores of *Uredo portulacae* and *Uredo candida* but was unable to get them to form swarmspores in the manner described by Prevost, finding only the germination by a tube.

Hoffman (1859: 210) was also unable to confirm Prevost as to the formation of swarm spores in Cystopus. He describes the germination of the spores by tubes in the same manner as described by Tulasne.

DeBary (1860: 236) studied the method of germination in *Cystopus cubicus* and *Cystopus candidus* and found the germination was by zoospores, as described by Prevost, but that germination might take place at any temperature between 5° and 25° C. He emphasized for the first time that the spores are really sporangia producing from five to eight zoospores in *Cystopus candidus* and from eight to twelve in *Cystopus cubicus*. DeBary describes the changes in the sporangium very clearly. The spores absorb water and swell when sown in a drop of water. On one side an obtuse papilla is developed and vacuoles form in the granular protoplasm which disappear later. At this stage of development, fine lines of demarcation divide the protoplasm into five to eight polyhedral portions leaving at the center a small feebly colored vacuole. When the division of the content of the sporangium is complete, the papilla swells, opens and the zoospores are pushed to the outside one by one, showing no sign of movement. Once outside the sporangium they become lenticular in form and group themselves before the opening of the sporangium in a spherical mass. Very soon the swarm spores begin to move, vibratile cilia appear and the globular mass is set oscillating. The zoospores ultimately become free and swim away singly. The motile spores are plano-convex or slightly concavo-convex having a small disk-like vacuole on one side. Attached near the vacuole are two cilia, a short one in front and a long one behind, both on the same side. In from one and a half to three hours after being placed in water the escape of the zoospores begins. They will develop either from sporangia freshly formed or from those which have been kept as long as six weeks.

DeBary (1863:14) found the conidia of Cystopus germinating on the leaves of the host plants. Zoospores were found in the drops of water on the leaves. Infection experiments with *Cystopus candidus* were made on various hosts. In the case of *Lepidium sativum* the zoospores readily entered the stomata of both leaves and cotyledons but produced infection only in the latter. Various species of Brassica showed the same tendency, though not to so marked an extent.

Farlow (1875:319) studied the germination of the conidia of *Phytophthora infestans* and observed that sometimes the contents of the spore discharged in one mass, and from this mass zoospores are produced as before. He believes that the production of zoospores is favored by darkness, whereas germination by a germ tube takes place more frequently in the light. He states, however, that he has repeatedly sown spores in watch glasses and both methods of germination resulted. The germinating power of the spores was retained for several weeks, but they did not germinate after a winter's exposure.

Farlow (1876:419) also describes the germination of the conidia of *Plasmopara viticola*. During the month of October when the disease is most prevalent, he found that in one and one-fourth hours zoospores were formed and began to make their way to the outside of the sperangium. The conidia might also germinate directly, i. e., by germ tubes. Darkness was more favorable for the germination of the conidia, whether directly or indirectly. He found the zoospores to swim about for fifteen to twenty minutes, after which the motion gradually became slower until they finally came to rest. In another quarter of an hour an outgrowth appeared on one side which rapidly developed.

Scribner (1886:10) states that temperature exercises a considerable influence over the germination of *Plasmopara viticola* from the grape. The most favorable temperature lay between 25° and 35° C. At a lower temperature, germination took place more slowly, but the temperature could be reduced to zero without destroying the vitality of the conidia. Under exceptional circumstances, Scribner states, another form of germination might occur in which a conidium may push out a tube. This method, he reports as undoubtedly rare.

The question of spore germination and physiological species in the genus Cystopus has quite recently been studied in considerable detail and with very interesting results by Eberhardt (1904:622). He began his work in June 1902 and continued it until the fall of 1903. *Cystopus candidus* on various cruciferous plants were used except in one experiment where he used *Cystopus cubicus* on *Tragopogon pratensis*.

Eberhardt (1903:655) found, as we have, considerable difficulty in germinating the conidia of Cystopus. He tried the different methods used by DeBary and Zalewski, but obtained only a low germination. To solve the question of spore germination,

Eberhardt turned his attention to methods of properly maturing the conidia. During all the month of May and the beginning of June, *Cystopus candidus* was available on Capsella, twelve experiments were made at as many different times of which the following may be taken as typical. Conidial pustules not opened were collected from time to time. The contents of the pustules were placed in a vial containing a small amount of rain water. This was called vial No. 1. In vial No. 2, containing rain water was placed the spores shaken from open pustules. In still another test a shoot was taken which bore many unopened pustules. It was wrapped in a moist cloth to be kept for further observations.

In vial No. 1 the conidia remained in chains, the protoplasm became granular and later bacteria developed decomposing the conidia. Vial No. 1 may show a small number of conidia germinated, but the larger part disorganized. In vial No. 2, containing spores shaken from open pustules one third of the conidia formed zoospores while the remainder decomposed. When taking spores from pustules just opened, more than one half germinated. It is permissible to think that the former were relatively old and that they had lost their capacity of germination whereas the later conidia were taken from pustules just opened and gave a germination of more than one half of the spores. The third test, in which the shoot containing pustules was wrapped in a moist cloth, gives further evidence in support of the above interpretation. After the pustules had been kept moist for one day a microscopic examination of three of the pustules showed the following conditions on being placed in a drop of water. The young pustule was found to contain the zoosporangia in chains which gave no germination. The second showed a small fraction of the spores empty or bottle-necked, ready to germinate. The other conidia were decomposing. The third presented all the conidia disassociated and about one third produced zoospores. The shoot was kept further until almost all of the pustules had opened liberating the spores that germinated in fairly large numbers. This same shoot was kept two weeks longer when it had wilted and dried up in places. The conidia that fell from it gave no germination.

These experiments show us that the zoosporangia are the organs of immediate infection, requiring for germination to be collected at the time when the pustules open. Infection occurs

when these spores fall upon the young host plants humid from rain or dew. In the numerous cultures made for infection purposes none were kept after the third day if germination had not already occured. It is well here to notice the variation in time required for germination of conidia. A few notes from Eberhardt's experiments will suffice.

Eberhardt used "room temperature" in every case except in one test made on the twenty-ninth of March. The exact temperatures are given in only two cases, however. In the first it varied from 11° to 17° C. In the experiment referred to, on March 29, the temperature varied from 2° to 8° C. The latter condition was obtained by placing the culture on a window sill where the influence of the out-door temperature had some effect. His germination experiments that are described were carried on from March 28 to August 9, 1902, on five different dates, all resulting in germination in from three to forty hours.

There was also another difficulty greater than that of the selection of the conidia, it was the choice of the proper time to inoculate the host plant. The task of growing and caring for the young plants, the delicacy of the material of infection and the considerable space required for growing the crucifers has made it impossible to often repeat the same infections.

In view of the fact that the following statements of the author are not clear we quote directly. "Un facteur important était la coïncidence qui doit toujours exister entre la récolte du parasite et l'état de germination propice de la plante à infecter. C'est pour ces raisons que nous ne pouvons poser en ce moment aucune règle basée sur l'expérience, relative à l'optimum de réceptivité du parasite par le végétal nourricier. Mais ce que nous pouvons certainement avancer, c'est qu'il ne suffit pas dans toutes les Crucifers d'avoir des cotylédons bien étalés. La question de l'optimum de réceptivité demande plusieurs années de recherches. Comme nos infections tendaient plutôt à prouver l'unité d'espèce, ce n'est que très tard que nous nous sommes aperu, après des insuccès nombreux, combien l'état du jeune plant influe sur la réussite de l'expérience. Ainsi nous avons vu que certains cotylédons, sortant de la gaîne refusent l'entrée du parasite, tandis que lorsqu 'ils sont bien étalés, ils sont susceptibles d'infections. Il nous a semblé que plusieurs de nos espèces qui avaient été infectées au moment où les cotylédons

étaient fanés peuvent recevoir l'endophyte par le jeune bourgeon foliaire déroulant ses feuilles. Mais nous ne pouvons encore rien affirmer à ce propos. Au reste, De Bary lui-même indique que *Holzophila erithmifolia* est apte à être infectée par les jeunes feuilles."

Comparatively speaking, but very little work has been done on the question of physiological species in the Oomycetes. Eberhardt (1904:714) has investigated this problem in *Cystopus candidus* occurring on various crucifers. Plants were inoculated by germinating the conidia and placing the liquid containing the zoospores on the lower side of the cotyledons of the plants to be infected or simply dipping the cotyledons to be infected in the water containing the zoospores. The seedlings were grown in large flower pots and small bunches were removed as needed for infection. His infection experiments were carried on both out of doors and in an ordinary laboratory. The conidia of five different species were used as material for infecting other cruciferous plants.

It is further interesting to note that Eberhardt made inoculation experiments with the oospores of *Cystopus candidus* from *Lepidium sativum*. In order to infect the plants of Lepidium and Capsella with the oospores from *Lepidium sativum*, the parts of the host containing the oospores were placed in small bags and hung out of doors in the open air during the winter months. In March and April the oospore material was distributed over the surface of pots where it might decay and liberate the oospores. Two of these pots were then seeded with *Lepidium sativum* and *Capsella Bursa-pastoris* and the young seedlings became infected. It should be noted in this connection that Eberhardt used no controls in this series of experiments.

His results may be summarized as follows: With the conidia of *Cystopus candidus* from *Capsella Bursa-pastoris* he infected *Capsella Bursa-pastoris*, *Lepidium sativum*, *Iberis amara*, and *Arabis alpina*. Conidia from *Capsella Heegeri* infected *Capsella Heegeri*, *Capsella Bursa-pastoris* and *Lepidium sativum*. Conidia from *Lepidium sativum* infected *Lepidium sativum* and *Capsella Bursa-pastoris*. Spores from *Brassica rapa* infected *Brassica rapa*, *Brassica oleracea* (varieties: *botrytis*, *capitata*, and *congylodes*), *Brassica nigra*, *Sinapis arvensis*, and *Diplotaxis tenuifolia*. The widest range of infec-

tion was obtained with the conidia from *Arabis alpina* which infected *Arabis alpina, Arabis hirsuta, Arabis Halleri, Arabis turritis, Lepidium sativum, Iberis amara, Cardamine pratensis, Cardamine amara, Capsella Bursa-pastoris,* and *Senebiera coronopus.* With the oospores from *Lepidium sativum* he infected *Lepidium sativum* and *Capsella Bursa-pastoris.* It was impossible to infect any of the Cruciferae with conidia from *Tragopogon pratensis,* but quite easy to infect *Scorzonera hispanica.* From these data Eberhardt believes that there are no biological species in the species, *Cystopus candidus.* It should be noted, however, that the above conclusions are based upon only one trial in some cases with each species or variety of plant. Eberhardt states in this connection that the laborious task of growing, inoculating and recording results, together with the fact that the ground used for growing the plants could not be had during the next year, made it impossible to duplicate any of the series of experiments performed.

The fact that Eberhardt's work was incomplete and not fully convincing, and that it is especially important that each group of parasitic fungi be fully understood as to the existence of physiological species, led me to study this problem.

EXPERIMENTAL STUDIES IN SPORE GERMINATION

METHOD

As has been previously stated, the major portion of the experiments recorded in this paper were carried on with the common white rust, *Cystopus candidus,* as it occurs on various garden plants. The culture work was all carried on either in the greenhouse or in an ordinary laboratory. Both distilled and tap water were used to germinate spores. The tap water in this case is unfiltered water drawn directly from Lake Mendota. The conidia were gathered from infected plants growing out of doors until frost, when they were taken from infected plants in the greenhouse and were sown the same day as gathered. The spores were sown in a drop of water placed near the center of a clean slide. It was always difficult to make the conidia sink, but by stirring the drop with a scalpel, a large per cent could be finally made to settle

to the bottom. The aim was always to add only a moderate number of spores to each drop, since too many spores make the water opaque and difficult to examine. Care was taken to obtain fresh spores in every case, which were stirred into the drop of tap water. Sometimes small pieces of leaves bearing pustules were dropped into water on a slide.

The first experiments were made at room temperature, but the irregularity of the results and the small per cent of the total which germinated even in the most favorable cases led to experiments with low temperatures. With this idea in mind, the slides sown with spores were placed in an ice box of the usual construction. A Richard's self-registering thermometer was also placed in the ice box so that a complete record could be had of the temperatures to which the spores were exposed. In the case of the controls at room temperature, the stands holding the slides sown with spores were placed on a wet earthen plate and a small bell glass placed over them to prevent rapid evaporation. The effect of darkness was also tested by placing similar cultures in the dark room. The temperature of the dark room was noted when the experiment was started and stopped and the average taken. The cultures at room temperature, not in the dark room, were kept in a greenhouse where another self-registering thermometer recorded the temperature. Here also the temperature was noted when the experiment was started and stopped. For convenience in tabulating, the average of the two extremes was taken as the prevailing temperature.

Cultures in the ordinary Van Tieghem cell were also used. The cell was partially filled with tap-water and a hanging drop made containing the spores. Vaseline was used to prevent evaporation and to hold the cover glass in place. The cells were laid on a stand as described above for the slides. Watch crystals were used when it was desired to secure large quantities of spores in different stages of germination.

RESULTS OF GERMINATION EXPERIMENTS

My earliest experiments in germination of the spores had the double aim of providing material for the cytological study of the processes of nuclear and zoospore formation in the germination of the conidia and of obtaining a reliable method for germination of the conidia for infection experiments in determining whether

physiological species are to be found in the group. As noted earlier, it was found difficult to obtain germination and a long series of one hundred experiments was made, testing the general questions as to the effect of age and maturity of the spores; the weather conditions under which they were collected; possible influence of rain water, dew, tap water and distilled water; and the effect of gathering the spores at different times of the day. Attention was turned to the possible effect of artificial media and a number of attempts were made to germinate the spores of Cystopus in artificial media, these experiments being made in a greenhouse the temperature of which varied from about 33° C. in the day time to 22° C. at night. First ordinary nutrient agar was tried (3 gr. meat extract, 10 gr. agar, 3 gr. salt, 1000 cc. water). The conidia were sown on this agar in petri dishes and kept under observation for twentyfour hours, but no germination resulted in any of the ten experiments tried. Beef bouillon (3 gr. meat extract, 3 gr. salt, 1000 cc. water) was tried in two experiments, but gave no germination. Following this, some special media were tested. Ten experiments were tried with lima bean agar, as prepared by Clinton (1908:904) for growing Phytophthora, and eight experiments with his pumpkin agar; none of the cultures showed any signs of germination at the end of twenty-four hours. Various other forms of artificial media were tested, including mustard leaf decoction, four trials; corn meal agar, five trials; a two per cent sugar solution, six trials. The cultures were kept under observation in each case for twenty-four hours. In no case did germination result either by germ tubes or by zoospores. The above experiments were made during July and August, 1909. The conidia used were from *Cystopus candidus*, *C. bliti*, *C. cubicus* and *C. portulacae*. They were collected at various times of the day ranging from seven o'clock in the morning until seven in the evening, the great majority being gathered about eight o'clock in the morning. The infected leaves were cut off from the host plants and carried to the laboratory, where the material was used immediately. A number of times the infected leaves were immediately placed in a damp chamber after they were removed from the host plant. A large number of tests were made with both young and old conidia, before and after the epidermis of the pustule had ruptured.

These conidia were, of course, also tested in water on a slide or in a hanging drop in a so-called Van Tieghem cell. The slides

were placed on a small stand on a wet earthen plate under a bell jar in the greenhouse. In all, fifty-four trials were thus made in water and in no case was germination observed. Each experiment lasted for twenty-four hours and several observations were made during that time.

The effect of chilling was next tried, both on various nutrient media and in tap water. No germination was obtained in this way when using nutrient media but with water, germination was secured. Thus, when four slides were prepared as before and placed on a metal stand in an ice box, the conidia had germinated by the production of zoospores in the course of 1½ hours. A large number of cultures were subsequently made by this method to secure material for cytological study and with controls kept at room temperature to show the exact value of the chilling in influencing germination. The results are strikingly uniform and in strong contrast with those obtained before without chilling.

SUMMARY OF TABLE I

The first experiment with chilling in germinating the conidia of Cystopus was made on August 10, 1909. From that date up until April 9, 1910, experiments were carried on as indicated in the table. In all, 197 experiments were made, giving germination in 147 cases. From the number of those which showed no germination should undoubtedly be subtracted the results obtained on August 12 and 15 with conidia of *Cystopus portulacae*. The plant from which the conidia were obtained had been dug up, potted and taken to the green house July 25. It died in the course of ten days and the vitality of the conidia may have been reduced by its condition on August 2. If these experiments are omitted the number of failures to germinate is reduced by ten and we should have about 78 per cent of the experiments showing germination and about 22 per cent negative.

As shown in the table the conidia of four different species of *Cystopus* were used in these experiments, including *Cystopus candidus* 78 trials, *Cystopus cubicus* 60, *Cystopus bliti* 45, and *Cystopus portulacae* 14. Until October 19 the conidia were taken from live plants growing out of doors and used immediately. The terms "old" and "young" as used in the table indicate only the approximate age of the conidia. The conidia were called young, although the epidermis of the pustules had ruptured, as long as

TABLE I.—EFFECT OF LOWERING TEMPERATURE ON THE GERMINATION OF THE CONIDIA OF CERTAIN SPECIES OF CYSTOPUS.

Date.	No. of cultures.	Species tested.	Age of spores.	Period of refrigeration.		Result.	Out-door temperature. Minimum.
				Hrs.	Temp.		
Aug. 10..	4	Cystopus bliti.....	Young ..	1.5	21	+	17
Aug. 11..	4	C. candidus.....	Young ..	2	21	+	15
Aug. 12..	2	C. candidus.....	Young ..	1	21	+	19
Aug. 12..	2	C. bliti.....	Young ..	1	21	+	19
Aug. 12..	2	C. candidus.....	Young ..	1.5	21	+	19
Aug. 12..	2	C. bliti.....	Young ..	2	21	+	19
Aug. 12..	2	C. bliti.....	Old.....	2	21	+	19
Aug. 12..	4	C. portulacae.....	Young ..	2.5	21	—	19
Aug. 13..	4	C. bliti.....	Young ..	2.66	20	+	18
Aug. 14..	6	C. portulacae.....	Old.....	3.5	14	—	24
Aug. 16..	2†	C. candidus.....	Young ..	1.5	.....	—	18
Aug. 16..	3	C. candidus.....	Young ..	2	18	+	18
Aug. 18..	8†	C. candidus.....	Young ..	2	18	+	17
Aug. 20..	3†	C. candidus.....	Young ..	2.5	.....	+	19
Aug. 24..	4	C. candidus.....	Young ..	11.75	18	—	12
Aug. 23..	4†	C. candidus.....	Old.....	2	17	—	17
Aug. 23..	4†	C. candidus.....	Young ..	24	17	+	17
Aug. 23..	5	C. bliti.....	Young ..	2.5	17	+	17
Aug. 23..	4	C. bliti.....	Young ..	4.5	17	+	17
Sept. 25..	2	C. candidus.....	Young ..	24	7	—	6
Sept. 27..	3	C. candidus.....	Young ..	7	6	+	6
Sept. 27..	1*	C. candidus.....	Young ..	7	6	+	6
Sept. 27..	1*	C. candidus.....	Young ..	24	6	+	6
Oct. 1...	4*	C. cubicus.....	Young ..	36.5	11	+	6
Oct. 1...	3*	C. cubicus.....	Young ..	28	10	—	6
Oct. 28..	2	C. candidus.....	Young ..	4.5	8	—	8
Oct. 28..	1*	C. candidus.....	Young ..	4.5	8	+	8
Sept. 23..	2*	C. cubicus.....	Young ..	5.75	8	+	8
Sept. 23..	1†	C. bliti.....	Young ..	4.5	8	+	8
Oct. 4...	1†	C. bliti.....	Young ..	3	8	+	6
Oct. 4...	1*	C. bliti.....	Young ..	3	8	+	6
Oct. 4...	3*	C. cubicus.....	Young ..	4	9	+	6
Oct. 9...	2*	C. candidus.....	Young ..	3.5	10	+	13
Oct. 9...	2*	C. candidus.....	Young ..	16	10	+	13
Oct. 14...	2†	C. bliti.....	Young ..	15	13	+	2
Oct. 14...	3*	C. bliti	Young ..	2.66	12	+	2
Oct. 28..	2	C. candidus.....	Young ..	7.5	12	+	4
Oct. 8...	2†	C. bliti.....	Young ..	6	11	—	11
Oct. 19..	4	C. cubicus.....	Young ..	6.25	10	—	1
Oct. 10..	3	C. cubicus.....	Young ..	3	10	+	.....
Oct. 10..	3	C. bliti.....	Young ..	3	10	—	.....
Jan. 24...	4	C. candidus.....	Young ..	47	11	—	.....
Jan. 25...	3	C. candidus.....	Young ..	10	10	+	.....
Jan. 26...	4	C. candidus.....	Young ..	22	11	—	.....
Jan. 26...	3	C. candidus.....	Young ..	12	11	+	.....
Aug. 25..	1	C. bliti.....	Young ..	5.5	18	+	20
Aug. 28..	4	C. candidus.....	Young ..	6	14	+	16
Aug. 30..	4	C. portulacae.....	Young ..	3	15	+	11
Aug. 31..	3†	C. bliti.....	Young ..	4.25	11	+	8
Aug. 31..	1	C. cubicus.....	Young ..	18.5	11	+	8
Sept. 2..	1†	C. cubicus.....	Young ..	2.12	10	—	7
Sept. 4..	4	C. cubicus.....	Young ..	10.75	10	+	12
Sept. 4..	2	C. bliti	Old.....	2.5	10	+	12
Sept. 4..	1	C. candidus.....	Young ..	2.5	10	+	12
Sept. 6..	1	C. cubicus.....	Old.....	5.25	8	+	9
Sept. 6..	1	C. cubicus.....	Old.. ..	5.25	8	—	9
Sept. 7..	2	C. cubicus.....	Old.....	6.5	9	+	9
Sept. 10..	4†	C. cubicus.....	Young ..	4.25	11	+	13
Sept. 10..	1	C. candidus..	Young ..	7	11	+	13
Sept. 13..	3	C. cubicus.....	Young ..	2.33	8	+	18
Sept. 14..	4	C. cubicus.....	Young ..	2.75	8	+	14
Sept. 14..	4	C. cubicus.....	Young ..	3	9	+	14
Sept. 16..	1*	C. cubicus.....	Young ..	4.87	10	+	9
Sept. 20..	1*	C. cubicus.....	Young ..	5.5	.....	+	15
Sept. 21..	4†	C. candidus.....	Young ..	9.25	12	+	16

* Experiments carried on in watch crystals.
† Pieces of leaves, on which there were pustules, were laid on the slide.

TABLE I. Continued.—EFFECT OF LOWERING TEMPERATURE ON THE GERMINATION OF THE CONIDIA OF CERTAIN SPECIES OF CYSTOPUS.

Date.	No. of cultures.	Species tested.	Age of spores.	PERIOD OF REFRIGERATION.		Result.	Out-door temperature. Minimum.
				Hrs.	Temp.		
Sept. 21..	1	C. candidus.....	Young ..	23	14	+	16
Sept. 21..	1*	C. candidus.....	Young ..	23	14	+	16
Sept. 21..	1	C. cubicus.	Young ..	4.87	12	+	16
Sept. 21..	3	C. bliti.....	Young ..	29	14	+	16
Sept. 22..	4*	C. cubicus.....	Young ..	6	11	+	13
Sept. 24..	3*	C. cubicus.....	Young ..	3.75	8	+	7
Sept. 25..	4	C. cubicus.....	Old.....	23	7	—	6
Sept. 25..	2*	C. cubicus.....	Young ..	3.25	7	+	6

*Experiments carried on in watch crystals.

a considerable number of spores remained in the pustules; and old after the pustules were nearly empty. The conidia were either taken out of the pustule and placed in a drop of water or small pieces of leaves with pustules were laid in a drop of water. If the pustules on the pieces of leaves were not already open when they were laid in the drop of water the epidermis was broken with a needle.

An ordinary ice box was used and in it was kept a self registering thermometer. By referring to the table it can be seen that the temperatures from August 10 to August 25 ranged from 15° to 21° C. The temperature was usually above 18° C. The ice box was kept in a rather warm room adjoining the green house, and it was also used for other purposes so that the doors were opened and closed quite often. The temperature curve was very irregular. There were fluctuations of 10° C. in five hours in some cases although usually it was less. Since the fluctuations were too numerous to explain in connection with each test, the average of the maximum and minimum temperature has been taken as the prevailing temperature and is that recorded in the table. This, in some experiments and especially in those before August 13, does not give the correct temperature conditions. In the tests after August 13, there was much less variation and the average of the two extremes is much nearer the prevailing temperature condition. The exact temperatures during two tests are given in detail to show more clearly the existing conditions. For example, the temperature varied as follows during the experiment on August 25: The test was started at 9:15 a. m. with a temperature of 20° C. The temperature remained constant until 10 o'clock. Thirty minutes later the temperature was 21° C. At 11 o'clock

it was 19° C. where it remained until 12 o'clock noon. At 1 o'clock it was down to 14° C.; at 2 o'clock it was 13° C. and at 2:45 p. m. it again rose to 14° C. During 5 1-2 hours, the temperature varied 8° C. After August 25, the temperature of the ice box was much more constant and often fluctuated only one degree during the time of an experiment. The period of refrigeration on September 2 started at 9:30 a. m. with a temperature of 10½° C. and stopped at 2:35 p. m. with a temperature of 11° C. During the five hours of refrigeration, the temperature only varied ½ degree.

It is to be noted also that the temperature grew gradually lower until October 9. This was due to the fact that before this time, no artificial heat of any consequence was used in the green house; while after this date it was heated. For comparison with the temperature in the ice box, the minimum out door temperature is given for each day on which an experiment was made, as published by the local weather bureau of Madison in their monthly meteorological summary. It will be seen that the temperatures in the ice box varied two degrees or less from the minima out doors in 72 per cent of the tests until about October 14. This suggests that germination can readily take place at temperatures equal to or varying two degrees or less from the minima for the outdoors. The length of time required for germination varied from one to thirtysix hours. The one hour period required for germination was on August 12 and the thirty-six hour period was on October 10. All of the experiments that required an unusual length of time for germination were examined from one to six times before the final observation was made. Water was added to replace the amount that evaporated. It should be said, however, that the usual period required for germination in the majority of the cases was less than six hours. From August 10 to 31 the average length of the period of refrigeration necessary to produce germination was about 3½ hours. The longest period was 18½ and the shortest period, one hour.

During the month of September experiments were made on twentyone different days, one more than in August, and the average length of the period of refrigeration was about 7½ hours; here the longest period was twentynine hours and the shortest period two hours and five minutes. In October, experiments were made on nine different days. The average of the periods of refrigeration used where germination resulted was nine

hours, the longest period being 36½ hours, and the shortest two hours and forty minutes. From these facts it is strongly suggested that the period of refrigeration is longer in the fall than in the summer as has already been pointed out by Zalewski (1883:215). No germination experiments were carried on from October 28 to January 24, 1910. However, on January 24, 25, 26, 1910, fourteen trials were made in which six germinations occurred. The time required was ten hours in the trials on January 24 and twelve hours in the three successive tests on the following days. Although only a small number of tests were made during the month of January, it was quite evident that the conidia responded differently at this time than in the summer. In the tests made in January the zoospores lost their motility in less than one hour and developed long germ tubes. In none of the tests made before that time had germ tubes been seen. The different behavior of the conidia in the late fall and winter as compared with spring and summer, are attributed to the loss of vitality of the host and fungus or to the improper maturing of the spores.

TABLE II.—EFFECT OF LOWERING TEMPERATURE IN GERMINATION OF CONIDIA OF CERTAIN SPECIES OF CYSTOPUS, WITH CONTROLS AT ROOM TEMPERATURE.

Date.	Species tested.	EXPERIMENT AT LOW TEMPERATURE.				CONTROLS AT ROOM TEMPERATURE.			
		No. cultures.	Period of refrigeration.		Results.	No. cultures.	Time continued.		Results.
			Hrs.	Temp.			Hrs.	Temp.	
Aug. 11..	Cystopus bliti..	4	1.5	21	+	4	1.5	25	—
Aug. 18..	C. bliti.....	8	2	18	+	8	2	27	—
Aug. 20..	C. bliti.....	3	2.5	19	+	3	2.5	27	—
Aug. 21..	C. bliti.....	4	10	18	—	4	10	25.5	—
Aug. 23..	C. bliti.....	9	7.3	17	+	9	7.3	27	—
Aug. 16..	C. candidus.....	5	1.6	18	+	5	2	28.5	—
Sep. 15..	C. bliti.....	2	5.5	10	+	2	5.5	22	—
Sep. 25..	C. candidus.....	2	24	8	—	2	24	22	—
Oct. 9..	C. candidus.....	2*	5.5	11	+	2*	23.75	22	—
Oct. 9..	C. candidus.....	2	28	10	+	2	28.5	21	—
Aug. 13..	C. bliti.....	4	2.6	20	+	4	14	27	—

*Conidia placed on watch crystals, instead of slides.

SUMMARY OF TABLE II

As noted, no control experiments were kept in connection with the trials reported in Table I. The conidia were germinated as material for cytological study which will be reported on later. A second set of similar experiments (Table II) with controls, was

carried on to demonstrate beyond question that chilling is necessary for abundant germination. In this series of forty five cultures subjected to low temperatures, six failed to germinate. That is, about 85 per cent of the tests gave germination. In the controls, no germination was observed. The temperature during the period of refrigeration was very high for ice-box temperature in most of the experiments, due to the conditions explained in the summary of Table I.

In all of these experiments the conidia were placed in tap water on the slides. In order to prevent too rapid evaporation in the trials at room temperature, the slides were laid on a metal stand placed on a wet plate under a small bell jar. The final observations were made in both sets of experiments at the same time and the results recorded. The average room temperature at which the experiments were made varied from 21 to 28.° C., as can be seen in Table II; while the temperature during the period of refrigeration varied from 8° to 21° C. Otherwise the conditions were the same in both sets of experiments. These results show that a slight lowering of the temperature stimulates the conidia of Cystopus to germinate with the production of zoospores.

TABLE III.—EFFECT OF LIGHT ON THE GERMINATION OF THE CONIDIA OF CERTAIN SPECIES OF CYSTOPUS.

Controls Chilled.						Culture at High Temperatures.					
			Period of refrig'ra'n.			Period in dark room.			Period in light.		
Date.	No. of cultures	Species tested.	Hours.	Temp.	Results.	Hours.	Temp.	Results.	Hours.	Temp.	Results.
Aug. 11..	4	Cystopus candidus	2.	21	+	8.75	26	—	.....	...	.....
Aug. 13..	4	C. bliti.....	2.66	20	+	14.	24-27	—	14.	24-27	—
Aug. 20..	3*	C. bliti.....	2.5	18	+	1.5	28	—	2.5	24-27	—
Aug. 23..	4*	C. bliti.....	9.	17	+	4.	24-27	—	7.33	24-27	—
Sept. 15..	2	C. bliti.....	5.5	10	+	5.5	24-27	—	5.5	24-27	—
Sept. 25..	2	C. candidus.....	24.	8	—	24.	24-27	—	24.	24-27	—
Oct. 9....	2†	C. candidus.....	5.5	11	+	6.	27.5	—	23.75	24-27	—

* Pieces of leaves on which there were pustules, were laid on the slide.
† Experiments carried on in watch crystals.

SUMMARY OF TABLE III

The possible effect of light on the germination of the conidia of Cystopus was also tested. Controls were kept to ascertain the viability of the conidia and were chilled as described above. Twenty-one cultures were exposed to light and seventeen were

kept in darkness, in each case without chilling. All failed to germinate. The controls all germinated except two. These results are shown in Table III. All the experiments were carried on simultaneously and all the conditions were the same except that no bell jars were used to cover the controls while bell jars were used in the experiments in the light and in the dark. The conidia were taken from freshly matured pustules and placed in a drop of water on slides that were well cleaned. One series of cultures was kept in the diffused light of an ordinary laboratory where no direct sunlight fell upon them; the other series was kept in a dark room. The high temperatures, from August 11 to 25, have been explained in connection with Table I. The question might naturally be raised as to whether germination of the conidia in the ice box were not due to the dark moisture saturated atmosphere of the ice box rather than to the low temperature. These experiments answer this question. The series of seventeen cultures kept in the dark room were in a saturated atmosphere the same as the controls in the ice box. The only difference was in the temperature which varied from 26° to 28° C. in the dark room and from 8° to 21° C. in the ice box. The ice box cultures all germinated except two; while none of the seventeen cultures in the dark room germinated. From these results it is clear that light is not a determining factor in germination. It is also clear that a saturated atmosphere at high temperatures will not cause the germination of the conidia of Cystopus.

TABLE IV.—EFFECT OF USING STILL LOWER TEMPERATURES IN GERMINATION OF CONIDIA OF CERTAIN SPECIES OF CYSTOPUS.

Date.	No. of cultures	Species tested.	Age of spores.	Period of refrigeration.		Results.	Out-door temperature. minimum.
				Hours.	Temp.		
Sept. 9..	1	Cystopus bliti....	Young....	1.16	§	—	15
Sept. 9..	2	C. cubicus.....	Young....	8	§	+	15
Sept. 9..	1	C. candidus.....	Young....	8	§	+	15
Sept.13..	3*	C. cubicus.....	Young....	2.5	—1	—	18
Sept. 8..	2	C. cubicus.....	Young....	4.5	§	+	12
Sept. 4..	1†	C. bliti.....	Young....	19.5	§	+	14
Sept.27..	1†	C. cubicus.....	Young....	27	§	—	6
Sept.27..	1†	C. cubicus.....	Young....	27	§	+	6
Oct. 4..	1	C. cubicus.....	Old.....	10.75	§	+	6
Oct. 4..	1	C. bliti.....	Young....	10.75	§	+	6
Oct. 12..	3	C. bliti‡.....	Young....	3	10	—	—4
Oct. 12..	3	C. cubicus‡.....	Young....	3	10	+	—4
Oct. 16..	2	C. cubicus‡.....	Old.....	3	12	+	0
Oct. 15..	4	C. bliti‡.....	Old.....	3	12	+	0

*This experiment was made with a Van Tieghem cell and hanging drop.
†Pieces of leaves with pustules in water on watch crystal.
‡Conidia taken from frozen leaves.
§The slides in these experiments were laid on a block of ice in the ice box.

SUMMARY OF TABLE IV

The effects of lower temperatures than those ordinarily obtained in the ice box were also tried. The results of these experiments are shown in Table IV. The slides were laid on blocks of ice, except in the case of the experiments on September 13 and October 12 and 16, which are further described below. Twelve cultures were made. In nine, the spores germinated while in three, there was no germination. These results are somewhat at variance with DeBary, who found the minimum to be 5° C. There can be no doubt from the above results that the minimum for germination is very near 0° C.

To test the effect of still lower temperatures, three Van salt giving a temperature of 1° C. At the end of thirty minutes no germination had occurred. These slides were allowed to remain in the laboratory at 28° C. for ten hours after being removed from the freezing mixture, but no germination resulted. This indicates that a change from high to low and then back to high does not lead to germination.

The effect of frost on the conidia of Cystopus outdoors was also tested. The conidia were collected October 12 and 16 from frozen leaves which had been allowed to thaw out in the laboratory. Twelve cultures were kept at 10° to 12° C. for three hours. Nine of the twelve cultures germinated and three did not, indicating that the conidia were not killed by a frost.

SUMMARY OF TABLE V

In view of the fact that germination was obtained in the ice box at temperatures above 20° C. in some cases, it was thought advisable to make a further study of the relation of temperature to spore germination at room temperatures. During the latter part of March and first part of April, 1910, further experiments were made to determine whether the conidia of Cystopus would germinate at room temperatures. As previously described, the experiments with cultures at green house temperatures in the summer of 1909 had given only a low percentage of germination. In fact germination was observed in only one or two cases. In the new series of experiments, seventythree cultures were made from March 27 to April 8, at temperatures varying from 17¾° to 25° C. Controls were kept at ice box temperatures in cases

TABLE V—FURTHER EXPERIMENTS ON GERMINATION OF CONIDIA OF CYSTOPUS AT ROOM TEMPERATURE FROM MARCH 27 TO APRIL 8.

Date.	No. cult.	Species Tested.	Age past. days.	Period of incubation.		Atmosphere.	Amt. Evap.	Result.	Controls Chilled.			
				Temp.	Time.				No. cult.	Time.	Temp.	Result.
March 27..	4	Cystopus candidus.....	9	23	4	Saturated.....	None.....	+*	5	3.5	12	+
March 27..	5	C. candidus.....	9	25	4	Saturated.....	None.....	+*	5	3.5	12	+
March 27..	3	C. candidus.....	9	24	3.5	Saturated.....	None.....	+	5	3.5	12	+
March 27..	1	C. candidus.....	9	24	3.5	Saturated.....	None.....	−	4	2	13	+
March 8..	4	C. candidus.....	10	25	19	Not saturated.....	Nearly dry	−	2	2	15	+
March 30..	2	C. candidus.....	12	23	2.5	Not saturated.....	Some.....	−	2	2	15	+
March 30..	2	C. candidus.....	12	23	2.5	Saturated.....	None.....	−	4	2	13	+
March 28..	4	C. candidus.....	10	25	19	Saturated.....	None.....	−	.....	.....	.....	.....
March 30..	2	C. candidus.....	13	18	2	Not saturated.....	Some.....	+	.....	.....	.....	.....
March 30..	2	C. candidus.....	13	18	2	Saturated.....	None.....	+	.....	.....	.....	.....
April 1..	2	C. candidus.....	14	18.3	2	Saturated.....	None.....	+	.....	.....	.....	.....
April 1..	2	C. candidus.....	14	18.3	2	Not saturated.....	Nearly dry	+	.....	.....	.....	.....
April 1..	2	C. candidus.....	14	18.3	2	Saturated.....	Little	+	.....	.....	.....	.....
April 1..	2	C. candidus.....	14	18.3	2	Not saturated.....	Little	+	.....	.....	.....	.....
April 2..	2	C. candidus.....	15	17.75	1.33	Not saturated	Nearly dry.....	+	.....	.....	.....	.....
April 2..	2	C. candidus.....	15	17.75	1.33	Saturated.....	None.....	+	.....	.....	.....	.....
April 2..	2	C. candidus.....	15	17.75	1.33	Not saturated.....	Some.....	+	.....	.....	.....	.....
April 2..	2	C. candidus.....	15	17.75	1.33	Saturated.....	None.....	+	.....	.....	.....	.....
April 2..	2	C. candidus.....	15	20	1.5	Saturated.	Nearly dry	+	.....	.....	.....	.....
April 2..	2	C. candidus.....	15	20	1.5	Saturated.....	Little	+	.....	.....	.....	.....
April 2..	2	C. candidus.....	15	20	1.5	Saturated.....	Some.....	+	.....	.....	.....	.....
April 2..	2	C. candidus.....	15	20	1.5	Saturated.....	None.....	+	.....	.....	.....	.....
April 4..	1	C. candidus.....	3	19.5	2	Not saturated.....	Nearly dry	+*	.....	.....	.....	.....
April 4..	1	C. candidus.....	3	19.5	2	Not saturated.....	.....	+*	.....	.....	.....	.....
April 4..	1	C. caddidus.....	3	19.5	2	Saturated.....	Little	+*	.....	.....	.....	.....
April 4..	1	C. candidus.....	3	19.5	2	Saturated.....	Little	+*	.....	.....	.....	.....
April 4..	2	C. candidus.....	3	19.5	2	Saturated.....	None.....	+*	.....	.....	.....	.....
April 4..	2	C. candidus.....	3	19.5	2	Not saturated.....	Some.....	+*	.....	.....	.....	.....
April 6..	2	C. candidus.....	5	19	1	Saturated	Nearly dry	+	.....	.....	.....	.....
April 6..	2	C. candidus.....	5	19	1	Saturated.....	None.....	×	.....	.....	.....	.....
April 7..	4	C. candidus.....	5	18	45 min.	Saturated.....	Little	−	4	45 min.	10	+
April 8..	4	C. candidus.....	5	19.5	1.5	Saturated.....	Little	−*	.....	.....	.....	.....

*Ice water used. ×Two cultures germinated and two did not.

where the temperature of the room was about 20° C. The room where this series of experiments was carried on was lighted by a skylight. It was steam heated and had only one door. The temperature during any one experiment never varied more than four degrees. The temperature was noted when the experiment was started and stopped and the average of the two readings are given in Table V. The conidia were obtained from stock cultures kept growing in the green house during the winter. The age of the conidia was determined as far as practicable by noting the time of appearance of the pustules and each time using conidia from the marked pustule. The plants serving as the source of conidia were placed on a separate bench where no wind could strike them, so that the conidia could not be blown away. Care was exercised in watering the plants not to jar the infected leaves. In this way it was possible to obtain conidia from pustules of known age. The spores were placed in tap water except on March 27 and April 4 when ice water was used. Twenty tests were made without covering the cultures with bell jars; while the remaining fiftythree were kept in a saturated atmosphere obtained by placing the cultures under bell jars placed on a wet earthen plate. The approximate amount of evaporation in the cultures was observed and recorded in each case. In this case of seventythree tests at room temperature varying from 17¾° to 25° C., twenty-six failed to germinate. Controls were kept at lower temperatures all of which germinated readily, indicating that the conidia used were normal. The time required for germination varied from forty-five minutes to four hours. The former being the shortest period at which germination was observed. Twenty tests were made in a non-saturated atmosphere and fifty-three in a saturated atmosphere. Nine of the cultures that were subjected to ordinary room conditions failed to germinate, and eleven of the cultures that were in a saturated atmosphere failed. This suggests that a saturated atmosphere is not necessary so long as the conidia are in water. The germination apparently took place as readily in cultures nearly dry as when no evaporation occurred. It should be noted still further that twenty-five cultures were made at temperatures above 20° C. in which 58 per cent germinated. The remaining forty-eight cultures in the series were at temperatures between 17¾° and 20° C. or below and 60 per cent germinated. From these results it is again clear that temperature is an important factor

in the germination of the conidia of Cystopus. These results would have been still more striking had the temperatures in the last series been nearer the optimum, 10° C.

TABLE VI. OBSERVATIONS ON OUTDOOR GERMINATION OF CYSTOPUS ON RADISH AND SALSIFY.

Date.	Time of day a. m.	Species of plant	Results observed. Zoospores. Present.	Absent.	Outdoor weather conditions. Max. Temp.	Min. Temp.	Precipitation.	Character of day.
Sept. 9..	8	Salsify....	abundant..	.....	16	8	0	Clear.
Sept. 11..	8	Salsify....	abundant..	.....	26	10	.04	Cloudy.
Sept. 15..	8	Salsify....	abundant..	.....	20	11	0	Clear.
Sept. 20..	5	Radish...	abundant..	.....	21	10	0	Clear.
Sept. 20..	5	Salsify....	abundant..	.....	21	10	0	Clear.
Sept. 28..	8	Salsify....	abundant..	.....	19	8	0	Clear.
Oct. 7..	9:15	Salsify....	.....	absent..	19	6	0	Clear.
Oct. 21..	8	Salsify....	abundant..	.....	10	5	.22	Cloudy.

SUMMARY OF TABLE VI

Since it was found that the spores of Cystopus germinated readily in the laboratory when chilled, my attention was directed to the possibility that chilling also favored germination on the host plant out doors when a dew was present. Observations were made on seven different days from September 9 to October 21. The observations were made on the leaves of salsify (*Tragopogon porrifolius*) and radish (*Raphanus sativus*) which were badly infected with Cystopus. The leaves were gathered from 5 to 9:15 a. m. and were carried to the laboratory and examined for motile zoospores. The zoospores were found in every case except on October 7. On this date the observation was made at 9:15 a. m. rather than at 8:00 a. m., which was the usual time. It should be noted that no observations were made after 9:15 a. m. when the leaves were wet. This should undoubtedly be done to fully prove that germination takes place only in the morning or when the temperature is low. The minimum for the days on which observations were made varied from 5 to 11 2-3° C. as seen from the table. The lowest temperature for the days in question came about sunrise as is usually the case. It is quite clear from these observations that germination outdoors does take place at the time of lowest temperature for the day. The question as to whether germination takes place at any other time of the day will be further investigated this coming summer.

RESULTS WITH OTHER SPECIES OF PERONOSPORACEAE

It has been found that the favorable effect of chilling is not restricted to the conidia of Cystopus, but is manifest as well in the case of the conidia of other Oomycetes. I have incidentally tested the germination of the conidia of *Plasmopara viticola* at two different times and each time found that the conidia germinated readily at a temperature of about 10° C. Four tests were made with *Peronospora effusa* and germination resulted in two to four hours when kept at 10° C. Two trials were made with the conidia of *Peronospora parasitica* and germination resulted in two and one half hours at 12° C. The conidia of *Phytophthora infestans* also germinated when chilled for 2½ hours at 12° C. The controls for each of the above experiments failed to germinate at room temperature. These results are based on only one to four trials with each species, but show that chilling favors the germination of conidia of other Oomycetes as well as those of Cystopus. Further experiments are in progress to determine more fully the effect of chilling on the germination of the conidia of Plasmopara, Peronospora and Phytophthora.

GROWING CYSTOPUS IN STOCK CULTURES UNDER GREEN HOUSE CONDITIONS

It might naturally be supposed that so vigorous a parasite as *Cystopus candidus* would maintain itself quite easily under green house conditions, but it was soon evident that this is not the case. New infections were very reluctant to appear even when the host plants were well infected in the beginning and the old infections after a time would die out. In order to maintain through the winter vigorous stock cultures several methods were tried.

I first attempted to learn the affect of varying the light in the case of well infected plants. In this experiment ten radish, three Amaranthus and two white mustard plants, all of which were well infected with Cystopus were placed on an isolated bench in the green house where only diffuse light was accessible. Over these plants a large bell jar was placed, but free ventilation was provided by allowing the bell jar to rest on two bricks, one on each side of the pots containing the plants. On September 24, 1909, fourteen other plants for controls were placed where they

could get direct sunlight, also under similarly ventilated bell jars. All these plants were grown in three inch pots, and were four to six inches high and had four to eight leaves when the experiment was begun. Daily observations were made when the plants were watered. The experiments were continued from September 24 to October 29.

It was quite evident that internal growth of the fungus took place both in the diffuse and in the direct light because hypertrophy of the tissues about the infected areas occurred on the leaves of the radish plants as well as the production of the oospores in the leaves of the specimens of *Amaranthus retroflexus*. At no time did new pustules appear on any of the plants used. It was thus evident that Cystopus would grow under bell jars in the green house both in direct and diffuse light but at no time did new conidial pustules form. Some condition necessary for the production of conidia was lacking.

Further experiments were made to learn whether the fungus could be made to spread from an infected to a non-infected plant when in close contact and under the same conditions of moisture, light, and temperature described in connection with the above experiment.

On September 27 a three inch pot that had three infected plants in it was buried in sand on a bench in the sunlight. Around the infected plants were seven pots containing twentyone plants not infected. A large bell jar was placed over the whole in such a way that plenty of air was accessible. On October 13, new infections were found on seven new leaves which had become infected during a period of sixteen days. The plants were much healthier and more vigorous than the plants that were kept in the diffuse light in the above experiment. The spores were possibly carried from plant to plant by aphids or by slight currents of air that might enter under the bell jar. The culture was maintained for twenty days more but the fungus gradually disappeared.

In the following experiment attempts were made to produce new infections on a large number of plants and thus to retain and propagate the conidial stage. Six inch pots, in which there were about two hundred plants, were used. A more crowded condition was thus produced which showed itself more favorable for the fungus. When the plants were eight or ten days old, with only two cotyledons, they were inoculated with spores from *Cystopus candidus*. The conidia were placed in water and sprayed on with

an atomizer. It was in this way easily possible to inoculate every cotyledon. The method of chilling for abundant spore germination was also tried on these stock cultures. The pots containing the plants were placed in the ice box on a wet earthen plate over which a bell jar was inverted. The plants were usually left in the ice box from two to twelve hours, so that ample time might be provided for the conidia to germinate. December 6, two pots, A. and B., were treated as outlined above and became heavily infected by December 17. On this date the pots were placed on an isolated bench where plenty of sunlight was available for normal development of the plants, to learn whether or not the fungus would maintain itself under green house conditions when the plants were closely crowded together. On December 28 about half of the plants in the pots had died. These were carefully pulled out and more seed sown in these same pots among the living plants. In a short time a new crop of plants was at hand in the place where the dead plants had been. A supply of young host plants for the fungus was thus provided. On this same date, a third pot, C., thirteen days old and not infected, but otherwise exactly like the two described, was placed as close to the other two as possible.

Observations were made on January 8 and it was found that five small pustules had appeared on the plants in pot C. On January 11, no changes were observed in the extent of infection. Three pots, D., E., and F., of mixed radishes eight days old and not infected were put with the above three, making six in all. The next day two six inch pots of mixed radishes, G. and H., nine days old, were placed with the rest, all in a group around the infected plants. Observations on January 21 showed that pot C. had about 10 per cent of its leaves infected. Four more pots, I., J., K., and L., containing young seedlings eighteen days old, thickly sown in large pots, were added to the group. January 24 two of the pots, eight days old, D., and E., showed two pustules each. The following day the third, F., showed one pustule. On the same day the pots nine days old, G. and H., showed infection, each pot having four pustules.

On February 5, two pustules had appeared on each of the two pots, I. and J., placed on the bench on January 21. On the following day the third one, K., showed five pustules, and the fourth, L., nine pustules. Daily observations were made of the pots of plants as they were watered. The disease spread gradually, the

number of infected areas increasng as well as the number of pustules in each area. As the cotyledons died the young leaves became infected. On February 26, it was evident that the disease had reached its maximum. It was estimated that 15 per cent of the leaves were infected. The spread of the fungus, however, had hardly kept pace with the growth of the plants. The leaves increased in number faster than the fungus spread.

The disease gradually decreased from this time on. On March 20, it was found that about 5 per cent of the leaves were infected. The plants now were six inches high with six to ten leaves each and the number of pustules had decreased considerably. At least 40 per cent of the plants in the six inch pots were pulled out and seed sown among the remaining plants.

Observations were made from time to time on the twelve pots of plants and the fungus was found to be gradually decreasing in amount. On April 8, a damping off fungus attacked the young plants just at the surface of the soil, causing them to fall over and dry up. The young crop had become infected in every pot, but because of the damping off fungus, the infection was not as extensive as had been the case before. Three pots were so badly injured that they were discontinued. The large leaves of the plants in the pots were shading the surface of the soil too much for healthy growth of the young plants. To relieve this condition, the tops of all the plants in the pots were cut down even with those of the young plants. It is quite evident that this is a method that can be used to maintain the white rust under green house conditions. Two infected pots of crowded seedlings infected ten more standing near them. At no time was more than 15 per cent of the leaves infected and as the plants grew older, the amount of white rust decreased. The inoculation period in these experiments varied from eleven to sixteen days, due possibly to lack of optimum conditions for germination. This was longer than was required when the stock cultures were chilled.

An attempt was made to show whether or not the white rust could be maintained directly on the green house benches without chilling the seedlings in the beginning as was done in the above experiment. The radish seed was sown directly on the bench and not in pots. It was thought that possibly the disease might do better under these conditions. On November 1, four varieties of radish were sown in a box built on the bench, which was 8 inches wide and 3 feet long. When the plants were nine days old and

showed two cotyledons they were sprayed with water and inoculated with *Cystopus candidus* spores from "Early scarlet globe" radish. On November 18, one leaf was found having one pustule. November 24, eleven cotyledons showed infection. From that time on the disease increased gradually. On December 16, it was estimated that about 300 of the 2000 plants showed white pustules.

December 29, the pots of plants that had been inoculated but not chilled and the plants in the box on the bench showed about the same degree of infection.

A record of the temperature was kept by placing a self registering thermometer as near the box of plants as possible. The range of temperature was about 12° C. for the first thirteen days. During the day the temperature was about 27° C. and at night about 15° C., showing that temperature conditions are sometimes favorable in the greenhouse.

The plants in this experiment were given the most favorable conditions possible and they showed normal growth. This was evident from the dark green color on the cotyledons and leaves. When the radish plants are sickly and not doing well in the green house the infected cotyledons and the leaves tend to become variegated and fall off. None of these symptoms were noted in the plants studied in this experiment. The experiment indicates that it is impossible to get any such development of the disease as was secured by chilling the plants at the time of inoculation. Still the disease appeared without chilling and increased in amount until about 300 of the plants became infected. Therefore, it is possible to maintain the white rust under green house conditions by the method suggested in this experiment.

TABLE VII.—RECORD OF MAINTAINING TEN INFECTED STOCK CULTURES.

Name Cult.	Variety of radish.	Age Cult.	Time in ice box.	Date inoc.	Date inf.	Date re-planted.	Results.
		days	Hrs.				
J.	Ne Plus Ultra.....	5	11½	Sept. 29	Oct. 6		Heavy inf.
		18	6	Oct. 12		Oct. 12	Young leaves well infected.
		42	6	Nov. 5			Well infected.
		46			Nov. 9		Second crop heavily infected.
		77				Dec. 11	
		83	19	Dec. 17			Well infected.
		118			Jan. 24	Jan. 13	Heavy inf.
		148	12	Feb. 23			Old plants with little inf.; younger plants, plenty of inf.
		164				Mch. 11	Old pl. about to blossom. Tending to shade young pl. Not well inf.
		192			Apr. 8		Two old pl. in blossom. Pot filled with soil.
K.	Crimson Giant.....	9	6	Dec. 25	Nov. 9		Well infected.
		43				Dec. 7	Well infected.
		53	19	Dec. 17	Dec. 31		Young heavily inf.
		80				Jan. 13	Well infected.
		121	12	Feb. 23			Low in inf.
						Mch. 11	Young crop well inf.
					Mch. 24		6 big plants about to blossom. other pl. shaded. Poor inf.
		165			Apr. 8		One pl. in flower. Others in bud. Poor inf.
L.	New White Chinese.....	6	6	Dec. 17	Dec. 29		Well infected.
		27	20	Jan. 13	Jan. 13	Jan. 13	Disease increased in amount.
		41	12	Feb. 23		Feb. 23	Well infected.
	Ne Plus Ultra.....	57				Mch. 11	Young crop well inf.
M.	Scarlet turnip, white tip.	5	6	Dec. 17	Jan. 2	Nov. 1	Well infected.
	Ne Plus Ultra.....					Jan. 13	Coty dead. Young pl. becoming inf.
			12	Feb. 23		Mch. 11	Young pl. well inf. Infection decreased.
	Ne Plus Ultra.....	86					Young plant inf.. old also tho not as much as young.
N.	Old's Snowball.....	9	8.5	Dec. 21	Jan. 2		Well infected.
						Jan. 13	Well infected.
		83	12	Feb. 23			Well inf. Pot broken.
O.	Cincinnati Market	7	23	Dec. 13	Dec. 26		Well infected.
	Ne Plus Ultra.....	95				Mch. 11	Pl. 5 in. high. 5% of pl. inf.
P.	Mixed radish.....	8	7	Jan. 11	Jan. 22		Well infected.
			22	Feb. 23		Feb. 23	Well infected.
	Ne Plus Ultra.....	67	22	Mch.			Infection low.

TABLE VII Continued.—MAINTAINING TEN INFECTED STOCK CULTURES

Name Cult.	Variety of Radish.	Age Cult.	Time in ice box.	Date inoc.	Date inf.	Date re-planted.	Results.
Q.	Old's Twenty Day.....	7	24	Dec. 13	Dec. 23	Jan. 22	Well infected.
			9	Feb. 25		Jan. 13	Less than ½ of pl. inf.
		95	22	Mch. 11			Young crop well inf.
R.	Winter			Jan. 3	Jan. 12		Well infected.
	China Rose.....	11	24	Feb. 23		Feb. 25	
		78	22	Mch. 11			
S.	Mixed Radish	9	24	Jan. 12	Jan. 20		Well infected.
			24	Feb. 23			75% of leaves inf.
						Feb. 25	75% of leaves inf.
		67	7	Mch. 11			Decreased in inf.

SUMMARY OF TABLE VII

On the basis of the experiment with stock cultures just described the following method for maintaining stock cultures was marked out. Good infections were secured by spraying the spores on the young seedlings in a crowded six inch pot with an atomizer and then setting the pot on a wet earthen plate over which a bell jar was inverted. Under these conditions the pots of plants were placed in the ice box where the temperature was about 10° C., long enough to allow the conidia to germinate. After this they were placed on a bench in the green house. As the fungus continued to kill the young seedlings, others were supplied by sowing more seed among the remaining plants. When this new crop came up, the pots were again placed in the ice box as described above so that the young plants might become infected. It will be convenient in the following work to speak of a six inch pot thickly sown with radish plants infected with *Cystopus candidus*, as a stock culture. Nine of these stock cultures were maintained in this experiment, each designated by a capital letter from J to S. In all the stock cultures from J to S good infections were maintained by chilling and reseeding whenever the disease showed signs of disappearing. These stock cultures became the source for the conidia used in the subsequent experiments up to the time when the plants were about to bloom. In stock cultures J and K good infections were kept in the same pot for 192 and 165 days respectively. On March 8, as a result of the pots becoming so filled with soil from continued reseeding, it was impossible to grow more new plants among the old ones.

At this time the old plants in the stock cultures were in blossom and the leaves were large and broad. This condition was not conducive to the growth of young plants in the same pot. Even in this stage of development, the plants were not free from disease although the pustules were much less abundant than when the plants were younger. The stock cultures from L to S were all younger than J and K, and on March 11, all the plants were about to blossom. The white rust was evident in every stock culture but was not as plentiful as when the plants were smaller. Had it not been that it was desired to make a study of the production of oospores, the larger plants might have been cut off, and the culture inoculated again whenever the disease showed signs of marked decrease in amount.

Stock culture J was chilled in the ice box five times, reseeded four times and maintained a good infection for 192 days or until the plants blossomed.

Stock culture K was chilled in the ice box three times and reseeded three times during the 165 days. Infected plants were always abundant until the old plants blossomed.

The remaining stock cultures L to S were not as old, but in every case they were chilled at least twice and reseeded from one to three times. Here also, well infected stock cultures were maintained until March 11, when the plants grew tall and began to blossom. This is undoubtedly a method by which white rust can be maintained abundantly under green house conditions with the use of only a few pots. The full data as to these cultures are given in Table V.

To fully test the value of chilling in maintaining stock cultures of Cystopus, parallel cultures, chilled and not chilled, were made during a period of 116 days. Chilling at the start a crowded stock culture of *Raphanus sativus* such as is described above and inoculated with *Cystopus candidus* from the radish undoubtedly causes a larger per cent of infection than when the stock cultures are not chilled. Six inch pots were thickly seeded with radish so that each pot contained about two hundred seedlings when it was about ten days old. The plates except VII and VIII are photographs of such cultures. At this time the stock cultures consisting of two hundred seedlings, were inoculated with conidia of *Cystopus candidus* from *Raphanus sativus*. The conidia were sprayed on the young seedlings with an atomizer. Following this treatment, the stock cultures were placed on a wet

TABLE VIII.—EFFECT OF CHILLING STOCK CULTURES OF *Raphanus sativus* INOCULATED WITH CONIDIA FROM *Cystopus candidus* WITH CONTROLS NOT CHILLED

No. of exp.	Date.	Variety of radish.	Source of conidia.	Period of refrigeration.		Date inf.	Ext. inf.	Date photographed.	Control not chilled.		
				Hours.	Temp.				Date inf.	Ext. inf.	Date photographed.
1	Oct. 29...	White Icycle.....	Ne Plus Ultra.....	8.5	16	Nov. 5....	Much.....	Nov. 17.	Nov. 15...	Little....	Nov. 17.
1	Dec. 21....	Triumph.....	Ne Plus Ultra.....	7	11	Dec. 31....	Much.....	Jan. 5..	Dec. 31....	Little....	Jan. 5.
2	Jan. 3....	Triumph.....	Ne Plus Ultra.....	8	10	Jan. 12....	Much.....	Jan. 18.	.....	Little....	Jan. 18.
3	Jan. 3....	China Rose Winter.....	Ne Plus Ultra.....	8	10	Jan. 12....	Much.....	Jan. 18.	Jan. 12...	Little....	Jan. 18.
4	Jan. 11....	Mixed	Ne Plus Ultra.....	7	13	Jan. 24....	Much.....	Jan. 25.	Jan. 24...	Much	Jan. 25.
4	Nov. 8....	NePlus Ultra..	Ne Plus Ultra.....	24	.....	Nov. 15...	Much.....	Nov. 17.	Nov. 18...	Little....	Nov. 29.
2	Nov. 8....	NePlus Ultra.....	Ne Plus Ultra.....	24	.....	Nov. 15...	Much. ...	Nov. 17.	Nov. 18...	Little....	Nov. 29.
1	Dec. 3....	NePlus Ultra.....	Ne Plus Ultra.....	6	19	Dec. 12....	Much.....	Dec. 19..	Dec. 12...	Little....	Dec. 19.
3	Dec. 8....	NePlus Ultra.....	Ne Plus Ultra.....	6	15	Dec. 17....	Much.....	Dec. 27..	Dec. 19...	Little....	Dec. 27.
16	Jan. 10....	Olds' Golden Globe.....	Ne Plus Ultra.....	23	10	Jan. 22....	Much.....	Jan. 25.	Jan. 22...	Little....	Jan. 25.
2	Dec. 13....	Olds' Twenty Day.....	Ne Plus Ultra.....	22	10	Dec. 23....	Much.....	Dec. 27..	Dec. 26...	Little....	Dec. 27.
1	Jan. 14....	Mixed	Ne Plus Ultra.....	7	13	Jan. 24....	Much.....	Jan. 25.	Jan. 24...	Little....	Jan. 25.
2	Dec. 3....	NePlus Ultra.....	Ne Plus Ultra.....	6	19	Dec. 12....	Much.....	Dec. 29..	Dec. 12...	Little....	Dec. 29.
2	Dec. 8....	NePlus Ultra.....	Ne Plus Ultra.....	6	15	Dec. 17....	Much.....	Dec. 27..	Dec. 19...	Little....	Dec. 27.
1	Feb. 22...	NePlus Ultra.....	Ne Plus Ultra.....	15.1	10	Mar. 3....	Much. ...	.....	Mar. 1....	Little....	.....
2	Feb. 22...	NePlus Ultra.....	Ne Plus Ultra.....	15.1	10	Mar. 1....	Much.....	.....	Mar. 8....	Little....	.....
3	Feb. 22...	NePlus Ultra.....	Ne Plus Ultra.....	15.1	10	Mar. 3....	Much.....	.....	Mar. 8....	Little....	.....
4	Feb. 22...	NePlus Ultra.....	Ne Plus Ultra.....	15.1	10	Mar. 1....	Much.....	.....	Mar. 5....	Little....	.....
3	Jan. 12....	Mixed	Ne Plus Ultra.....	7.66	10	Jan. 18....	Much.....	.....	Jan. 22....	Little....	.....
4	Jan. 12....	Mixed	Ne Plus Ultra.....	7.66	10	Jan. 18....	Much.....	.....	Jan. 22....	Little....	.....
1	Jan. 12....	First and Best.....	Ne Plus Ultra.....	22	10	Jan. 19....	Much.....	.....	Jan. 24....	Little....	.....
16	Dec. 28....	NePlus Ultra.....	Ne Plus Ultra.....	17	11	Jan. 7.....	Much.....	....	Jan. 10....	Little....	.....
18	Dec. 28....	NePlus Ultra.....	Ne Plus Ultra.....	17	11	Jan. 7.....	Much.....	.....	Jan. 7.....	Little....	.....

earthen plate under a bell jar and placed in the ice box for a period varying from six to twentyfour hours. When the stock cultures were removed from the ice box they were placed on a bench in the green house and in from seven to twelve days the fungus made its appearance. The controls used in each experiment were treated in the same manner as the stock cultures except that they were not chilled but placed directly on a bench in the green house. The results were photographed when the pustules were fully developed and about to burst.

The extent of infection secured by the chilling method can best be seen by referring to the plates. Plates I and II show two stock cultures of radish seedlings that were inoculated when the seedlings were ten days old. In seven days, the cultures began to show infection. Four days later, or eleven days after inoculation, the conidial pustules were fully developed and the results photographed to show the difference in the extent of infection between cultures chilled and not chilled. Pustules developed on both the upper and lower side of the cotyledons but only on the lower side of the first true leaves, which can be seen in the plates. November 8, two more stock cultures were inoculated and became infected November 15. The results were photographed November 17, two days later (Plates III and IV). Here, again, it will be noted that chilling produces the more abundant infection. These two stock cultures were kept as were many others as a source of conidia for future inoculation. November 29, or twentyone days after inoculation, the stock cultures shown in Plates III and IV were photographed again to show the further development of the fungus as well as the effects on the host plants (Plates V and VI). The radish seedlings in the chilled culture were being killed rapidly by the fungus while in the control (Plate VI) the plants are healthy with no marked increase in the amount of white pustules. It was very evident in this experiment as well as in all the others that more abundant infection had occurred in the cultures that were chilled. The same striking results were secured when the radish seedlings were grown in small pots (Plate VII). In these experiments, radish seedlings were grown in three-inch pots and treated as described above. The effect of chilling can be readily seen. The results shown in Plate VII suggest that an extensive infection is not dependent upon the crowded condition of seedlings in the stock cultures, but upon chilling. Not only do the coty-

ledons become heavily infected by the chilling method, but also the leaves. The curled and hypertrophied leaves can be seen in Plate VIII. The leaves are as readily infected as the cotyledons of the radish. In *Lepidium sativum* only the cotyledons can become infected according to DeBary (1863; 27).

Twenty-four tests with a control for each trial were made, between October 29, 1909, and February 22, 1910. Experiments have been continued since the last named date with the same striking results. Plates I and X show stock cultures made from October 29, 1909, to June 6, 1910, and photographed when the fungus was well developed. In every case in which the stock cultures were chilled, a heavy infection resulted; while the controls, except in one case, showed but little development of the white rust. The control in experiment No. 4 on January 11, showed an exceptional development of infection. It was as heavily infected as the stock culture that was chilled which again shows that the conditions favorable for infection do occasionally occur in the green house, as has already been pointed out. It might naturally be asked whether chilling has the same effect in the spring and summer as in the fall and winter. Experiments have been carried on continuously since February 22 (although the results are not tabulated) until June 6, 1910, with the same marked results as were obtained during the fall and winter of 1909. The above data lead me to conclude that chilling strongly favors the infection of radish plants with *Cystopus candidus*.

THE RELATIVE SUSCEPTIBILITY OF COTYLEDONS AND LEAVES TO *CYSTOPUS CANDIDUS*

In the preceding experiments it was quite impossible to determine whether any marked degree of difference of susceptibility existed between cotyledons and leaves of the radish. DeBary found that it was only the cotyledons that showed any marked degree of susceptibility. In order to test this point for the cotyledons and leaves of radish, shepherd's purse, white mustard, and garden cress, the following experiments were planned.

All the plants utilized in this series of experiments were grown in the green house and were in all cases free from infection at the outset. Twelve three-inch pots were seeded with radish on

October 15, each pot containing only two plants. On November 11 the plants had lost their cotyledons and the first leaves were two to three inches long. At this time the twentyfour plants were inoculated and chilled and on November 23, twelve of the plants showed infection. On the following day twenty of the twentyfour plants inoculated were infected. There was not the slightest evidence that the infection was abnormal as the pustules were abundant on each leaf and the leaves were becoming badly distorted. On September 15, five plants of Capsella that had grown from seed collected outdoors and planted in the green house were in blossom. At this time the plants were inoculated with conidia from Capsella and September 26, three of the plants were infected on both stem and leaves. On the following day the leaves and young fruits of the two remaining plants also became infected. A stock culture of at least fifty white mustard plants that were planted September 1 in the green house were inoculated September 23 when the plants were six inches tall. They were healthy and the cotyledons had fallen off. On the last named date, the culture was inoculated with spores from the white mustard and chilled. On October 12, at least 75 per cent of the leaves on the plants were infected.

I have never collected Cystopus on garden cress so it was impossible to test this host with the Cystopus growing on it outdoors; but I have inoculated garden cress with spores from Capsella. Three three-inch pots of garden cress containing eighteen plants that were four inches high and without cotyledons were inoculated October 15. Infection resulted October 27. Twelve of the plants became infected. From the above results it is clear that the leaves as well as the cotyledons of radish, shepherd's purse, white mustard, and garden cress are readily infected with *Cystopus candidus*.

SUSCEPTIBILITY OF DIFFERENT VARIETIES AND SPECIES OF RAPHANUS TO *CYSTOPUS CANDIDUS*

One object in developing a method of germinating the conidia of Cystopus with certainty and in abundance was to provide means for attacking the question as to the existence of so-called physiological species in the genus. It seemed desirable also to test the relative susceptibility of different varieties of radish

to Cystopus. For this latter purpose twentytwo varieties of radish were grown and inoculated. In these experiments on different varieties of radish, three-inch pots were used and about ten seeds of the same variety were planted in each of the three pots. When the plants were eight to twelve days old all of the plants in each pot except three of the healthiest were pulled out. At this time each plant was about 1½ inches high and had two well developed cotyledons but no true leaves. These plants were then inoculated. In testing each variety, at least nine plants were inoculated. In Table IX are given the results obtained from inoculating twentytwo varieties of radish with conidia taken from the varieties Ne Plus Ultra, White Icicle, or Crimson Giant. In every experiment except those started November 9 and November 12, Ne Plus Ultra was used as the source of conidia. In all, ninetyseven inoculations were made and in ninetyfive cases, infection appeared. Controls of Ne Plus Ultra of the same age and under the same conditions were kept in every experiment and always showed abundant infection. 97½ per cent of the cotyledons inoculated became infected. This would suggest very little immunity for any of the twentytwo varieties of radish tested, to *Cystopus candidus*. It is quite evident that the same form of *Cystopus candidus* can grow on all the varieties of radish.

Tests were next made to determine whether or not a different species of Raphanus *(Raphanus caudatus)* could be infected with conidia of Cystopus from *Raphanus sativus*. Nine plants growing in three different pots were inoculated and all became infected showing that *Cystopus candidus* on *Raphanus sativus* is not limited to this species, but can also infect *Raphanus caudatus*. Many tests have been made with the above species since this experiment with similar results.

TABLE IX.—RELATIVE SUSCEPTIBILITY OF DIFFERENT VARIETIES OF *Raphanus sativus* TO *Cystopus candidus* FROM *Raphanus sativus*, VARIETY NE PLUS ULTRA AND OTHERS.

No. cult. tested.	Date.	Host plant.	Source of conidia.	No. of plants inoculated.	No. of controls inoculated.	Period of refrigeration.		Results.				Controls.
						Hours.	Temp.	Date.	No. coty.			
									Inf.	Not inf.	Dead.	
3	Oct. 24	Scarlet turnip white tip	Ne Plus Ultra	9	3	19.	9.89	Nov. 5	17	1	0	Inf.
3	Oct. 24	New Leaf less	Ne Plus Ultra	9	3	19.	9.89	Nov. 5	17	0	1	Inf.
3	Oct. 25	New white Chinese	Ne Plus Ultra	9	3	7.5	.....	Nov. 5	15	1	0	Inf.
3	Oct. 25	Olds' Snowball	Ne Plus Ultra	9	3	7.5	9.89	Nov. 5	5	1	0	Inf.
3	Oct. 29	White Icicle	Ne Plus Ultra	9	3	8.5	16.66	Nov. 5	18	0	0	Inf.
3	Oct. 29	Early Scarlet Globe	Ne Plus Ultra	9	3	6.5	16.66	Nov. 15	16	2	0	Inf.
3	Oct. 30	Cincinnati market	Ne Plus Ultra	9	3	6	15.56	Nov. 11	5	0	0	Inf.
4	Nov. 1	Crystal Beauty	Ne Plus Ultra	12	5	23	16.11	Nov. 20	20	4	0	Inf.
2	Nov. 1	Cincinnati market	Ne Plus Ultra	6	5	21	16.11	Nov. 9	9	3	0	Inf.
2	Nov. 1	New white Chinese	Ne Plus Ultra	6	5	21	16.11	Nov. 20	9	1	2	Inf.
1	Nov. 1	New white Chinese	Ne Plus Ultra	3	5	21	11	Nov. 20	4	2	0	Inf.
1	Nov. 1	Brightest long scarlet	Ne Plus Ultra	3	5	21	11	Nov. 20	5	0	1	Inf.
2	Nov. 1	Scarlet turnip white tip	Ne Plus Ultra	6	5	21	11	Nov. 10	10	1	1	Inf.
2	Nov. 1	Brightest long scarlet	Ne Plus Ultra	6	5	21	11	Nov. 20	10	2	0	Inf.
2	Nov. 1	New leafless	Ne Plus Ultra	6	5	21	11	Nov. 13	6	2	4	Inf.
3	Nov. 9	Olds' Improved French Breakfast	White Icicle	9	5	5	11	Nov. 19	18	0	0	Inf.
3	Nov. 9	Olds' Snowball	White Icicle	9	5	5	11	Nov. 19	4	2	0	Inf.
3	Nov. 9	Olds' Snowball	White Icicle	9	5	5	11	Nov. 19	11	6	1	Inf.
2	Nov. 9	Brightest long scarlet	Crimson Giant	6	5	5	11	Nov. 17	1	7	4	Inf.
3	Nov. 12	Olds' Snowball	Ne Plus Ultra	9	5	5.5	11	Nov. 20	17	1	1	Inf.
3	Nov. 12	Olds' Improved French Breakfast	Ne Plus Ultra	9	5	5.5	11	Nov. 21	16	0	2	Inf.
3	Nov. 12	Triumph	Ne Plus Ultra	9	5	5.5	.....	Nov. 21	11	7	0	Inf.
4	Nov. 12	Long black Spanish	Ne Plus Ultra	12	5	5.5	11	Nov. 22	20	4	0	Inf.
3	Nov. 12	Long white Vienna	White Icicle	9	5	5.5	11	Nov. 23	14	0	0	Inf.
4	Nov. 12	China Rose Winter	White Icicle	12	5	4	11	Nov. 21	23	0	1	Inf.
4	Nov. 17	White Strassburg	Ne Plus Ultra	9	5	5.5	11	Nov. 26	14	2	2	Inf.
4	Nov. 17	Olds' Golden Globe	Ne Plus Ultra	9	5	5.5	11	Nov. 26	7	9	0	Inf.
4	Nov. 17	Mixed radish	Ne Plus Ultra	9	5	5.5	11	Nov. 26	14	4	0	Inf.
4	Nov. 19	Early long scarlet	Ne Plus Ultra	12	5	7.5	11	Nov. 27	18	4	2	Inf.
1	Nov. 23	Olds' Twenty-day	Ne Plus Ultra	3	5	2.5	17.22	Dec. 1	6	0	0	Inf.
2	Nov. 23	White Delicious	Ne Plus Ultra	6	5	2.5	17.22	Nov. 30	11	1	0	Inf.
1	Nov. 23	Olds' Twenty-day	Ne Plus Ultra	3	5	2.5	17.22	Dec. 1	4	1	1	Inf.
1	Nov. 23	White Delicious	Ne Plus Ultra	3	5	2.5	17.22	Nov. 30	6	0	0	Inf.
1	Nov. 23	Olds' Twenty-day	Ne Plus Ultra	3	5	2.5	17.22	Dec. 1	5	1	0	Inf.
2	Nov. 23	Olds' Earliest Scarlet Turnip	Ne Plus Ultra	6	5	2.5	17.22	Dec. 1	9	3	0	Inf.
2	Nov. 23	First and Best	Ne Plus Ultra	6	5	2.5	17.22	Nov. 30	10	2	0	Inf.
1	Nov. 23	Olds' Earliest Scarlet Turnip	Ne Plus Ultra	3	5	2.5	17.22	Dec. 1	5	1	0	Inf.
1	Nov. 23	First and Best	Ne Plus Ultra	3	5	2.5	17.22	Nov. 30	5	1	0	Inf.
3	Jan. 22	Raphanus caudatus	Triumph	9	5	26.5	12.77	Feb. 5	18	0	0	Inf.

SUSCEPTIBILITY OF OTHER CRUCIFERS TO *CYSTOPUS CANDIDUS* FROM THE RADISH

Tests were made to learn if the spores of *Cystopus candidus* from the radish can infect turnips *(Brassica rapa)*. The turnip plants used in this series of experiments were grown in the same way as the radish plants, as explained in connection with Table VII. Three healthy plants were allowed to grow in each pot and these were inoculated by spraying them with the spores of *Cystopus candidus* from the radish. The spores were sprayed on with an atomizer and the plants chilled in the usual manner.

As shown in Table X, ten varieties of turnips *(Brassica rapa)* were inoculated with conidia of *Cystopus candidus* from the radish, variety Ne Plus Ultra. In each variety, at least nine plants were inoculated, while in the case of Snowball, eighteen plants were tested. This would mean that eighteen or more cotyledons were inoculated. These experiments extended from November 13 to January 28, and at no time did any infection result, although in every trial the controls were infected. It was impossible to infect any of the ten varieties of turnips with *Cystopus candidus* from *Raphanus sativus*, variety Ne Plus Ultra. These results suggest that there may be a physiological species of *Cystopus candidus* occurring on radish and turnip respectively.

TABLE X.—RELATIVE SUSCEPTIBILITY OF DIFFERENT VARIETIES *Brassica rapa* TO *Cystopus candidus* FROM *Raphanus sativus*, VARIETY NE PLUS ULTRA.

No. of cult. tested.	Date.	Host Plant.	Source of conidia.	No. pl. inoc.	No. controls inoc.	Period refrigeration.		Results.		
						Hour.	Temp.	Date.	Inf. coty.	Controls.
3	Nov. 30.	Yellow aberdeen	Ne Plus Ultra.	9	5	4	16	Dec. 17..	0	Inf.
3	Nov. 30.	Olds' heavy copper	Ne Plus Ultra.	9	5	4	16	Dec. 17..	0	Inf.
3	Dec. 4..	Golden ball	Ne Plus Ultra.	9	5	5	17	Dec. 22..	0	Inf.
3	Dec. 6..	White flat dutch	Ne Plus Ultra.	9	5	8	11	Dec. 17..	0	Inf.
3	Dec. 28.	Early white milan	Ne Plus Ultra.	9	5	17	9	Jan. 13..	0	Inf.
3	Dec. 28.	Cow horn	Ne Plus Ultra.	9	5	17	9	Jan. 13..	0	Inf.
3	Dec. 28.	Extra early purple top milan.	Ne Plus Ultra.	9	5	17	9	Jan. 13..	0	Inf.
3	Dec. 28.	Purple top white globe	Ne Plus Ultra.	9	5	17	9	Jan. 13..	0	Inf.
3	Dec. 28.	White egg	Ne Plus Ultra.	9	5	17	9	Jan. 13..	0	Inf.
3	Jan. 10.	Snowball	Ne Plus Ultra.	9	5	23	10	Jan. 22..	0	Inf.
3	Jan. 22.	Snowball	Ne Plus Ultra.	9	5	26.5	12	Feb. 15..	0	Inf.
2	Jan. 28.	Early white milan	Ne Plus Ultra.	6	5	26.5	12	Feb. 15..	0	Inf.
1	Jan. 28.	Golden ball	Ne Plus Ultra.	3	5	26.5	12	Feb. 15..	0	Inf.

SUMMARY OF TABLE XI

An attempt was made to infect three different varieties of rutabaga (*Brassica campestris*), namely: Olds' Improved Purple Top, New Necklace, Olds' Large White, with the conidia from the radish, variety Ne Plus Ultra. Three seedlings were grown in two-inch pots in the same manner as described in connection with Table IX. Controls were kept in every experiment and always became infected. Final observations were made in the case of the different varieties of rutabaga several days after the controls had become infected. This was done so as to exclude any possibility of overlooking the disease, which possibly might take longer to develop on the rutabaga.

The age of the seedlings inoculated varied from eleven to twentynine days. The plants had one to five leaves. Both cotyledons and leaves were inoculated. In all, sixteen separate trials were made. In each trial, three plants were inoculated, making a total of fortyeight plants inoculated, and in no case did infection result. These experiments extended from September 25, 1909, to January 28, 1910. Plants of different ages were tested. It is quite evident that *Cystopus candidus* occurring on *Raphanus sativus*, variety Ne Plus Ultra, will not grow on *Brassica campestris*, variety Olds' Improved Purple Top, New Necklace and Olds' Long White.

TABLE XI.—RELATIVE SUSCEPTIBILITY OF DIFFERENT VARIETIES *Brassica campestris* TO *Cystopus candidus* FROM *Raphanus sativus* VARIETY NE PLUS ULTRA.

No. cult. tested.	Date.	Host plant.	Source of conidia.	No. plants inoculated.	No. controls inoculated.	Period of refrigeration.		Results.		Controls.
						Hrs.	Temp.	Date.	No. Coty inf.	
3...	Dec. 4..	Olds' Improved purple top.	Ne Plus Ultra.	9	5	5	17	Dec. 22.	0	Inf.
3...	Jan. 10.	New neckless.....	Ne Plus Ultra.	9	5	24	10	Jan. 22.	0	Inf.
3...	Jan. 10.	Olds' large white.....	Ne Plus Ultra.	9	5	24	10	Jan. 22.	0	Inf.
3...	Jan. 22.	Olds' large white.....	Ne Plus Ultra.	9	5	26.5	12	Feb. 5..	0	Inf.
3...	Jan. 22.	New neckless.....	Ne Plus Ultra.	9	5	24.5	12	Feb. 5..	0	Inf.
1...	Jan. 28.	Olds' large white.....	Ne Plus Ultra.	3	5	20	12	Feb. 15.	0	Inf.
2...	Sept. 23.	Olds' large white.....	Ne Plus Ultra.	6	5	9.5	12	Oct. 8...	0	Inf.
1...	Sept. 25.	Olds' large white.....	Ne Plus Ultra.	3	5	3	9	Oct. 9...	0	Inf.

SUMMARY OF TABLE XII

Experiments were undertaken to determine whether *Cystopus candidus* from the radish could be made to grow on different varieties of cabbage *(Brassica oleracea)*. About twenty plants were grown in each three-inch pot. Fifteen different varieties of cabbage were tested from January 10 to March 13, 1910. Inoculations were made in the usual way by spraying the spores on with an atomizer and placing the pots containing the plants in the ice box under a bell jar on a wet plate. Three tests were made on each variety, so that at least sixty plants of each variety were inoculated. Four of the varieties tested became infected. All Head Early showed two cotyledons infected out of a total of sixty plants tested. The Volga was the most susceptible to *Cystopus candidus*. Nine cotyledons became infected out of a total of sixty. One cotyledon became infected out of sixty plants of Olds' Selected Early. Of the sixty plants of Olds' Ballhead inoculated, two cotyledons were infected. In all the fifteen varieties of cabbage tested, nine hundred plants and of course nearly twice that number of cotyledons were inoculated and only fourteen cotyledons became infected. The results show that it is possible to infect at least four of the fifteen varieties of cabbage tested with *Cystopus candidus* from the radish, yet not to any marked extent.

The fungus had a more marked effect on the cotyledons of the cabbage than on those of the normal host, the radish. The infections found were always on sickly looking cotyledons and in two or three days after the pustules appeared the cotyledons would dry up and drop off. In the radish plants used as controls the cotyledons lived for two or three weeks after becoming infected. This would mean that the fungus was more virulent on the cabbage, and immediately killed the host when it was able to establish itself, or that it attacked only the sickly, weak cotyledons. I am inclined to believe that the fungus attacked all of the cotyledons alike, but the further development of the fungus was overcome by the host in all the cases except in the few infections described above. This is of course a point that needs further investigation before definite information can be had.

To still further determine the susceptibility of different varieties of *Brassica oleracea* to *Cystopus candidus* of the radish, an-

TABLE XII.—RELATIVE SUSCEPTIBILITY OF DIFFERENT VARIETIES OF *Brassica oleracea* TO *Cystopus candidus* FROM *Raphanus sativus*, VARIETY NE PLUS ULTRA.

No. cult. tested.	Date.	Host plant.	Source of conidia.	No. plants inoculated.	No. controls inoculated.	Period of refrigeration.		Results.		Controls.
						Hours.	Temp.	Date.	No. Coty. Inf.	
3	Mar. 25.	All Head Early	Ne Plus Ultra...	60	Stock culture ...	7	16	Apr. 6	2	Inf.
3	Mar. 25.	The Volga	Ne Plus Ultra...	60	Stock culture....	7	16	Apr. 7	3	Inf.
2	Mar. 25.	Sure Head	Ne Plus Ultra...	40	Stock culture....	7	16	Apr. 7	0	Inf.
1	Mar. 25.	Olds' Select Early	Ne Plus Ultra...	20	Stock culture....	7	16	Apr. 10	1	Inf.
2	Mar. 25.	Olds' Select Baldhead	Ne Plus Ultra...	40	Stock culture....	7	16	Apr. 7	2	Inf.
1	Mar. 25.	Extra Early Express	Ne Plus Ultra...	20	Stock culture....	7	16	Apr. 7	0	Inf.
2	Mar. 25.	Olds' Bridgeport Drumhead	Ne Plus Ultra...	40	Stock culture....	7	16	Apr. 7	0	Inf.
3	Mar. 26.	Henderson Succession	Ne Plus Ultra...	60	Stock culture....	6	15	Apr. 10	0	Inf.
2	Mar. 26.	Extra Early Express	Ne Plus Ultra...	60	Stock culture....	6	15	Apr. 10	0	Inf.
1	Mar. 26.	Early Summer	Ne Plus Ultra...	20	Stock culture....	7	15	Apr. 14	0	Inf.
2	Mar. 26.	Olds' Select Early	Ne Plus Ultra ..	40	Stock culture....	6	15	Apr. 10	0	Inf.
1	Mar. 26.	Henderson's Early Summer..	Ne Plus Ultra...	20	Stock culture....	6	15	Apr. 10	0	Inf.
1	Mar. 26.	Olds' Bridgeport Drumhead	Ne Plus Ultra...	20	Stock culture....	6	15	Apr. 10	0	Inf.
1	Mar. 26.	Sure Head	Ne Plus Ultra...	20	Stock culture....	6	15	Apr. 10	0	Inf.
1	Mar. 26.	Olds' Select All Head	Ne Plus Ultra...	20	Stock culture....	6	15	Apr. 10	0	Inf.
2	Mar. 30.	Premium Late Flat Dutch	Ne Plus Ultra...	40	Stock culture....	6	16	Apr. 14	0	Inf.
3	Mar. 30.	Early Spring	Ne Plus Ultra...	60	Stock culture....	6	16	Apr. 14	0	Inf.
1	Mar. 30.	Fottler's Improved Brunswick	Ne Plus Ultra...	20	Stock culture....	6	16	Apr. 14	0	Inf.
1	Mar. 30.	Olds' Bridgeport Drumhead	Ne Plus Ultra...	20	Stock culture....	6	16	Apr. 14	0	Inf.
3	Mar. 30.	Early Summer	Ne Plus Ultra...	60	Stock culture....	6	16	Apr. 14	0	Inf.
3	Jan. 10	Premium Late Flat Dutch	Ne Plus Ultra...	8	5	23	10	Jan. 22	0	Inf.
5	Jan. 28	Premium Late Flat Dutch	Ne Plus Ultra....	15	5	21	12	Feb. 15	0	Inf.
2	Sep. 23	Cabbage	Ne Plus Ultra....	6	5	9.5	9	Feb. 15	0	Inf.
2	Mar. 25	Fottler's Improved Brunswick	Ne Plus Ultra....	40	11	?	16	Feb. 15	0	Inf.
3	Mar. 25	Large Wakefield	Ne Plus Ultra....	60	11	?	16	Feb. 15	0	Inf.
1	May 3	Brussel's Sprouts	Radish	20	20	4	11	May 15	0	Inf.
1	May 3	Extra Early Express	Radish	20	20	4	11	May 15	0	Inf.
1	May 3	Sure Head	Radish	20	20	4	11	May 15	0	Inf.
1	May 3	Fottler's Improved Brunswick	Radish	20	20	4	11	May 15	0	Inf.
1	May 3	Olds' Select Dan. Ballhead	Radish	20	20	4	11	May 15	0	Inf.
1	May 3	All Head Early	Radish	20	20	4	11	May 15	0	Inf.
1	May 3	Fottler's Improved Brunswick	Radish	20	20	4	11	May 15	0	Inf.
1	May 6	Henderson Succession	Radish	20	10	6	10	May 18	0	Inf.
1	May 6	All Seasons	Radish	20	10	6	10	May 18	0	Inf.
1	May 6	Olds' Select Early Jersey Wakefield	Radish	20	10	6	10	May 18	0	Inf.

TABLE XII.Continued.—RELATIVE SUSCEPTIBILITY OF DIFFERENT VARIETIES OF *Brassica oleracea* TO *Cystopus candidus* FROM *Raphanus sativus*, VARIETY NE PLUS ULTRA.

No. cult. tested.	Date.	Host Plant.	Source of conidia.	No. plants inoculated.	No. controls inoculated.	Period of refrigeration.		Results.		Controls Inf.
						Hrs.	Temp.	Date.	No. Coty Inf.	
2	May 6	All Season	Radish	20	10	6	10	May 18	0	Inf.
1	May 6	Olds' Bridgeport Drumhead	Radish	20	10	6	10	May 18	0	Inf.
1	May 6	Old's Select Danish Ballhead	Radish	20	10	6	10	May 18	0	Inf.
1	May 6	Fottler's Improved Brunswick	Radish	20	10	6	10	May 18	0	Inf.
1	May 6	Brussel's Sprouts	Radish	20	10	6	10	May 18	0	Inf.
1	Apr. 30	The Houser (cabbage)	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	The Volga	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Sure Head	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Dan. Summer Ballhead	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Olds' Bridgeport Drumhead	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Olds' Danish Sureheader	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Perfection Drumhead Savoy	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Dan. Summer Ballhead	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Olds' Danish Sureheader	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30	Red Dutch	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Danish Summer Ballhead	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Brussel's Sprouts	Radish	20	20	6	13	May 13	2	Inf.
1	Apr. 30.	Olds' Bridgeport Drumhead	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Olds' Selected Danish Ballhead	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Large Wakefield	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Olds' Danish Sureheader	Radish	20	20	6	13	May 13	0	Inf.
1	Apr. 30.	Large Wakefield	Radish	20	20	6	13	May 13	0	Inf.
1	May 3...	Olds' Select Danish Ballhead	Radish	20	20	4	11	May 13	0	Inf.
1	May 3...	Henderson's Succession	Radish	20	20	4	11	May 13	0	Inf.
1	May 3...	Olds' Select Early Jersey Wakefield	Radish	20	20	4	11	May 13	0	Inf.
1	Aug. 24.	Kohlrabi	Radish	90	100	14	12	Sept. 6	0	Inf.
1	Aug. 24.	Kale	Radish	90	100	14	12	Sept. 6	0	Inf.
1	Apr. 30.	Brussel's Sprouts	Radish	90	20	6	13	May 12	0	Inf.
1	Apr. 30.	Cauliflower	Radish	90	40	6	13	May 12	0	Inf.

other series of experiments was made from April 13 to August 24, 1910. The fifteen varities of cabbage were all tested again as well as Kale, Kohlrabi, Brussel's Sprouts, and Cauliflower as shown in Table XII. From twenty to sixty plants were inoculated and tested as described above. No infections were obtained in this series except on one plant of Brussel's Sprouts. One plant became infected and showed five pustules on its two cotyledons, which again indicates only very slight susceptibility in all the varieties of *Brassica oleracea* tested.

SUMMARY OF TABLE XIII

The mustards, both *Brassica nigra* and *Brassica alba* were next inoculated with spores of *Cystopus candidus* from the radish. The seedlings were grown in three-inch pots in the same way as described for the experiments on the turnips, and inoculated by spraying the spores on with an atomizer. The plants were placed under a bell jar on a wet earthen plate as previously described and placed in the ice box for various periods of time, varying from 3 to 23½ hours.

Six cultures of three plants each were tested. In all, eighteen plants of black mustard were inoculated with *Cystopus candidus* from the radish, variety Ne Plus Ultra. None of the eighteen plants became infected. A variety of white mustard, New White Chinese, was tested eight times. Each time using three plants in the same manner as in the case of the black mustard. Again no infections were secured. Further thirteen tests of three plants each or a total of thirtynine plants of white mustard were inoculated with *Cystopus candidus* from the radish, variety Ne Plus Ultra. In five of the trials infection occurred; three cotyledons and six leaves became infected. The infections secured on the white mustard were not as vigorous as those secured on the controls in the same experiment. The susceptibility of white mustard was further tested in the spring of 1910 on April 11 and May 6. On April 11, three seven-inch pots containing both radish and white mustard growing together, were inoculated when they were nine days old. Infection occurred in seven to eight days. The amount of infection varied from 10 to 40 per cent of the cotyledons. On May 6, eight tests were made in which the seedlings were grown in three-inch pots. The number of plants per pot varied from seven to forty and when the plants

TABLE XIII.—SUSCEPTIBILITY OF *Brassica alba* AND *Brassica nigra* TO *Cystopus candidus* FROM *Raphanus sativus*, VARIETY NE PLUS ULTRA.

No. cult tested.	Date.	Host Plant.	Source of conidia.	No. plants inoculated.	No. controls inoculated.	Period of refrigeration.		Results.		Controls.
						Hours.	Temp.	Date.	Coty inf.	
3	Nov. 23	Black Mustard	Ne Plus Ultra	9	5	4	17	Dec. 4	0	Inf.
1	Nov. 25	Black Mustard	Ne Plus Ultra	3	5	4	17	Dec. 6	0	Inf.
1	Nov. 25	New White Chinese	Ne Plus Ultra	3	5	4	17	Dec. 6	0	Inf.
1	Nov. 25	Black Mustard	Ne Plus Ultra	3	5	4	17	Dec. 6	0	Inf.
1	Nov. 25	New White Chinese	Ne Plus Ultra	3	5	4	17	Dec. 6	0	Inf.
1	Nov. 25	Black Mustard	Ne Plus Ultra	3	5	4	17	Dec. 6	0	Inf.
1	Nov. 25	New White Chinese	Ne Plus Ultra	3	5	4	17	Dec. 6	0	Inf.
3	Dec. 6	Black Mustard	Ne Plus Ultra	9	5	8	11	Dec. 17	0	Inf.
3	Dec. 6	New White Chinese	Ne Plus Ultra	9	5	8	11	Dec. 17	0	Inf.
2	Dec. 28	New White Chinese	Ne Plus Ultra	6	5	17	9	Jan. 13	0	Inf.
1	Sept. 16	White Mustard	Ne Plus Ultra	6	5	5	11	Jan. 13	0	Inf.
4	Sept. 20	White Mustard	Ne Plus Ultra	3	5	5	11	Dec. 5	0	Inf.
1	Sept. 20	White Mustard	Ne Plus Ultra	3	5	4	11	Dec. 5	0	Inf.
1	Sept. 20	White Mustard	Ne Plus Ultra	6	5	3.75	11	Dec. 9	0	Inf.
1	Sept. 25	White Mustard	Ne Plus Ultra	3	5	3	11	Dec. 6	0	Inf.
1	Sept. 29	White Mustard	Ne Plus Ultra	3	5	23.5	10	Dec. 8	2	Inf.
1	Sept. 29	White Mustard	Ne Plus Ultra	3	5	23.5	10	Dec. 10	1	Inf.
1	Sept. 29	White Mustard	Ne Plus Ultra	3	5	22	11	Dec. 10	1	Inf.
1	Sept. 29	White Mustard	Ne Plus Ultra	3	5	22	11	Dec. 10	2	Inf.
1	Sept. 29	White Mustard	Ne Plus Ultra	3	5	22	11	Dec. 10	3	Inf.
1	Nov. 19	White Mustard	Ne Plus Ultra	3	5	5	11	Dec. 10	0	Inf.
1	Nov. 19	White Mustard	Ne Plus Ultra	3	5	3.5	13	Dec. 10	0	Inf.
1	May 6	White Mustard	Radish	40	10	5	10	May 18	10%	Inf.
1	May 6	White Mustard	Radish	40	10	5	10	May 18	10%	Inf.
1	May 6	White Mustard	Radish	7	10	5	10	May 18	40%	Inf.
1	May 6	White Mustard	Radish	18	10	5	10	May 18	20%	Inf.
1	May 6	White Mustard	Radish	40	10	5	10	May 18	25%	Inf.
1	May 6	White Mustard	Radish	20	10	5	10	May 18	50%	Inf.
1	May 6	White Mustard	Radish	30	10	5	10	May 18	10%	Inf.
1	Apr. 11	White Mustard and Radish	Radish	300	10	24	12	Apr. 18	15%	Inf.
1	Apr. 11	White Mustard and Radish	Radish	300	10	24	12	Apr. 18	18%	Inf.
1	Apr. 11	White Mustard and Radish	Radish	300	10	24	12	Apr. 19	25%	Inf.

were eleven days old they were inoculated with conidia from the radish. All of the experiments showed infection on May 18. From 35 to 50 per cent of the total number of cotyledons were infected as shown in Table XIII. Only a small number of leaves became infected due to the fact that not many of the plants had leaves when they were inoculated. The results in these experiments show clearly that the white mustard is susceptible to the cystopus that occurs on the common radish.

SUMMARY OF TABLE XIV

During the spring and summer of 1910, various other hosts of Cystopus were grown from seed in the green house and inoculated as already described. The plants were grown in three inch pots and were vigorous and healthy when inoculated. In every species, at least two separate tests were made. The number of plants in each pot varied from one to twenty. If the plants to be inoculated were large before inoculated, enough were removed to allow the remainder to develop normally. Twenty-six plants of *Capsella Bursa-pastoris,* nineteen plants of *Sisymbrium officinale,* twenty plants of *S. altissimum,* forty plants of *Lepidium sativum,* forty plants of *Nasturtium officinale,* sixty plants of *Brassica nigra,* and thirty plants of *Iberis coronata* were inoculated and no infections were observed. Inoculations on radish were used as controls in every case and infections were always obtained. Further tests must be made with these plants before positive statements can be made as to their susceptibility to the spores from Cystopus from the radish.

It should be noted that a larger percentage of infection was obtained in the spring than in the fall. Thirteen tests were made in the fall of 1909 from October 20 to December 28 in which thirtynine plants were inoculated and only three cotyledons and six leaves became infected. While in the tests in the spring from 10 to 50 per cent of the cotyledons became infected. The difference in extent of infection was possibly due to a difference in the host plants. The white mustard seedlings grown in the fall were not as vigorous as those obtained in the spring.

TABLE XIV.—SUSCEPTIBILITY OF OTHER CRUCIFERS TO *Cystopus candidus* FROM RADISH.

No. cult. tested.	Date.	Host Plant.	Source of Conidia.	No. plants inoculated.	No. controls inoculated.	Period of refrigeration.		Results.		Controls Inf.
						Hours.	Temperature.	Date.	Coty Inf.	
3	June 9.	Capsella Bursa-pastoris	Ne Plus Ultra.	21	20	8	14	June 24.	0	Inf.
3	June 9.	Sisymbrium officinale...	Ne Plus Ultra.	9	20	8	14	June 24.	0	Inf.
3	May 25.	Sisymbrium officinale...	Ne Plus Ultra	10	200	22.5	17	June 8.	0	Inf.
2	Apr. 29.	Lepidium sativum.....	Ne Plus Ultra.	49	12	27.5	9	May 25.	0	Inf.
3	Apr. 29.	Brassica nigra.....	Ne Plus Ultra.	60	12	27.5	9	May 25.	0	Inf.
2	Apr. 29.	Capsella Bursa-pastoris.	Ne Plus Ultra.	5	12	27.5	9	May 25.	0	Inf.
2	May 6.	Nasturium officinale....	Ne Plus Ultra.	40	10	5	10	May 21.	0	Inf.
3	Aug. 24.	Iberis umbellatta.....	Ne Plus Ultra.	30	200	14	12	Sep. 9.	0	Inf.
3	Apr. 30.	Sisymbrium altissimum.	Ne Plus Ultra.	40	20	6	13	May 13.	0	Inf.

DISCUSSION AND CONCLUSION

GERMINATION OF THE CONIDIA

These studies with various species of Cystopus and other Oomycetes have shown that germination of the conidia is controlled by certain factors of which temperature is the most important. Prevost (1807:33) in his studies made over a century ago states that at a temperature of 12° to 16° R. *Cystopus candidus* spores sometimes germinated in 40 to 45 minutes, whereas they ordinarily required from one to two hours. DeBary (1863:14) found the conidia of Cystopus to germinate at temperatures ranging from 5° to 25° C. My own results have led me to conclude that chilling the spores to a relatively low temperature is necessary for the most vigorous germination. In our preliminary series of over one hundred cultures of Cystopus spores at green-house temperature, 22 to° 33° C. during the summer, only a very low percentage of germination occurred. In none of the cultures were zoospores observed and the only indication of germination was the presence of an occasional empty sporange which may possibly have been empty before the spores were brought into the laboratory.

In a later series (Table II) during the summer, including fortyfive cultures chilled with controls at room temperature, it

was found that 85 per cent of the chilled cultures germinated, whereas none of the cultures kept at higher temperatures germinated. In a third series (Table V) of seventythree cultures during the following spring where about one third were held at room temperature (i. e. above 20° C.) scanty germination occurred in 48% of the cultures, whereas 69% of the cultures which were kept at a temperature below 20° C. showed abundant germination. This last series shows that germination may occur at room temperatures and above, as has already been pointed out by DeBary (1863:14), Zalewski (1883:215), Büsgen (1882:22), and Eberhardt (1904:614). But it was also clear that the percentage of germination was much increased by using lower temperatures as was further shown in the behavior of the stock cultures described in connection with Table VIII. The chilled stock cultures became heavily infected while the controls not chilled showed only a low percentage of infection as is conclusively shown by referring to plates I and II.

Two things are clearly indicated from our results. First, and most important; temperature exercises a marked influence upon germination; second, this influence was more marked with spores obtained in the late summer and autumn than with those developed in the spring. We interpret this latter difference as probably due to the greater vigor of host and fungus during the spring months. Be this as it may, it was at all seasons evident that comparatively low temperatures were necessary to induce strong normal germination.

Various media were used for germination trials with spores of Cystopus; such as rain water, distilled water, tap water, extracts of the host, sugar solutions and certain nutrient media. No germination was obtained in any medium except water and no marked difference was noted in the percentage of germination obtained whether it was rain water, distilled water or tap water from Lake Mendota. In all of the subsequent tests this tap water was used. Where spores were immersed in a drop of water, the condition of the surrounding atmosphere, whether saturated or dry, and the amount of evaporation had no effect on the percentage of germination. Other physical changes in the culture containing the spores, such as diffusion of the drop and changes in surface tension, were of no consequence.

No attempt has been made to determine with exactness the optimum temperature for germination. Indeed, the variations

associated with seasonal and host conditions as just noted and with maturity of the spores may preclude perfectly definite conclusions upon this point. It was at least strongly suggested by the large number of experiments made in the laboratory and from the observations made out doors that the optimum for normal spores produced under the best conditions was about 10° C.

Results as to the maximum temperature of germination tend to substantiate DeBary, who found, as previously noted, that the maximum temperature was 25° C. In a series of over one hundred cultures carried on in the greenhouse during the months of July and August of 1909 at temperatures varying from 22° to 33° no germination was obtained. Again in a later series (Table V) in the spring of 1910 scanty germination was secured at 25° C. Although these experiments were not planned especially to test this point, yet they show that the maximum temperature of germination is about 25° C.

DeBary (1860:236) concluded that the minimum temperature for germination was 5° C. My results show that the conidia of Cystopus will germinate at temperatures below 5° C. Twelve cultures were made and laid on blocks of ice in the ice chamber of the ice box and nine of the cultures germinated in 4½ to 27 hours. The temperature of the cultures was between 0° and 10° C. In view of these facts it is quite clear that the minimum is very near zero.

Not only do the conidia germinate at low temperatures in the laboratory but also out of doors. Observations were made on seven different days between 5 and 9 o'clock A. M. in the autumn of 1910, and in every case except one, zoospores were found on the infected leaves of both radish and salsify. The minimum early morning temperatures on the days when observations were made, varied from 5 to 11 2-3° C. as is shown in Table VI. DeBary also records having found the motile zoospores in the morning dew. Since the motile zoospores were thus found in the morning dew by both DeBary and myself, it suggests that the conidia germinate in the coolest part of the day when moisture is at hand. According to Salisbury (1908:556) it is a well established fact that the surface of the earth is the coolest at about sunrise, a condition that leads to the formation of dew and thus moisture and low temperature naturally asso-

ciate themselves in the environment of the fungus and may well have come to have a correlated influence on its development.

Somewhat similar conditions have been reported in the rusts by Jaczewski (1910:21), who found that in the cereal rusts of Russia, both the uredospores and aecidiospores germinated in the morning when the foliage was wet with dew and the temperature was low. The relation of dew to the asparagus rust has been pointed out by Smith (1904:19) in California. He found that the rust spreads most rapidly when heavy dews are prevalent. It should be noted, however, that Smith (1904:19) mentions no temperature factor as especially important.

The relation of light to the germination of the spores of Cystopus was not as marked as it has been reported for Plasmopara and various species of Phytophthora. Farlow (1875:319) concluded from his studies with *Plasmopara viticola* and *Phytophthora infestans* that the conidia germinated better in the dark than in the light. Coleman (1910:59) who has recently studied *Phytophthora omnivora* states that light is a very important stimulus to germination. I have found in Cystopus that the conidia do not germinate in the light at high temperatures and that they do germinate in the light at low temperatures. My conclusion on the first point is based upon seventeen experiments tabulated in Table III, while the latter conclusion is evident from my outdoor observations (Table VI) and also from laboratory studies not tabulated. I have also incidentally tested *Phytophthora infestans* and *Plasmopara viticola* as to the relation of light to germination and have found no such marked difference as has been reported by Farlow (1876:419).

Zalewski (1883:215) concluded that the time of the year had an effect on the time required for the germination of the spores of Cystopus. He found that the time required for germination in the summer was two or three hours, while in the fall it required from one to three days. I have found that during August the average length of the period of refrigeration was 3½ hours; September, 7½; and October, 9 hours. My results show without doubt that the time of the year has a direct influence on the time required for germination.

It may well be due to the different host reaction on the fungus in spring and fall. And again, the different weather conditions of spring and fall, may have a direct influence on

the conidia. The cause of this increase in time required for germination, I do not know, and it cannot be definitely determined, until we are able to absolutely control all of the factors influencing the host and fungus.

A more direct comparison of my results with those of Zalewski would be possible if we knew the method he used in his germination experiments and the number of tests made.

In none of my experiments did the conidia of Cystopus germinate by the production of germ tubes as described by Tulasne (1854:77) and Hoffmann (1859:210). Zoospores were always produced when the conidia germinated. It should be noted that not all of the conidia germinated. Some of the spores were dead or immature.

Eberhardt (1904:614) considers the proper maturing of the spores as the most important factor in securing germination. As noted above, his method was to carefully collect infected leaves with unopened pustules, wrap them in moist cloth and place them in a damp chamber until the pustules were about to burst. With these precautions Eberhardt experienced no trouble in germinating the conidia. I have never found it necessary with the method of chilling described to exercise such precaution. To be sure, it is quite necessary in spore germination to have a goodly supply of ripe spores, but in Cystopus, where the spores are produced acropetalously and borne in a pustule, no such precautions were required in order to secure plenty of ripe spores. Had Eberhardt been studying *Phytophthora infestans* or *Plasmopara viticola,* where the spores are borne on long, much branched conidiophores standing out from the surface of the leaf, such a procedure might have been more important. Conidia from pustules in all stages of development have been used and the conidia readily germinated when chilled. In some of the experiments described above and in many of my preliminary experiments (See page 34) not chilled, conidia from pustules just about to open were used without securing germination. In only five cases does Eberhardt (1904:624) record the data of his experiments. In two of his tests the temperature varied from 2° to 17° C. In another test the spores were placed in water at 6 o'clock in the afternoon and germination was observed the next morning at 7 o'clock. Under ordinary conditions, the room temperature during the night would be lower than in the day

and may well have been between 15° and 20° C., which is sufficient chilling to cause germination.

The two remaining tests were made on June 6 and August 8. No record is made of the temperature conditions. On the basis of such data and the microscopical examination of the conidia from pustules just opened in which he found the conidia swollen and bottlenecked, Eberhardt concludes that germination depends upon the proper maturing of the conidia. In view of the fact that in two of the tests made by Eberhardt, the temperature was much below 17° C., and in another the temperature was that of night time, it seems to me probable that temperature may have been a more important factor in Eberhardt's experiments than he realized.

Since I conclude that temperature is a controlling factor in spore germination of Cystopus, it is worth while to make a comparison with results obtained in the case of other fungi.

In the Myxomycetes, Jahn (1905:489) has found that if the spores are soaked in water for 36 hours and then allowed to dry out, they will germinate in about 30 minutes when again moistened. High temperatures for short periods and then normal temperatures tend to hasten the germination of the spores of Reticularia. More recently Kusano (1909:8) has shown that a weak acid solution is possibly the normal stimulus and that a temperature below 20° C. retards germination. It is at once apparent that in the Myxomycetes thus far investigated, there is no correlation of low temperature and spore germination. In certain of the fleshy Basidiomycetes investigated by Duggar (1901:38) and Miss Ferguson (1902:16) they found that temperature changes had only a slight tendency to increase the percentage of germination. In the rusts or parasitic Basidiomycetes, on the other hand, the relation of temperature to germination is more marked, as is evident from the work of Ericksson and Henning (1896:73) and Jaczewski (1910:21). The former found that fresh accidiospores sown in water at room temperature gave a very low percentage of germination, but when the spores were placed on blocks of ice for a while and then returned to water of room temperature, a much higher percentage of germination was obtained. It should be pointed out in this connection, however, that the above investigators made too few experiments to draw definite conclusions. Their results are fur-

thermore misleading in that such extreme temperatures were used. The germinations obtained by Ericksson and Henning were possibly not due to the temperature of melting ice, but rather to the slightly higher temperatures obtained after the slides were removed from the blocks of melting ice. In similar experiments performed with the spores of Cystopus this has been found to be the case. The observations of Jaczewski (1910:21) further substantiate my conclusions. He found that the aecidiospores of *Puccinia graminis* germinated in the morning dew outdoors and at temperatures considerably below normal in the laboratory. The uredospores were found to germinate best also at temperatures slightly below normal (18° C.).

It is evident from the above facts that the spores of the saprophytic Basidiomycetes and parasitic Basidiomycetes respond differently to temperature at the time of germination and we would naturally expect that forms so different in habit and environmental relations would respond differently. In the later cases, it is purely an adaptation to environmental conditions in much the same way as I have found it to be in the Oomycetes, although the relation is less marked in the rusts than it has been found to be in Cystopus and various other Oomycetes. In the Ascomycetes there has been no correlation of low temperature and spore germination revealed up to the present time but too little is known of the factors influencing germination in this group to draw any conclusions. In the Fungi Imperfecti, on the other hand, also little studied as to the factors influencing spore germination, it has been noted in one instance that low temperature has a direct relation to spore germination. It is very important that parasitic fungi belonging to the two above mentioned groups should be investigated as to temperature relations because of their direct bearing on remedial measures.

In order to inoculate various hosts with Cystopus, Eberhardt (1904:625) germinated the spores as described above and placed the water containing the zoospores on the cotyledons of the plants to be infected. In some cases the plants were immersed in water containing zoospores. The method of inoculating plants with the conidia of Cystopus that has been used in my experiments was based on the relation of chilling to germination of the conidia. The spores were placed in water and sprayed on the plants

with an atomizer, then the plants were covered with a bell jar and placed in the ice box long enough to insure germination. The method I have used is more nearly that which occurs in the normal environment of the fungus than that used by Eberhardt.

No difference in the susceptibility of the cotyledons and leaves has been noted in any of my infection experiments, although DeBary (1863:24) concluded from his experiments that in Capsella and Lepidium the cotyledons only were susceptible to infection and that in various species of Brassica, both cotyledons and leaves were susceptible but usually only the cotyedons. Still further tests were made as to the susceptibility of the leaves of the above hosts. Twentyfour radish plants were used, two in each of twelve pots, which had been grown in the greenhouse and had at no time shown any infection. After these had lost their cotyledons they were inoculated. Thirteen days later twenty of the plants, i. e., all but four, showed leaf infection at many points. A pot culture of at least fifty white mustard plants having lost their cotyledons and at no previous time showing infection were inoculated and every plant showed infection on at least several of its leaves. Five plants of Capsella in blossom were inoculated and four of the plants became infected, developing large white pustules on both the stems and young fruits. We have never tested *Lepidium sativum* with Cystopus from the same host but have succeeded many times in infecting the leaves with Cystopus from Capsella. The same care was exercised in growing the two named hosts free from infection as was noted for radish and white mustard. The details of these experiments are given in an earlier part of this paper. There can be no doubt of the susceptibility of the leaves of the various hosts described. I have noted, however, that the leaves of radish plants about to blossom or in blossom seldom become infected and when infection does occur a marked hypertrophy results. This was evident from stock culture J, which may be taken as typical of the nine described in connection with Table VII. It was started September 29, 1909, and became infected October 6. The pustules until March, 1910. Then the stronger of these plants sent up flowering stalks, bearing scattered leaves and blosplants sent up owering stalks, bearing scattered leaves and blossoms. From this time on the fungus development on the basal leaves started to disappear and the upper leaves remained practically free from infection, not only in culture J, but also in the

other eight cultures listed in Table VII. This same condition has been repeatedly observed on plants growing outdoors, and I believe that the leaves of the radish plants are not less susceptible at the time flowers are developing than earlier, but that the decrease in extent of infection is due to less moisture being deposited on the upper leaves. The flowers and young fruits, on the other hand, may become the seat of systemic infection at this stage of the host plant, in which case oospores are produced.

With the method of infection well established my attention was directed to determining the relative susceptibility of the different varieties of radish. Twentytwo varieties were found to be susceptible with no marked degree of variation. Another species, *Raphanus caudatus* (rat-tail radish), was tested to determine whether different species in the same genus were susceptible to the conidia from *Raphanus sativus* (common radish). It was found that *Raphanus caudatus* was readily infected. Further tests were made with the conidia of *Cystopus candidus* from radish on species of other genera; *Brassica rapa* (turnip), *B. campestris* (rutabaga), *B. napus* (rape), *B. nigra* (black mustard), *B. oleracea* (varieties: cauliflower, kohlrabi, and kale), *Capsella Bursa-pastoris* (shephard's purse), *Lepidium sativum* (garden cress), *L. virginicum* (wild pepper grass), *Sisymbrium officinale* (hedge mustard), *S. altissimum* and *Iberis umbellata* (candy-tuft). In none of the above cases did infection occur. Infection was secured on both cotyledons and leaves of *Brassica alba* (white mustard) and on the cotyledons of *Brassica oleracea* (four varieties of cabbage). My results show that it is possible to inoculate several other crucifers with the spores of Cystopus obtained from the radish, which tends to preclude the possibility of so called physiological species in accordance with Eberhardt's conclusions; yet it may well be that limited specialization exists when further cross inoculations with the spores from other hosts have been made. Eberhardt has already raised the question as to the existence of a biological form on each of the groups: Lepidium—Capsella—Arabis and Brassica—Sinapis Diplotaxis. My results show further that the spores of Cystopus on the various species of Raphanus are quite limited but it may be that *Brassica alba* serves as the bridging species. These are questions that can be fully determined only by a large

number of cross inoculations with the spores from various hosts of Cystopus.

As has been pointed out, the infections that were secured on *Brassica alba* and *B. oleracea* with the conidia from radish differed in appearance from those usually occurring on the radish. The infection on the radish is vigorous, causing marked hypertrophy and developing large, white, plump pustules. On the white mustard and cabbage this was not the case. No hypertrophy occurred and the pustules were small, showing none of the signs of vigor evident on the radish. Not only was there a marked difference in the appearance of the fungus pustules on the hosts in question but also in the effect upon them. The fungus killed the host tissues very much faster on the white mustard and cabbage than on the radish. A possible explanation of these results would be that the infection of the white mustard and cabbage occurs only in the most vigorous cotyledons; that in these the fungus is able to overcome the host cells and persist in only a few cases and that in such, the host cells when overcome die immediately.

In my observations, plants infected with aphids or thrips seem to be quite immune to Cystopus. At no time was I able to get infection on a plant that was badly infected with insects. Reed (1907:381) also found that it was quite impossible to infect grain seedlings with mildew that were already infected with thrips. This was more evident in the case of wild plants such as Capsella, Lepidium and Sisymbrium than in the case of such cultivated plants as radishes and mustards. The lack of infection can not be attributed to the aphids eating the spores, since some of the plants were fumigated, killing the insects and then inoculated, with similar results. These facts lend support to Cook's (1911:624) view that plants injured by plant or animal parasites develop an excess of tanin which causes more or less immunity. Not only was it quite impossible to infect plants attacked by insects, but likewise, plants that showed signs of not being vigorous and healthy from other causes. It was also impossible to infect wild and cultivated seedlings that showed yellowing of the cotyledons and first true leaves. This was also true of the more mature plants. If for any reason a stock culture of radishes showed signs of not being healthy and vigorous the extent of infection was at once reduced. As has been

stated earlier, Eberhardt believed that the various hosts of Cystopus do not at all stages of development show the same susceptibility for the fungus. Nowhere does Eberhardt have any data to substantiate this conclusion, nor has he taken into consideration host abnormalities as a factor influencing the question of susceptibility. From my results it is at least very evident that Cystopus reacts differently to healthy and sickly plants respectively.

It is impossible to infect *Capsella Bursa-pastoris, Lepidium virginicum,* or *Sisymbrium officinale* when the plants are not vigorous and healthy. Many attempts were made during the fall of 1909 to infect *Sisymbrium officinale* with *Cystopus candidus* from the same host but the infections were very scanty. Out of the fourteen experiments on fiftysix plants, only eight plants became infected. I attribute this to the weakness of the plants that were grown at that time. In every case, it was the largest and healthiest looking plants in the lot which took the disease. Although I have not succeeded in proving entirely to my own satisfaction that the extent of infection is dependent upon the vitality of the host; yet it seems highly probable that this is the case. Reed (1907:381) has fully described a similar relation between the host and fungus in the grain mildews. Since there is this evidence in both the mildews and white rusts that sickly hosts do not readily become infected, in testing a species for socalled physiological species, all possible care should be exercised in cases where plants are used as hosts that are at all difficult to grow. Failure to infect may be due to weakness of the host plant.

SUMMARY

The studies outlined in the preceding pages were carried on chiefly with *Cystopus candidus* as it occurs on the common radish, *Raphanus sativus*. The leading problems considered are: Conditions influencing germination of the conidia; conditions influencing infection; and, the occurrence of so-called physiological species of *Cystopus candidus* on the various crucifers.

GERMINATION OF CONIDIA

When the conidia are placed in water they germinate better at strikingly low than at high temperatures. The optimum was not definitely determined, but the results tend to show that it was 10° C. The minimum temperature of germination was very near zero, while the maximum was, as DeBary has shown, about 25° C.

It was found that water is the most favorable medium for germination. No germination was obtained on various nutritive culture media.

The time required from the immersion of the conidia to the escape of the zoospores usually varied from two to ten hours. The shortest period in which such germination was observed was 45 minutes.

Environmental factors, season and host vitality, seemed to influence the time required for the spores to germinate. It was strongly suggested that the time required in spring and summer is shorter than in the late fall and winter.

No difference was observed in the time or percentage of germination which occurred in light as compared with darkness.

Spores obtained from leaves after a killing frost germinated.

Such factors as evaporation, surface tension, and diffusion of the drop containing the conidia did not influence the percentage of germination. The conidia germinated as readily in a non-saturated as in a saturated atmosphere.

CONDITIONS OF INFECTION

Chilling was also found to have a very marked effect on the degree of infection secured, as can be seen by referring to plates I to X. Ninetyfive per cent of the seedlings chilled became in-

fected while the controls not chilled usually showed less than 5 per cent of infection and never more than 15 per cent. This difference in extent of infection, I believe was due to the increased percentage of spore germination. It should be noted, however, that the chilling process may have had some effect on the host, possibly making it more susceptible. This is a point that needs further investigation.

The favorable effect of chilling on the conidia of Cystopus is plainly an adaptation to the environment of the fungus. The spread of a fungus by zoospore infection is directly dependent upon the presence of water on the foliage of the host. DeBary found the motile zoospores of Cystopus in the dew drops in the morning on the host plant and I have often made the same observation. The fall in temperature which leads to the deposition of dew and thus provides a medium in which the zoospores may develop serves at the same time as the necessary stimulus to the germination of the conidia.

The results obtained suggest that a close relation exists between host vigor and susceptibility in that healthy plants are more susceptible than sickly or abnormal ones.

No marked difference in the susceptibility of leaves and cotyledons of the radish, shepherd's purse, white mustard and garden cress was observed.

SO-CALLED PHYSIOLOGICAL SPECIES

Repeated infection experiments were made using conidia of *Cystopus candidus* from the common radish, *Raphanus sativus*, upon this same and other cruciferous hosts to learn whether there is any difference in susceptibility.

A large number of experiments were made testing the susceptibility of twentytwo different varieties of radish, and it was found that no marked difference in their susceptibility existed. It was also readily possible to infect *Raphanus candatus* with the conidia from *Raphanus sativus* which shows that species of the same genus are susceptible to the form of Cystopus that occurs on the common radish.

Species of crucifers from other genera known to be hosts of the white rust were investigated as to their susceptibility to *Cystopus candidus* from the common radish. Infection was secured on the white mustard, *Brassica alba*, and cabbage, *Bras-*

sica oleracea. At no time was it possible to infect more than 50 per cent of the cotyledons or leaves of white mustard which were inoculated. With the cabbage, it was even more difficult to secure infection, although fifteen varieties were tested. Less than 1 per cent of the plants inoculated became infected.

No infection could be secured on any of the other crucifers tested. These included turnip, *Brassica rapa*, ten varieties; black mustard, *B. nigra*, rutabaga, *B. campestris*, three varieties; shepherd's purse, *Capsella Bursa-pastoris;* garden cress, *Lepidium sativum;* wild pepper grass, *Lepidium virginicum;* hedge mustard, two species *Sisymbrium officinale* and *S. altissimum;* candy tuft, *Iberis umbellata;* water cress, *Nasturtium officinale*, and wall flower, *Cheiranthus cheiri.*

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DESCRIPTION OF PLATES

The following plates are all from photographs of radish plants grown in six inch pots, taken from above. In some cases the entire culture is shown with a slight reduction. In the rest only a portion of the culture is shown, but so selected as to be fairly representative. The plates illustrate the advantage of chilling in securing optimum spore germination and favorable conditions for infection with *Cystopus candidus*. The seedlings were inoculated, covered with bell jars and either placed in an ice box or kept at room temperature.

I. Radish. Var.—Ne Plus Ultra. Sowed May 16. Inoculated May 26. Infected June 2. Photographed June 6. This culture was chilled.

II. Control. Radish. Var.—Ne Plus Ultra. Sowed May 16. Inoculated May 26. Infected June 2. Photographed June 6. This culture was not chilled.

III. Radish. Var.—Ne Plus Ultra. Sowed Nov. 1. Inoculated Nov. 8. Infected Nov. 15. Photographed Nov. 17. This culture was chilled.

IV. Control. Radish. Var.—Ne Plus Ultra. Sowed Nov. 1. Inoculated Nov. 8. Infected Nov. 15. Photographed Nov. 17. This culture was not chilled.

V. Same culture as shown in III. Photographed twelve days later, Nov. 29.

VI. Same culture as shown in IV. Photographed twelve days later, Nov. 29.

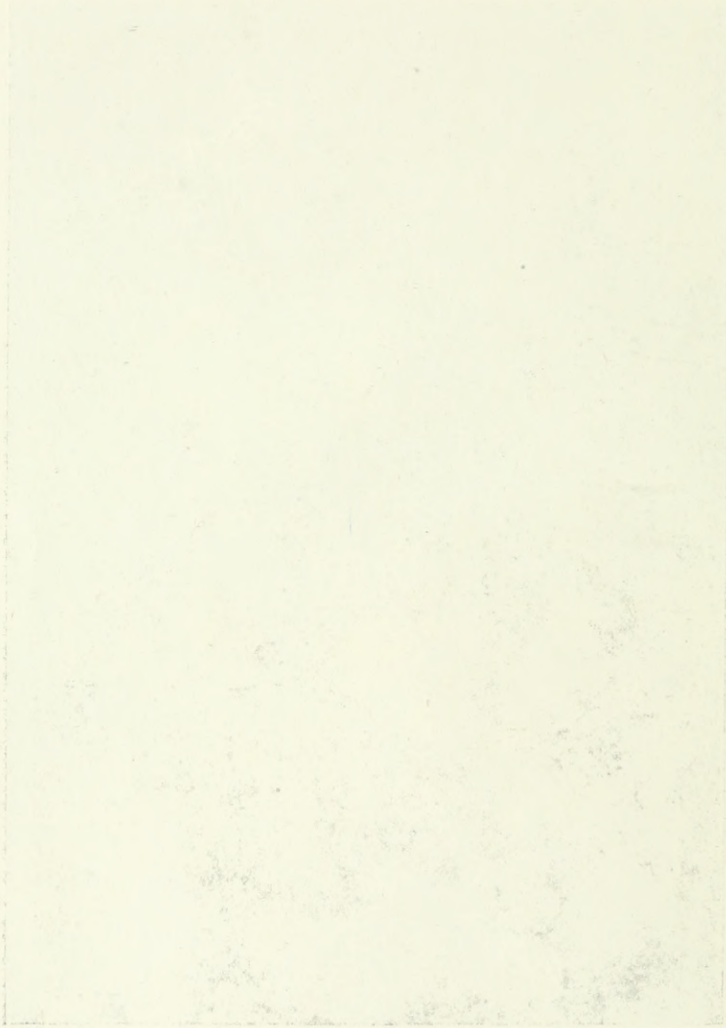
VII. Radish. Var.—Ne Plus Ultra. Sowed Nov. 6. Inoculated Dec. 3. Infected Dec. 12. Photographed Jan. 1, 1910. Cultures at right of page chilled; at left of page controls, not chilled.

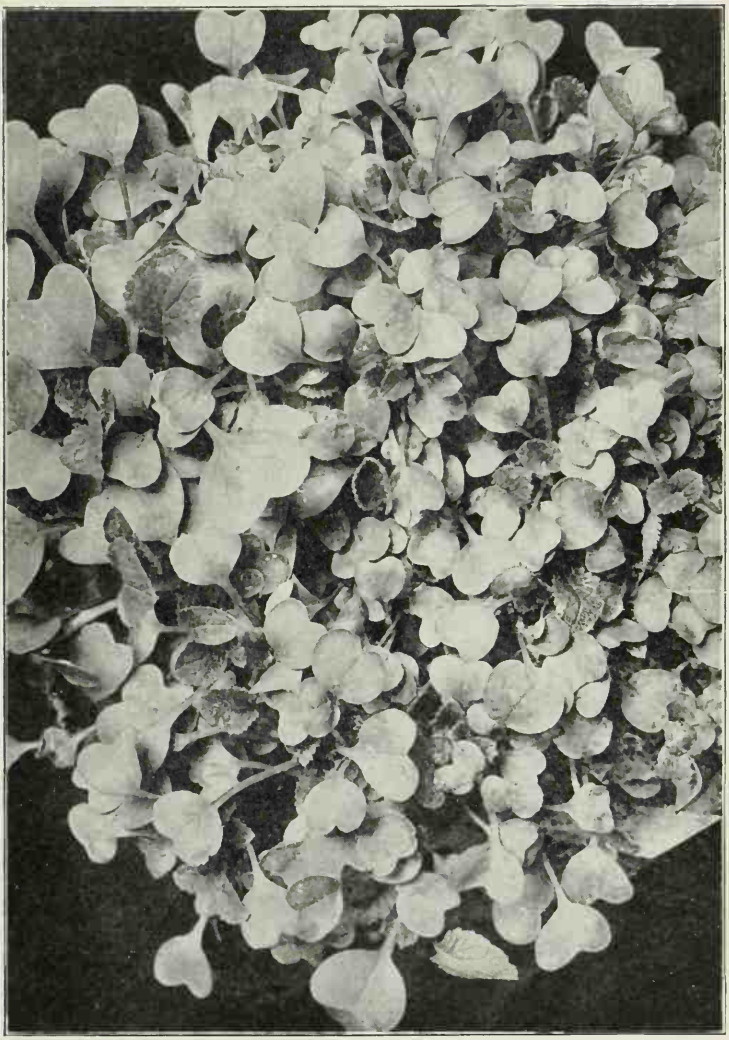
VIII. Radish. Var.—Triumph. Sowed Dec. 12, 1909. Inoculated Jan. 3, '10. Infected Jan. 12, 1910. Photographed Jan. 18, 1910. Upper culture chilled. Lower culture control, not chilled.

IX. Radish. Var.—Ne Plus Ultra. Sowed May 26, 1910. Inoculated June 6. Inf. June 12. Photographed June 15. This culture was chilled.

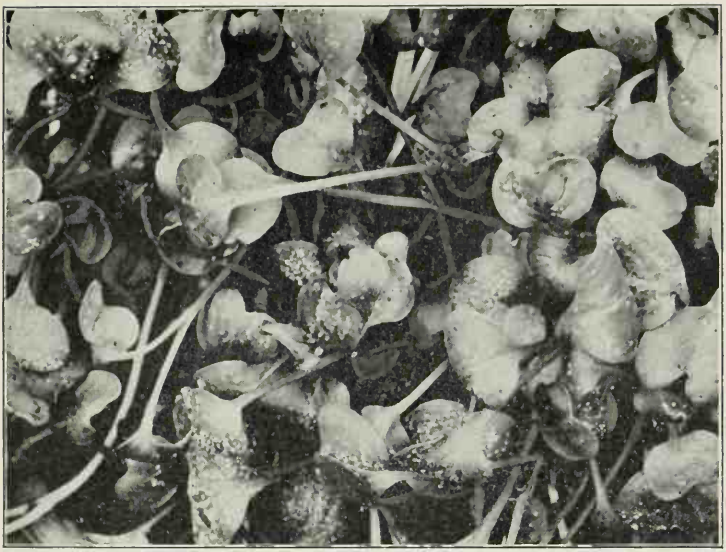
X. Control-Radish. Var.—Ne Plus Ultra. Sowed May 26, 1910. Inoculated June 6. Inf. June 14. Photographed June 15. This culture was not chilled.

I.—Radish seedlings inoculated with Cystopus, chilled. (Compare with II.)

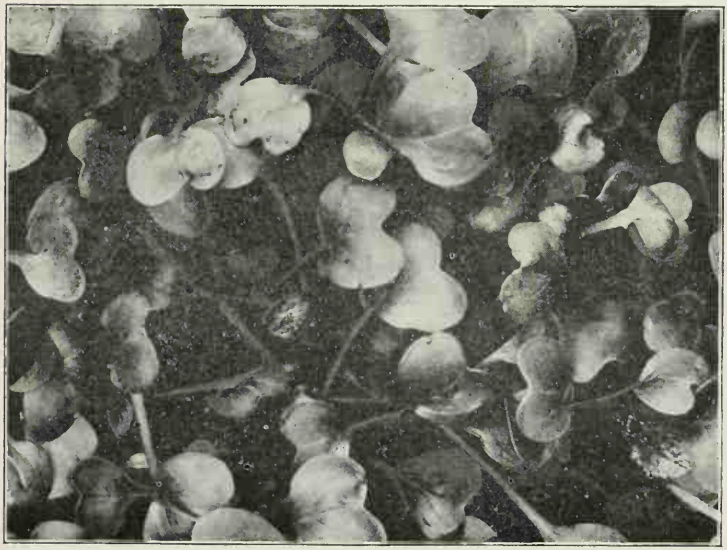




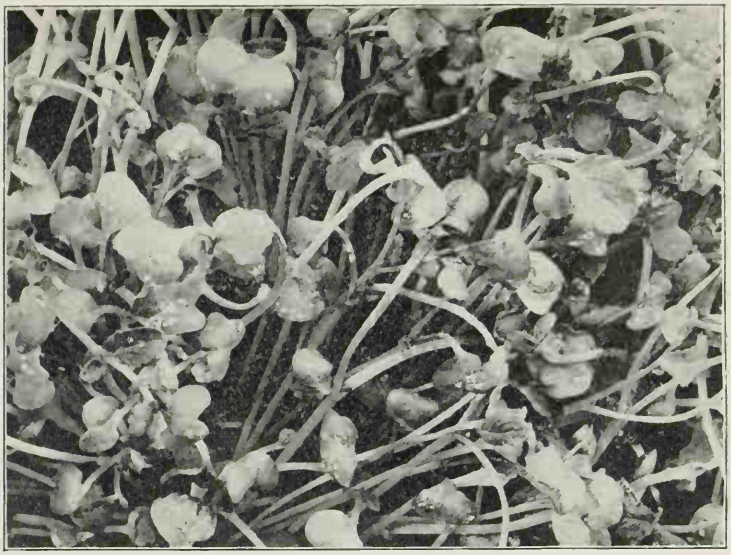
II.—Radish seedlings inoculated with Cystopus, not chilled. (Compare with I.)



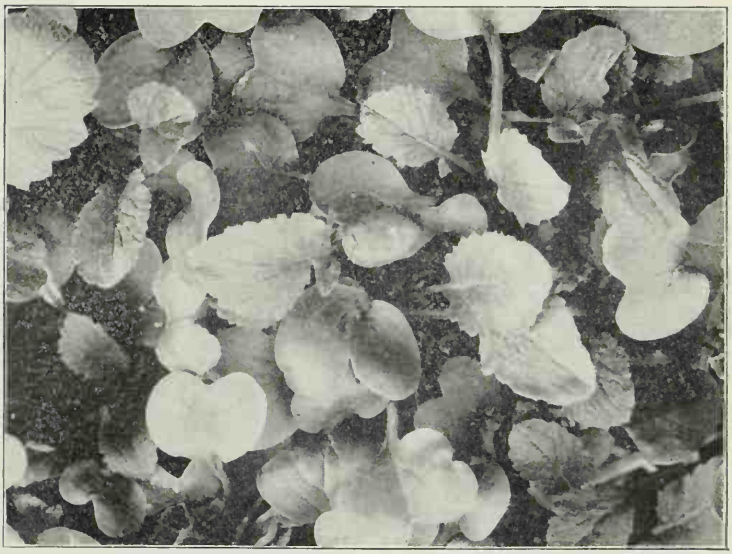
III.—Radish seedlings inoculated with Cystopus; chilled.



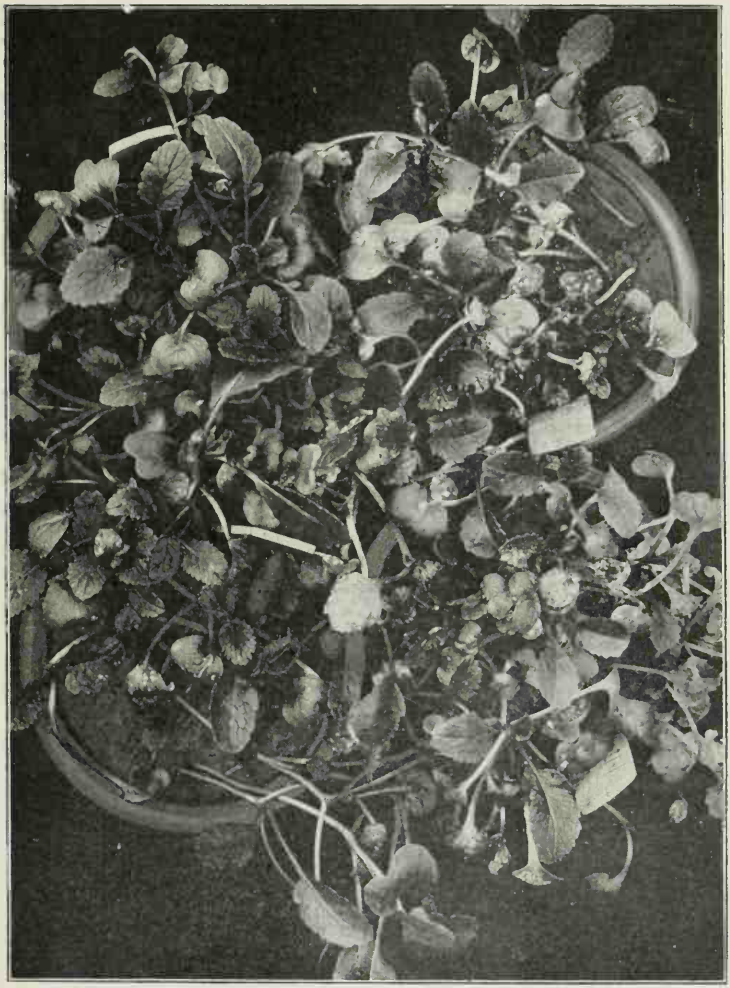
IV.—Radish seedlings inoculated with Cystopus; control not chilled.



V.—Radish seedlings inoculated with Cystopus; chilled.

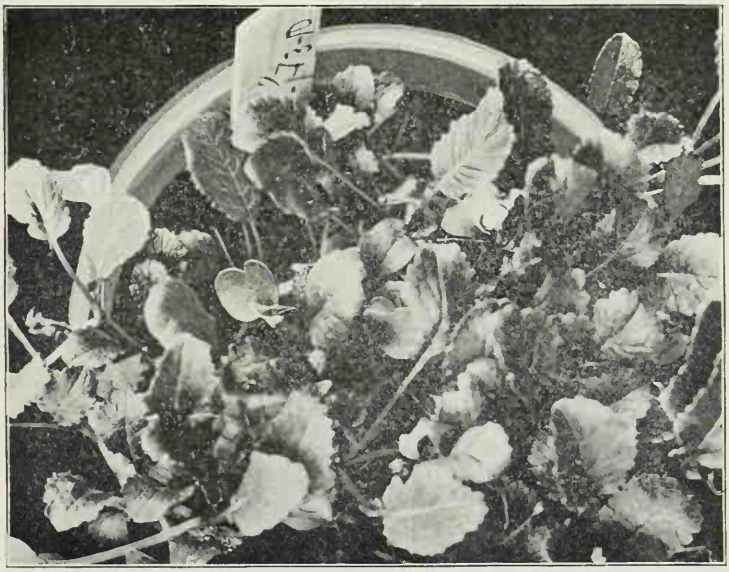


VI.—Radish seedlings inoculated with Cystopus; control not chilled.

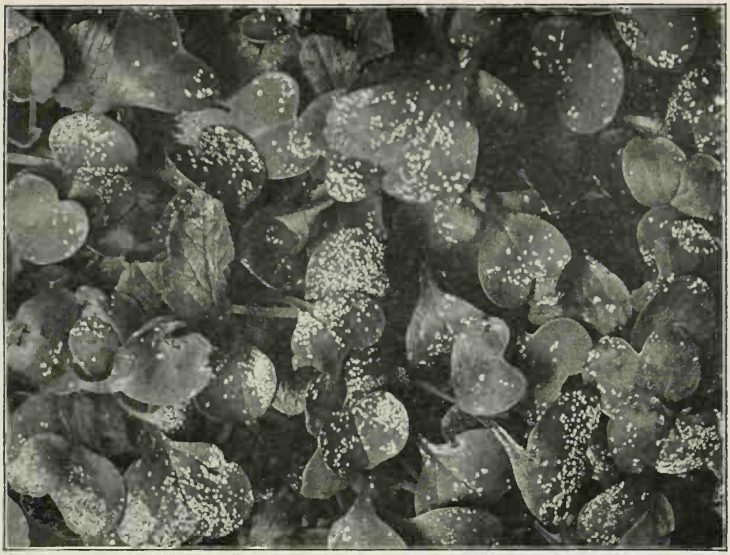


VII.—Four small cultures of radish seedlings; two at right of page, chilled; two, controls, at left of page, not chilled.

Larger radish plants inoculated with Cystopus; chilled.



VIII.—Large radish plants inoculated with Cystopus; control not chilled.



IX.—Radish seedlings inoculated with Cystopus; chilled.

X.—Radish seedlings inoculated with Cystopus; control not chilled.

A Sclerotium Disease of Blue Joint and Other Grasses[1]

A. B. STOUT

Introduction

During the summer of 1907 the writer observed a fungus which was appearing in the marsh meadows about Madison, Wis., as a parasite on the leaves of the grass commonly known as blue joint (*Calamagrostis canadensis*). The principal symptoms of this disease are as follows. The portions of the leaves attacked soon lose their green color and become dry and rigid. Often an entire culm is killed. When a large number of culms within a small area are infected, the general appearance of the dead and whitened leaves is somewhat similar to that often produced on young grain plants by a frost. A cursory examination, however, showed that there was present on the dying leaves a delicate gray felt of mycelium from which sclerotia often developed. When mature these sclerotia project into the air as small but conspicuous bead-like bodies.

These symptoms clearly indicated that this fungus was the cause of the death of the leaves and culms of the grass in question and the severity of the attack made it a matter of considerable economic importance inasmuch as blue joint is the most valuable of the wild hay grasses of Wisconsin.

Davis (1893, p. 183) has briefly described a fungus which produces sclerotia on *Calamagrostis canadensis* and which he found at various points in Wisconsin. He did not identify

[1] The investigations here reported were carried on in part under the guidance of Prof. R. A. Harper of the department of Botany of the University of Wisconsin. When Prof. L. R. Jones assumed the chair of Plant Pathology at Wisconsin in February 1910, the work was continued as a special pathological problem under his immediate direction. From each of these the writer has received helpful criticism and suggestions.

the fungus but stated that the sclerotia resemble those which he had found on Silphium, Helianthus, etc.

It was found at once that the fungus agrees with European specimens of *Sclerotium rhizodes* Auersw. growing on *Phalaris arundinacea*, and on *Calamagrostis arundinacea*. (Sydow, Mycotheca Germanica Nos. 298 and 299.) Later an examination of the exsiccati in the Ellis collection now in the herbarium of the New York Botanical Garden showed that the fungus *Sclerotium rhizodes* has been distributed as follows:

De Thümen, Fungi Austriaci, No. 1096, on *Poa pratensis*, 1873.

De Thümen, Mycotheca Universalis, No. 199 on *Poa pratensis*, 1873.

Eriksson, Fungi Parasitici Scandinavici, No. 82, on *Poa pratensis* 1882.

Rabenhorst-Winter, Fungi Europaei, No. 3,199, on *Phalaris arundinacea*, 1884.

Sydow, Mycotheca Marchica, No. 1339, on Poa leaves, 1887; No. 2698, on *Calamagrostis neglecta*, 1889; No. 2996, on *Digraphis arundinacea*, 1890; No. 3297, on *Poa trivialis*, 1891; No. 3298, on *Anthoxanthum odoratum*, 1891.

Krieger, Fungi Saxonici, No. 550, on *Phalaris arundinacea*, 1889; No. 600, on *Agrostis*, 1890; No. 1397, on *Brachypodium silvaticum*, 1896; No. 1398, on *Calamagrostis Halleriana*, 1898; No. 1399, on (a) *Holcus lanatus*, 1891; on (b) *Holcus mollis*, 1897.

Allescher and Schnabl, Fungi Bavarici, No. 200, on *Phalaris arundinacea*, 1891.

The examinations of these specimens established beyond question the identity of the Wisconsin fungus on *Calamagrostis canadensis* with the European species, and the writer has supplied specimens so named for distribution in Fungi Columbiani.

The first publication and description of this fungus was under No. 1232 in Klotzsch's Herbarium Vivum Mycologicum, a review of which appeared in the Botanische Zeitung Vol. 7, p. 294, 1849. The description reads as follows: "1232 *Sclerotium (Sarcidium) rhizodes* Awd. Mspt. Subglobosum, primum albovillosum, mox glabriusculum, nigrescens rugulosum, fibrillis albis seriatim insidens. Auf Blättern von *Calamagrostis Epigios* schon vor deren Entwicklung."

Frank (1881, p. 545, and 1896, vol. 2, p. 511) quite adequately describes the symptoms of this sclerotial disease of grass leaves. He records that the disease appeared as an epidemic in 1879 in the vicinity of Leipzig on *Phalaris arundinacea* and *Dactylis glomerata* and that a large part of one meadow appeared dry and white as a result of the attack of the fungus. He states that no conidia from the mycelium or fruiting bodies from the sclerotia had been observed.

Soraner (1886, p. 300) quotes briefly from Frank's description of 1881 but adds no new data.

Saccardo (1899, p. 1154) lists *Sclerotium rhizodes* with the fungi having sterile mycelia. He repeats the brief description quoted above. Otherwise he makes no mention of the occurrence of the fungus as given in the various exsiccati listed above.

Tubeuf (1897, p. 266) records this fungus with sclerotia of unknown affinity and mentions, only, that it occurs on living plants of *Phalaris arundinacea*, and Calamagrostis; also on dead leaves of *Dactylis glomerata*.

This summary of the literature pertaining to *Sclerotium rhizodes* indicates clearly the meagre and incomplete knowledge concerning its life history and its relations to its host plants.

In the region about Madison, Wis., the fungus is most abundant on *Calamagrostis canadensis*, although as explained later it occurs on various other species of grass. My studies have been made chiefly with material from this one host and all the statements which follow are to be so understood unless otherwise clearly specified.

Detailed Description of Symptoms

When the leaves of infected plants start to unfold, their tips remain more or less convolutely rolled as in the bud and soon become white, dead, dry, and rigid. The whitened tips, over badly infected areas, are conspicuous in mass effect and at first sight, as already stated, resemble frost injury. Examination, however, reveals the presence of a thin but dense felt of mycelium which is most marked on the inner surface of the infected leaves and along the inner edge of the leaf, especially when only a part of the leaf is rolled laterally. (See Figures

1 and 3, points marked *m*.) In the outermost leaves, the tip is usually the only portion affected and often only a lateral half of the blade is invaded. As the portion of the leaf attacked dies and fails to unroll, the tip of the leaf next in order is often caught and firmly held in the roll and it in turn holds in the same manner the leaf next in order. Meanwhile the growth of the basal portion of the leaves together with the elongation of the internodes tends to separate the lower halves of the leaves whose tips are thus held together and to produce peculiar and characteristic crooks as shown in Figures 1, 2 and 3. When the fungus develops vigorously, the inner leaves are completely penetrated by the mycelium which also extends into the culm below the growing point. In this case the death of the terminal bud results. During a season of vigorous development the majority of the infected culms never grow to be more than twelve inches high, while large numbers are less than six inches high. Entire groups of culms arising from a rhizome are often thin, puny, and dwarfed.

From these conditions it is evident that the mycelium first becomes virulent within the bud whence it spreads through leaf after leaf and that the individual leaves are thus infected before they unroll from the bud. The mycelium becomes most conspicuous on a leaf in the lowest part of its region of growth. The unaffected basal part of the blade becomes flattened out and just below the point where the next leaf in order emerges from the roll an area is usually covered with a felt of white mycelium.

In case of partial lateral infection a narrow zone with tufts and knots of mycelium appears along the margin of the fold or roll where the dead leaf tissue meets the green tissue. Soon rounded bead-like sclerotia are produced along the infected portions of the leaf or from the felt of mycelium at the base. (See Figure 1 points marked *s*). When mature, they vary in size from 1 to 5 mm. in diameter (See Figure 3). The sclerotia are formed on the leaves and are always superficial. They are seldom formed within the roll and never within the tissues.

The production of the conspicuous crooks and the development of the sclerotia as described, are features which make certain the identification of this fungus as it appears on *Calamagrostis canadensis*. The writer has found this fungus appearing in the region about Madison on the following addi-

tional grasses: *Phalaris arundinacea* L.; *Calamagrostis neglecta* (Ehrh.) Gaertn; *Poa pratensis* L.; *Panicularia nervata* (Willd.) Kuntze; *Phleum pratense* L.; *Hordeum jubatum* L.; *Bromus ciliatus* L.; *Eatonia Pennsylvanica* (DC.) A. Gray; *Agropyron caninum* (L.) R. & S.; *Agrostis hyemalis* (Walt.) B. S. P. All of these except the first three named are here reported as hosts for the first time. The occurrence of the fungus upon several European grass hosts not here mentioned has already been noted. In general the appearance of the fungus on all of these hosts is similar to that described for *Calamagrostis canadensis*. In each case the sclerotia produced are identical and infected plants of each species have been found growing by the side of infected plants of *Calamagrostis canadensis*.

It should be noted that in the case of perennial grasses a bud which grows upward into the air produces leaves only, for one or more years and then terminates its life as an individual culm by producing flowers and seed. There is considerable difference in the habit of growth of these leaf and fruiting culms in the different species that serve as host plants. In *Poa pratensis* and *Panicularia nervata* the vegetative culms, as I shall designate the culms bearing leaves only, are short and the leaves which they bear arise rather close to the ground. Here the infected leaves are not lifted up to the general level of the vegetation and although there may be many infected leaf culms, the general effect is not so conspicuous as it is in the case of *Calamagrostis canadensis*. The leaves of these two species are normally conduplicate in the bud and the infected leaves remain thus folded. Sclerotia are produced between the folds on the upper surface and are always superficial. Along the groove of the fold, a thin felt of mycelia develops. On *Poa pratensis* especially, the distribution of the fungus is rather irregular and not continuous in a single leaf.

During the seasons of 1907, 1908 and 1909, general observations were made in the marsh meadows about Madison as to the abundance and the course of development of the fungus. In 1910 and 1911 however, a single marsh meadow conveniently situated for observation was selected for a more intensive study of these problems. This meadow is almost circular in shape with a diameter of nearly half a mile. Although its elevation is but a few feet above the level of Lake Monona, which is near by and into which it is drained, it is usually sufficiently dry to

be cut over for hay. The statistical analysis which the writer made of the plant population of this marsh meadow showed that *Calamagrostis canadensis* was quite generally distributed over the marsh and that it constituted 18 per cent of the entire plant population. The counts were made rather late in a season in which the fungus was not especially vigorous except in certain areas through which the transect studied did not pass. The fungus was noticed at nearly all points, but no separate count was made of the infected culms.

Seasonal Development in the Field The development of the fungus was further studied in the field in the spring of 1910. It was an early spring. On March 24, the first grass buds were beginning to unfold and many of these showed the typical effects of the fungus. Sclerotia which had been formed during the previous year were found in numbers on the ground but none of them showed any signs of producing fructifications.

On April 15, the grass stood from four to six inches high. In certain areas where infection was most general in previous years, the fungus was especially abundant. In some areas of several square rods extent, it appeared that 75 per cent of the culms then unfolding their buds were infected.

On April 30, the grass averaged one foot high. The dead tips gave to the regions of worst infection a conspicuous whitened appearance as if the tips had been burned or frozen. Many of the infected culms were totally dead. Young sclerotia were forming in considerable number.

Throughout May and June there was no apparent increase in the number of culms showing the infection. The majority of culms already diseased had died to the ground. Others continued to grow and put forth new leaves which in turn showed the presence of the fungus in varying degrees of vigor. The season was one of unusual dryness and, as a result, few sclerotia were produced. By July 14 there were few areas where the fungus appeared abundantly on the growing culms. In areas of previous slight infection, healthy culms now stood about two feet tall overtopping the dead culms killed earlier in the season. Still during the remainder of the season, culms bearing infected leaves could always be found in considerable number. The grass itself made little growth after the middle of July and during the first week in August it was cut for hay.

On September 2, the new growth was nearly one foot high and in this, infected culms were scarce and the characteristic crooks were not well developed.

Observations in 1911 The spring of 1911 was nearly three weeks later than that of 1910. The fungus appeared as in previous years on the first leaves that opened and the subsequent development was as described above. There was a fair amount of rainfall through April and May and by May 27, it was evident from the number of affected culms that the infection was more vigorous and more general than in previous seasons. Sclerotia were abundant and many were mature on May 27.

Throughout June the dead and whitened tips of infected grass blades were abundant and conspicuous especially on culms which continued to develop new leaves. There was, however, no increase as the season advanced in the number of infected culms. Areas which were free from the fungus earlier in the season remained free from it. These observations made it clear that there is no spread of the fungus from culm to culm aerially and that a culm which does not show the fungus on its first leaves does not harbor it later in the season.

The effect of the fungus is especially marked on *Calamagrostis canadensis*. Many of the culms die to the ground early in the season. The remainder continue to put forth infected leaves, but are decidedly stunted. Very few infected culms produce flowers. Even apparently uninfected culms which arise from the same rhizomes with infected culms, are weak and stunted. Observations made year after year show a decrease in the number of plants of *C. canadensis* in the infected areas. Throughout the greater part of the marshes *C. canadensis* is associated with *Carex stricta* with which it is more or less in competition. The destructive effects of the fungus seem to be an important factor in favor of *Carex stricta*.

Observations made each year indicate that the fungus develops during the spring and early summer. It is most vigorous and conspicuous during the period of the most rapid growth of the host plant. It becomes less noticeable as the season advances due to the death of many culms and to the overtopping by unaffected culms and by associated vegetation. The fungus seems unable to make headway on fully developed leaves during June and July. Each leaf as it unfolds contains the fungus, but

the exposure to dry and heated air checks the development of mycelium and sclerotia.

ABUNDANCE AND DISTRIBUTION

To ascertain the amount of infection the following method was used upon the marsh selected for critical study. At intervals of fifteen paces along a line leading through the marsh a heavy wire hoop twelve inches in diameter was dropped down at random. All the vegetation inside the hoop was then cut close to the ground, the infected and the uninfected culms of *C. canadensis* were sorted out and counted and the results tabulated. The greatest injury appeared to be at the borders of the marsh meadow, especially on an area of about sixteen acres, which is somewhat isolated from the greater part of the marsh by a railroad embankment and a canal which passes through the marsh. This area is well drained and is always dry enough to be mowed by machine. Here the dominant species

TABLE I DISTRIBUTION OF FUNGUS ON *Calamagrostis canadensis*

Station	Number of infected culms	Healthy culms
1	18	6
2	19	4
3	21	6
4	17	7
5	49	8
6	22	26
7	87	31
8	14	5
9	45	8
10	36	7
11	21	32
12	58	29
13	18	6
14	0	9
15	0	7
16	1	18
17	10	6
Total	426	215

are *Carex stricta, Calamagrostis canadensis, Poa pratensis, Glyceria nervata and Spartina cynosuroides.* These are considerably intermingled. *Poa pratensis* is especially abundant along the edges near the upland. *C. canadensis* is quite uniformly distributed over the area except at the extreme border. Beginning at the margin, data on the distribution of the fungus were taken as described above at seventeen consecutive points fifteen paces apart. The results for *C. canadensis* are given in Table I.

From these data it appears that 66 per cent of the culms of *C. canadensis* growing on this area were infected. While the fungus was quite general in its distribution on this area there were patches of small extent, usually from one to two rods in diameter, that were almost entirely free from the fungus. Often these would be entirely surrounded by infected strips and in such cases the contrast was always marked. Data taken from typical areas show that on May 27 the healthy plants then stood on the average, 30 inches high, while the tallest of the infected ones nearby were but 12 inches, and the majority of them were 4 to 8 inches high. Even the culms that had escaped infection were smaller and poorly developed. These "islands" of uninfected plants in the otherwise destructively infected regions gave by comparison conspicuous evidence of the damage done by the disease.

Data on the abundance of the fungus over the greater part of the marshes given in Table II were obtained in the same

Table II Additional Data on Distribution of Fungus on *Calamagrostis canadensis*

Station	Infected culms	Healthy culms	Station	Infected culms	Healthy culms
1	0	0	19	13	24
2	0	9	20	5	12
3	6	3	21	0	6
4	2	5	22	0	13
5	7	7	23	0	0
6	2	18	24	0	0
7	0	0	25	0	4
8	14	7	26	3	12
9	6	10	27	6	19
10	1	2	28	5	28
11	8	22	29	0	0
12	1	37	30	0	0
13	14	15	31	1	12
14	17	6	32	1	10
15	7	2	33	0	19
16	8	27	34	0	8
17	27	21	35	6	24
18	8	45	36	19	22
			37	4	8
Totals for the 37 stations				195	467

manner. The transect began at the south edge and extended through the center of the marsh as far as the canal and thus coincided with the transect previously studied in determining the plant population of this meadow.

For this part of the marsh the infection averaged 29 per cent. Combining all the data obtained gives an average infection of 47 per cent for the two transects which represents fairly

the conditions on this particular meadow on May 27, 1911. One transect passed through a well drained marginal area. The other passed from the margin through the wettest part of the marsh meadow. The two give a fair average of the whole meadow.

In the central wet portions of the meadow *Carex aquatilis*, *Carex Sartwellii* and *Carex filiformis* were dominant and *C. canadensis* was either absent or sparse. It is noticeable that the percentage of infected culms was relatively lower under such conditions. (See data for stations 20-34 in Table II.) The sparseness of the grass evidently gives less opportunity for the infection to spread through the soil.

Throughout the entire marsh there were areas usually of small extent that were free from infection. There were also areas with more than 90 per cent of infection. The latter were uniformly located in areas where *C. canadensis* was dominant.

Range of the Disease in Wisconsin During June 1911, the writer spent three days in making observations on the occurrence of this fungus in the extensive marshes in the townships of Albion and Christiana, Dane county, Wis. Here several square miles of marsh meadow were traversed extending for nearly eight miles along Saunders' Creek. These meadows have for a number of years either been cut for hay or utilized as pasture. *C. canadensis* and *Carex stricta* were dominant over most of the area. The former grew in luxuriance over large areas and stood when in blossom 4 to 5 feet high. *Carex aquatilis* and *Carex riparia were* abundant in the wetter parts of the area and *Poa pratensis*, *Panicularia nervata* and *Poa flava* were common near the borders. In several areas *Phleum pratense* was abundant.

Over the entire area visited, the fungus was found to be conspicuously abundant on *C. canadensis*, although areas of several acres were found which were nearly free from the fungus. On others the fungus although abundant was much scattered and its effects not conspicuous. Over larger areas, however, the same degree of destruction was seen as has been described for the vicinity of Madison. For the season of 1911 the infection on this entire area was not less than 10 per cent of the culms of *C. canadensis*. Besides this there was a less conspicuous and less general injury to various other grasses by the same fungus.

In this region several small isolated patches of *C. canadensis*, located on high land, were found to be infected. In fact the most uniform and complete destruction seen anywhere was on such an area. A nearly pure formation of this species measuring 2½x2 rods was found by the roadside bordering a cultivated field and on high dry land with the nearest marsh three-fourths of a mile distant. The undisturbed dead culms of previous years formed a rich mulch. Except for a fringe at the ends of this formation, practically every culm was infected. Many were entirely dead and the short culms with the uniformly white tips appeared in sharp contrast to the surrounding green vegetation. The infected belt extended to the border of an oat field, but no trace of the fungus was found on the oat plants.

The occurrence of the fungus in such isolated areas of *Calamagrostis* suggests either that the fungus is a widespread soil or root fungus which does not always show parasitic development in the leaves, or that it is distributed by spores which cause a rapid infection of roots, stems, and leaves. As noted above I have so far found no spore stage.

Marshes were also visited in the region about Fort Atkinson and Lake Koshkonong in Jefferson county, Wis. In several small isolated marshes, no trace of the fungus was found, but in long stretches of marsh land along Bark River and Rock River, the fungus was abundant. Here again it was often found in roadside patches of *C. canadensis* on rather high land. The most general occurrence of the fungus found anywhere was near the mouth of Koshkonong Creek. Here a continuous marsh meadow of eighty acres was examined, June 23. A heavy growth chiefly of red top and *Carex stricta* covered the higher portions while *Phalaris arundinacea* stood five feet tall over the lower parts. *C. canadensis* was abundant over areas in which nearly every culm was infected with the fungus so that the entire *Calamagrostis* population was overtopped by *Carex stricta*, *Agrostis alba* or other plants usually of lower stature. The infection was so complete that not a single flowering culm of *C. canadensis* was observed on the entire eighty acres. *Phalaris arundinacea* was, however, but slightly affected with the fungus.

From various reports it seems that these conditions prevail throughout the greater part of the state. Dr. J. J. Davis states

in a letter to the writer: "I first noticed *Sclerotium rhizodes* on *C. canadensis* in 1892 and I have seen it on that host every year that I have been in the field since. I have collected it at both ends of the longest axis of the state and at various intermediate points so that I think that it may be said to be generally distributed through Wisconsin and to be a constant and general member of the parasitic fungus flora of the state."

In response to inquiries, George L. Peltier of the State Cranberry Experiment Station at Grand Rapids, Wis., writes as follows: "I have made observations on *Sclerotium rhizodes*, but have been able to find it only on blue joint (*C. canadensis.*) It is very widespread here and I have found it wherever I have looked for it. In a field just west of the station about 30 per cent of the stalks seemed affected. On one of my trips I found a whole field of many acres where almost every plant was affected. It had weakened the grass so much that none was able to head out."

It appears, however, that this fungus has not been reported in America outside of Wisconsin. This is most singular. Here in Wisconsin it is widespread, abundant and conspicuously parasitic. Its chief host, *C. canadensis* ranges from Newfoundland to Alaska south to North Carolina, New Mexico and California and other grasses which may serve as hosts increase the area in which the fungus may appear. It seems probable that this fungus is equally common in other sections besides Wisconsin, but has been overlooked.

Extent of Infection on Grasses Other Than Calamagrostis Canadensis

The frequency of the fungus on other grasses bears directly on the question as to the method of infection.

Phalaris arundinacea. Scattering groups of plants were found with the fungus in the marsh meadows along Albion Creek, Rock River near Ft. Atkinson, and Lake Koshkonong. These were always in the immediate vicinity of badly infected areas of *C. canadensis* and were usually at the border of a Phalaris formation. On the whole, this species was slightly infected in this region. This grass did not occur in the marsh meadows studied at Madison.

Calamagrostis neglecta The fungus was abundant and injurious over areas covered with this species. Infected *C. canadensis* was always found in the vicinity and usually the two species were intermingled.

Panicularia nervata Wherever this species grew intermingled with infected *C. canadensis* a small per cent. of its leaf culms showed infection. On this host however, the fungus did not appear to be seriously destructive.

Poa pratensis In the case of this species there was rather abundant and serious injury especially where infection of *C. canadensis* was vigorous in the immediate vicinity, but the fungus also appeared quite abundantly in the nearly pure formations of *Poa pratensis*, which thrive in the border and upland portions of marshes about Madison.

Phleum pratense Vigorous infection of this grass was seen in the border of a marsh near Albion. Only a few infected leaves and culms were found and in nearly every case these grew by the side of infected culms of *C. canadensis*.

Hordeum jubatum At Madison this grass was found growing in border areas of the marsh often with its roots intermingled with those of infected culms of *C. canadensis*. It was only under these conditions that culms were observed showing the characteristic effects of the fungus.

Bromus ciliatus, *Eatonia pennsylvanica*, *Agrostis hyemalis*, *Agropyron caninum*. A few culms of each of these species were found infected with the fungus. Infected plants of *C. canadensis* were always close at hand.

These observations suggest that while *C. canadensis* serves as the principal host for *Sclerotium rhizodes* the fungus may spread to various other grasses especially when they are in close proximity, a fact which is fully explained when the soil and root relationships of the host are considered. Several species of grasses, especially *Agrostis alba*, *Andropogon furcatus* and *Spartina cynosuroides*, appear to be immune, or at least no evidences of the fungus were found on plants of these species which grew within zones of infected *C. canadensis*.

It is of interest to note that on the stems of *Urtica gracilis*, which was growing within an area of vigorous development of the fungus on *C. canadensis*, there were found in one season sclerotia somewhat similar to those on *C. canadensis*.

CONDITIONS FAVORABLE FOR DEVELOPMENT

It has already been shown that the fungus usually developed most abundantly in the field during the early part of the season, but that during moist summers it was also vigorous later in the season. To determine the conditions which favor the development of the aerial mycelium and the production of sclerotia the following studies were made during April and May 1910.

Culms in the early stages of development were gathered in the field, immediately placed in sterile test tubes about 4x20 cm. in size and plugged about the stem with cotton. These were taken to the greenhouse and so placed that the cut end of the culm and the mouth of the test tube were in water thus forming a damp chamber of the test tube. Clumps of the plants with infected culms were also transplanted into pots and kept under bell jars. For comparison others were exposed to the air of the greenhouse. In all the latter the fungus developed slowly without any conspicuous show of mycelium and the sclerotia began to form in about ten days. Here the development and appearance closely resembled that observed in the field.

In the case of the infected culms placed in sterile test tubes there appeared within twenty-four hours an abundant mycelial growth which extended from the infected portions of the leaves out into the tube forming a cottony mass which often filled the tube for one-half or two-thirds its length. Soon numerous sclerotia began to form. Many of these were out in the aerial mycelium and were not directly attached to the leaves. At the end of fifteen days the culms and unaffected portions of the leaves were still green, the mycelium was still vigorous and many of the sclerotia were fully mature.

There was a less vigorous development of aerial mycelium on potted plants inclosed in a bell jar. Many infected culms died in ten to twenty-four days while noninfected culms remained green. Sclerotia on these plants were mature in about twenty days. These experiments show that increased humidity favors the development of the mycelium on the surface of the leaves and promotes rapid formation of the sclerotia, which agrees with the facts observed in the field as previously discussed.

Source of Infection

During the past three years hundreds of infected culms have been examined in all stages of the disease and throughout the entire period of its appearance, but no spores were found. Evidently the mycelium is prevailingly sterile as it occurs in nature.

The history of many other sclerotia-forming fungi suggests that the sclerotia may develop ascocarps with ascospores. Diligent search for germinating sclerotia has been made during each of the past three years. Each year as soon as the snow melted sclerotia were found on the ground in the areas of worst infection and they were found and examined *in situ* throughout the season. No evidence of germination was found.

Both sclerotia gathered from the grass in the field and those grown in cultures have been treated in a variety of ways in the endeavor to induce germination, but with no success at present writing. During the season of 1910 almost no sclerotia were matured in the field, but in 1911 they were produced in abundance and I have at least 500 now planted under a variety of conditions. In the case of a few sclerotia gathered from Urtica, germination was secured, but a discussion of these is reserved until the identity of the fungus is more certain.

So far as I can find the sclerotia do not germinate freely and abundantly each year. They are, in fact, not matured in abundance each year, for by far the greater number dry up and become shriveled, while immature. Yet the fungus is abundant year after year. It was noted that the infection reappeared year after year in the same areas and that patches near by were constantly free from infection. Examination also showed that in many cases the majority if not all of the culms arising from the same root stalk were infected. The primary development in every case seemed to be from within the bud for here the fungus appears when the first leaves unfold. All this evidence suggested that the mycelium may be perennial. To test this the following experiment was made.

March 5, 1910, while the snow stood nearly two feet deep on the marsh, rhizomes of the Calamagrostis were dug from an area where the fungus had been abundant during the previous season, taken to the greenhouse and potted in muck soil that had been in use in the greenhouse for some fifteen years. In the potting, the old culms and dead leaves were removed so that

the rhizomes and buds only were planted. The soil was kept well watered. March 14 the young culms were from two to four inches tall and in one of the opening buds the mycelium of the fungus was visible. March 25 this culm showed three leaves infected with the fungus and producing the typical crooks. At this date the opening leaves of three other culms showed the presence of the fungus, two culms were dead from the effects of the fungus, and eight were apparently free from infection. The culms were 12 to 15 inches tall on April 1, with as many as five leaves. The infected culms showed the typical development seen in the field[2]. These results are quite conclusive that the mycelium is present in the buds during the winter.

RELATION OF THE HOST AND THE FUNGUS

Method of Investigation Following the above experiment the infected areas were visited, and leaves, buds, portions of stems and rhizomes of infected plants were fixed in chrom-acetic and picro-formal fixing solutions. This material was imbedded, sectioned and stained with either iron-haematoxylin or with the Fleming triple stain. The sections showed that the mycelium is coexistent in and on the leaves, buds, stems, rhizomes, and roots of the same plants. The characteristics of the fungus in these different parts are such that a detailed description of each is necessary.

Character of the Aerial Mycelium On the matured foliage leaves, the mycelium is in part aerial as described above. The mycelium is white in mass. It is abundantly branched, is septate, and the hyphae anastomose to some extent. The ends of the hyphae are often enlarged. The walls are thin and the cytoplasm is slightly granular and much vacuolated. Figure 8 B shows the general appearance of the aerial mycelium taken from the surface of the leaf.

For a more careful study of the cell structure, the mats of mycelium produced in cultures on cooked potato were fixed in

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[2] This experiment has been repeated with the following data: November 24, 1911, Mr. A. G. Johnson chopped from the frozen ground at Madison rhizomes of *Calamagrostis canadensis* and *Poa pratensis*. These were sent to me at the New York Botanical Garden and immediately placed in pots which were kept in a greenhouse. As soon as the first leaves of the growing culms unfolded (January 8, 1912) the fungus was found with typical development in the buds of several culms of both species.

Fleming's weak solution and stained by either the triple method or by iron haematoxylin. This treatment showed that some of the cells of the mycelium were two-nucleated with a reticulated protoplasm as shown in Figure 7, *J*. I have not found division figures in any cells of the fungus and I can give no data on the constancy of the two-nucleated condition or its possible origin in conjugate division.

A few cells, however, had more than two nuclei and occasionally only one nucleus was present. The general character of the mycelium differed on the various culture media. This will be described later.

The Mycelium in the Leaves Figure 6 *A* shows a portion of an infected leaf in the condition shown in Figure 3 *A* the cross section being taken at a point indicated by *m*. The vascular bundles were all that was left of the leaf tissues in the infected portion at this stage and the fungal filaments ramified through all parts of the bundles except the phloem. All of the cells of the mesophyl had been totally destroyed and of the epidermal cells the outer walls alone remained. A cross section through an inner leaf of the roll showed almost complete destruction of the vascular bundle elements as is shown in Figure 6 *B*. In all of the tightly rolled and shriveled leaf tips (See Figure 3) the dead and dried remnants of vascular bundles are closely bound together by the mycelium which is itself dead at this point. During the early part of the season the vigorous destruction of the host tissues proceeds until the plant often appears as in Figure 2, when the entire roll of leaves is completely permeated by mycelium and the destruction is so complete that almost no tissues are recognized within the roll.

Action of the Fungus on Leaf Cells The action on the individual host cells is apparently rapid. Careful study of many sections perfectly fixed, sectioned and stained, fails to show the presence of hyphae within turgid mesophyl cells. There is some evidence that cells may collapse somewhat in advance of the actual penetration of the hyphae. Here the plasmolysis of cell contents preceding actual penetration by hyphae is not marked and may be due to other factors than the direct influence of the fungus. At any rate plasmolysis and disintegration of the mesophyl cells of the leaves occurs so rapidly that the successive stages in the process cannot readily be observed.

Although the mycelium develops abundantly on the leaves and thus extends beyond the point of internal infection, there are few cases of penetration from without into an expanded leaf either through stomata or through the epidermis. It is noticeable that the mycelium spreads in the leaves most readily from the tip toward the base in the direction in which the vascular bundles run. This is because the mycelium advances most rapidly in the mesophyl tissues which are arranged in strips separated from each other by the fibro-vascular bundles which extend from epidermis to epidermis and across which the mycelium passes less readily. This is clearly shown in the cross section of leaves exhibiting a strong lateral infection. Such sections reveal a clearly defined boundary of the invasion which presents for study various stages in the destruction of cells. In this zone of advance through the mesophyl the mycelium is chiefly intercellular. The ends and sides of the hyphae come in contact with the thin cell walls. In the first stages of penetration there appears to be a slight thickening of the host cell wall and a region about the point of dissolution often stains strongly. There is no tendency for the fungus to dissolve out the middle lamella. The hyphae simply pass through openings dissolved in the cell walls. Once inside, their work of destruction is rapid. The protoplast is plasmolized and the thin cell wall collapses. First the cell contents are digested and later the cell wall is also completely digested so that the entire mesophyl tissue soon disappears. In the outer epidermal layer the mycelium often travels from cell to cell destroying everything but the layer of cuticle.

In the region of fungal advance the mycelium is less destructive to the fibro-vascular bundles. Filaments soon ramify freely throughout the woody elements and there is a slight disorganization of the thick woody cell walls.

In portions of the leaves that have been infected longer and in the case of inner leaves more vigorously attacked, there is almost complete destruction of the vascular elements as is shown in Figure 6 B. Here the woody walls are relatively thin and collapsed, and the phloem distorted.

Distribution of the Mycelium in Aerial Buds Longitudinal sections were made of terminal buds exhibiting varying degrees of fungal destruction. In the early stages the mycelium extends over the surface of the rather tightly rolled leaves with

slight penetration in the youngest leaves and more or less penetration in the outer. It may even form a cushion of mycelium over and around the embryonic tissue of the growing point and yet not penetrate this tissue. Figure 6 *D* shows such a condition. Figure 6 *E* is a somewhat diagrammatic sketch of the same bud from which Figure 6 *D* was drawn. It shows that the mycelium is both upon and within the young leaves before they unroll from the bud. As young leaves develop from the meristematic apex they grow into this cushion of mycelium and are in turn coated by mycelium and thus exposed to infection. As noted, the mycelium does not penetrate the embryonic tissue. In culms which are vigorously attacked the mycelium penetrates into the tissues of the stem below the growing point, dissolving them and, if the process is not checked the death of the terminal bud may result.

The fungus seems first to become destructively parasitic in the mesophyl of the outer leaves as they are maturing and expanding, to which it gains entrance while the leaves are in the bud. Then the destruction extends to leaves within the bud roll and finally to the apical internodes. In many cases, however, the parasitic attack is confined to the tips of the outer leaves. As they develop each shows the dead tip and the felt of mycelium. All degrees in the rapidity and the amount of destruction may be observed.

The Mycelium in the Stems Plants were selected which showed abundant fungus infection of the leaves and pieces were cut from the culms at various points. These were sectioned and examined for the presence of the fungus in the successive nodes, internodes, and buds. To the eye the main portion of the stem, with the sheathes of the leaves and the buds enclosed by them showed no felt of mycelium such as has been described for the leaves. The stained sections however, revealed the presence of the fungus in greater or less abundance in all of these parts. Only a few strands of hyphae were found in the tissues of the hardened internodes. In the nodal regions, the strands were quite numerous in the peripheral tissues where they often appeared as knotted tangles of intracellular mycelium. Occasionally there was penetration to the internodal cavity. Here there was no evidence of destruction of cell walls other than at the points of penetration. In the old cortical cells the cytoplasm is

reduced to a thin film which is difficult to locate in any of the cells.

As a rule few buds are produced on the upper parts of a healthy culm, but late in the season lateral buds often develop from the upper nodes of tall culms whose terminal bud is badly affected with the fungus. The leaves from such buds may or may not be infected with the fungus.

The Mycelium in the Lower Buds and Underground Stems

A careful study was made of buds which arise low down on the culms and on the rhizomes and which would not develop into culms or rhizomes until another season. It is readily seen that the presence or absence of the fungus in the case of the buds is a crucial point in determining the life period of the fungus and the source of infection of the unfolding leaves. Horizontal and cross sections were made of buds of different ages and sizes which were variously situated on basal portions of plants whose aerial culms showed infection. In the majority of these buds the mycelium was found to be present. Figure 6 *G* is a drawing from such a bud which was situated just below the surface of the ground and which would develop as a culm in the following season. The successive sections showed, as did the longitudinal sections of other buds, that the fungus was rather irregularly distributed on and through the rudimentary leaves and that it was more abundant near the apex of the buds. Often but one side of a bud was infected by the fungus. When such a bud unfolds, but a lateral half of certain leaves will be diseased, a condition which explains the common partial and lateral infection of aerial leaves already described and illustrated. In these buds the hyphae pass through the cell walls freely in the manner shown in Figure 6 *G*. The cell walls in all cases appear normal but the cell contents have entirely disappeared.

The chief difference between the effects of the fungus in the foliage leaves and in the tissues of the leaves of dormant buds is that in the latter there seems to be no absorption of cell walls except at the points of penetration.

In the basal nodes which were close together and from which arose roots and buds producing aerial culms or rhizomes, the fungus was abundant. The mycelium was in part external. Here as in the aerial parts of the stem itself, the fungus did not penetrate far into the interior. In the leaf scales and in the cortical portion of the basal nodes there appeared within the

cells, coils or nests of mycelium and also rounded bladder-like enlargements of the mycelium, a feature not observed in the aerial portions of the host plant. These bladders often formed a belt or zone in the region of deepest penetration, as is shown in Figure 7 *A*. The mycelium was traced readily from these basal portions of the stems out into the buds. Figures 6 *G* and 6 *F* are from a bud and the stem from which it arose showing the relative positions of the two as they appeared in the same cross section. Figure 7 *B* is a section showing the mycelium passing directly from the stem out into a bud scale which enclosed a growing point. Thus the anatomical studies verify the experiments and field observations which indicated that the fungus exists in the underground buds.

Relations of the Fungus to the Roots In the stained sections the fungus was also traced from stems and rhizomes out into the roots, a fact which made a study of the roots desirable. Plants of *C. canadensis* produce a large number of fine fibrous roots which arise both from the rhizomes and from the basal portions of the culms. These form a dense tangled mass in the surface layer of soil, especially in the upper six inches. Certain roots may also penetrate to a depth of two feet. These deeply penetrating roots are rather straight, much branched, fibrous roots with side branches which are long and repeatedly branched. In contrast to these the strictly surface roots are somewhat smaller in diameter, shorter, more profusely branched, and much twisted and interwined.

Many of these roots live several years. Examination of a mass of roots in early spring shows that some of the roots are dead while others are putting out new branches. From the rhizomes and from the bases of living culms are also produced each spring new roots which grow rapidly to form either the deeply penetrating roots or surface roots. Young and rapidly growing roots do not harbor the mycelium and the deep roots do not contain the fungus to any considerable depth. Many but not all of the surface roots do contain the fungus and as a result are modified in a more or less characteristic manner. A typical infected surface root that is at least one year old is several inches long with its branches short, often twisted or curved, and usually slightly enlarged. Root hairs are seldom found on these spur branches, but they are numerous along the main

roots even at points between the side roots and several inches back of the growing tip. (See Figure 8 *D*.)

In making an external examination of these roots the mass was soaked in water and the soil was then washed out in gently running water. Then the roots were mounted in water for examination under the low powers of the microscope. By this method the mycelium could be seen rather sparsely distributed on the exterior of the roots, extending out from or penetrating into them, ramifying through the humus and passing from root to root. Few filaments come directly from the spur roots. Sections of infected roots similar to the one shown in Figure 8 *D* were placed in hanging drop cultures. In from three to five days considerable growth was made by the enveloping mycelium. Many filaments were traced directly from the roots and often several branches developed from the cut end of a root. Figure 8 *C* represents the mycelium as it thus develops outside a root. The drawing was made with a camera lucida using the same lenses which were used in sketching the aerial mycelium from the leaves which is shown in Figure 8 *B*. This mycelium appears to be almost indentical with that produced on the leaves.

In examining roots by this method mycelium was often found infesting the roots, which was somewhat different in character from the foregoing. It consisted in part of large coarse hyphae which were sparsely septate and which had heavy walls of unequal thickness. On short branches were borne terminal bladders or thick walled vesicles which were not cut off by a cell wall and which were filled with oil globules. These vesicles were similar to those produced by the mycelium internally in the roots, rhizomes and scales of the host plants, but were much larger. On casual examination this coarse mycelium bears little resemblance to the mycelium of *Sclerotium rhizodes* as described, but branches arising from it may be found which are thin walled, smaller in diameter, and more septate and almost identical with those which were secured from the cut roots in the hanging drop cultures. This heavy walled mycelium with the numerous large food vesicles was found on roots of infected plants collected from widely separated localities at various times throughout the spring, summer and autumn.

The evidence is conclusive that the fungus is in part soil inhabiting and this explains the vigorous local infection as noted and the infection of a number of grasses which may enter such

an area, especially the infection of an annual, such as *Hordeum jubatum*. A large number of roots of various sizes and ages were collected from infested *C. canadensis* plants at intervals throughout the season of 1910, fixed in picro-formal solution, imbedded in paraffin, sectioned with a microtome, and stained by the triple method. These sections revealed the presence of the fungus within nearly all of the older surface roots. Young rapidly growing roots did not contain the fungus.

So far as the behavior here is concerned there is no evidence that there is present a mycorrhizal relationship such as is commonly understood in which the fungus retains a constant position in reference to the growing points. In the roots, rhizomes, and stems the fungus attacks the older and hence weakened cells. This is in marked contrast to the action in the leaves where it attacks vigorously the active mesophyl cells but it is to be noted that the hyphae are unable to penetrate into the growing apex of the buds.

In larger roots such as shown in Figure 8 *D* there was an abundant but rather irregular distribution of the mycelium throughout the cortical tissue, with much the same characteristics as are seen in the cortex of the underground stems. The nests of mycelium and the bladders were formed within the cells although the latter were not grouped in a belt or zone. The mycelium was both inter-and intra-cellular. In the root cells of this region the cytoplasm forms an extremely thin layer which was difficult to identify even in uninfected cells. The cell walls were of normal thickness and were not collapsed. There was a strong tendency for the mycelium to extend longitudinally through the root yet there was here some evidence of its spread in a radial direction also. (See Figure 7 *D*.)

In the spur roots there is considerable variability in the behavior of the fungus. Here the mycelium is confined chiefly to the layer of cells immediately surrounding the central cylinder. It can be traced into this zone from the main root and its distribution is here decidedly in the direction of the growth of the rootlet. Here there is an opportunity to study the relations of the hyphae to the protoplast for they penetrate cells while the cytoplasm is conspicuous and the cell walls are thin. The fungus advances to the extreme tip which is devoid of a pronounced embryonic region. When a hypha passes into a living cell in this region it first becomes irregularly enlarged or lobed

and is somewhat coiled about or pressed upon the nucleus which in turn becomes rather irregular in shape. The cytoplasm becomes dense and granular or slightly stringy and stains a deep orange or red. (Figure 7 *C*, *E* and *G*.) Later the nucleus disappears in some cases evidently after fragmenting and the cytoplasm is transformed into irregular, dense, deeply staining particles scattered about within the cell wall. (See 7 *F*). In some instances the fungus filaments within such a cell seem also to disintegrate so that fragments of the mycelium are mingled with the debris of the protoplast. In the majority of infected cells, however, the mycelium remains intact while the cytoplasm and nucleus are undergoing disintegration. Adjoining but uninfected cells show the cytoplasm and nucleus as normal and faintly staining structures.

In certain of these infected cells these changes in the protoplast are accompanied by the formation of bladders which are intercalary or perhaps occasionally terminal swellings of the hyphae. They are always intracellular and are of various sizes. They begin to appear in the small cells of roots in the region of the advance of the fungus and they may be found fully developed in the older roots and in the basal nodes and the surrounding scales. When young they show a finely reticulated structure (Figure 8 *F*.) Later they are more dense and granular. The greater number found in old roots are entirely empty and possess rigid and unbroken cell walls although in a few cases they appear wrinkled or shriveled.

In early spring the vigorously growing roots are not infected. Soon these roots cease their rapid growth and begin to send out short slowly growing side roots. The fungus mycelium probably gains entrance both at the base from the stem and by direct penetration from the soil. It advances in the root toward the growing point. The lateral roots are infected directly from the main root soon after they are first formed and as a result are stunted and slightly hypertrophied. These infected roots may continue to live into at least the second season. Some side roots escape infection completely or for a longer time and these elongate in a normal fashion.

Summary of the Relations of the Host and the Fungus

These studies show that the fungus is coexistent in leaves, stems, buds, rhizomes and roots of the same plant. Its general distribution in underground perennial parts and its existence in buds

which are to develop in succeeding seasons make clear the perennial nature of the mycelium. It is of especial significance that the mycelium was found in greater or less abundance in the scales and young leaves immediately surrounding growing points. This provides for the distribution of the mycelium into the various branches as they are formed. As an infected basal bud develops into a culm the intercalary growth incident to the development of the internodes separates the leaves which are more or less infected in the bud. As the internodes elongate, the mycelium, which as has been noted, is chiefly confined to the young leaves, is carried with them and hence appears on the successive leaves. The tips however, being held together by the fungus form the series of characteristic crooks.

This development with the quite general distribution of the mycelium in the soil and in the underground portions of the host, insures most completely its perennial habit and accounts for its reappearance without spore formation in the same areas year after year, for the decided local infection so often observed, and for the infection of various other grasses which enter the infected areas. I have not yet worked out the relations of the fungus to the leaves, roots, and stems of the various other grasses upon which it has been found, so fully as in the case of *C. canadensis* but the evidence obtained indicates that the general relationships of host and fungus are the same in each instance.

There is in the case of the leaves of all infected species the same characteristic development of mycelium, production of sclerotia, formation of crooks and death to the leaves. The leaves of *Poa pratensis*, however, show a more decidedly local infection. Often two or more infected spots will be completely separated by green tissue. This is evidently due to an irregular infection in the bud and to the inability of the fungus to spread so rapidly in the leaves of this species as it does in other grasses.

In the roots arising from infected culms of *C. neglecta* there is a general distribution of the fungus with the same characteristics and effects described for *C. canadensis*.

Sections were also made through clusters of roots on infected culms of *Poa pratensis*, *Panicularia nervata* and *Hordeum jubatum*. In all of these the fungus was found in and on roots,

scales, and underground portions of the stem, although not to such an extent as was found in *C. canadensis*.

SCLEROTIA

The development of sclerotia was observed on the host plant and on various culture media. They originate in a small loose plexus of several mycelial strands which continue to branch, intertwine and anastomose until a rather compact mass of tissue is formed. The cells within become enlarged and irregularly rounded. The young sclerotium increases in size by repeated growth and branching from the periphery. Meanwhile large drops of clear liquid of a slightly yellow color are exuded. In maturing the color changes from white to dark brown or black and the outermost zone of filaments shrivels and forms a thin felted layer beneath which a dark colored rind develops. In this rind the cells are small and nearly isodiametic and possess heavy walls. The central mass is made up of intertwined hyphae whose cells are shortened and of greater diameter than those of the ordinary aerial mycelium. Although rather closely packed together there are many spaces between them. The structure appears homogeneous with no trace of primordia.

Two or more young sclerotia which are growing close together often unite to make an irregularly lobed compound sclerotium. In cultures on cooked potato and on potato agar the small sclerotia were so numerous that they formed a crusted stroma which did not round out into any definite shape. On the various other media tested there developed every stage from a matted mycelium forming an indefinite stroma to well rounded sclerotia. In form, many of these irregularly shaped sclerotia resemble those which Brefeld (1881 p. 115) obtained in his cultures of *Peziza sclerotiorum*. Brefeld (1881 p. 116) has also most clearly pointed out the two methods of origin of sclerotia. The sclerotia of Basidiomycetes (Agaricus, Coprinus, Typhula, etc.) start by the interlacing of branches from a single hypha, while the sclerotia of Ascomycetes which he investigated begin as a plexus of several filaments, as previously noted for this fungus.

The sclerotia which develop on the host plants are rounded and smooth on their entire surface except on the side which was appressed to the leaf and here the sclerotium is usually flattened and rugose to conform to the ridges in the surface of the leaf.

When fully mature they usually drop from the leaf. Attached to many of them are often short strings of dry and dead mycelium from which the sclerotium arose and in which the growing sclerotium is imbedded. These appear on the immature sclerotia especially somewhat like roots, or rhizoids, and hence probably suggested the specific name given by Auerswald.

It seems probable that spores when they are produced develop first saprophytically in the humus soil. Investigations and experiments are still in progress to determine more completely the ultimate fate of the sclerotia. As many as 500 sclerotia of the 1911 crop are now planted in pots and in bottles which are being handled in a variety of ways.

There is some evidence that sclerotia may sprout vegetatively. Fully mature sclerotia were taken before they had dried, and placed on sterile sand in small pots which were covered with glass lids and kept moist. A thin felt of mycelium developed from the sclerotia and spread over the sand where it continued to thrive for several months. When bits of this mycelium were transferred to various media the typical growth resulted. Other sclerotia were placed directly on media and in the majority of cases developed a growth typical to the various media. Old sclerotia which were thoroughly dried failed to produce growth of any kind when thus treated.

Infection Experiments

For these experiments healthy uninfected plants of *C. canadensis* were grown from rhizomes transplanted to pots of sand or garden loam. When the culms were from three to twelve inches in height masses of mycelium from various cultures were placed on leaves of various ages and the plants were then kept in bell jars. In no case did the mycelium establish itself on the leaves. The following method was then tried. Vigorously growing pure cultures were grown on hard potato agar and on lima bean agar in test tube slants. A test tube with a culture was inverted, the cotton plug removed and the open tube slipped over a culm and so adjusted in a clamp stand that the leaves came in contact with the mycelium. The plug was then replaced and thus the uninjured mycelium was brought in contact with leaves and the whole was enclosed in a moist chamber formed by the plugged test tube. In all, twenty-five experiments were tried on culms

of various ages and on leaves in various stages of development. The mycelium would develop over and about the leaves and although left for several days, when the test tube with its culture was removed and the culm enclosed in a moist chamber, the mycelium adhering to the leaves died, the leaves continued to develop normally and there was no evidence of infection.

Infected culms were brought in from the field and enclosed in a bell jar until there was abundant development of aerial mycelium and then placed in contact with healthy leaves. No infection resulted by this method. Experiments to test the possibility of the infection of seedlings through the soil are now being carried on and have not yet given any definite results. These experiments seem to indicate clearly that the mycelium can not penetrate into sound leaves after they have opened from the bud and that there is no spread of the fungus from culm to culm aerially.

CULTURAL STUDIES

The fungus was obtained in pure culture by placing fragments of infected leaves in Petri dishes poured with lima bean agar and with hard potato agar. Abundant growth of mycelium with formation of sclerotia resulted and transfers were readily made to media in test tubes. The fungus has been kept in culture since April 1910 and its behavior on various media studied. All cultures were grown in a refrigerator at a temperature of about 16° C.

Lima Bean Agar[3] On this medium there is luxuriant and rapid growth. At first a fine cottony layer of mycelium spreads over the surface of the medium and from this there is a copious aerial growth. Within from ten to twenty days sclerotia begin to form. When mature many of these are rounded and similar in size to those formed on the host in the field, but the individual sclerotia may grow together to make an irregular mass, often 1.5 cm. in diameter. Several cultures were kept for a period of 15 months and although the medium had shrunken to one-third of its original volume the mycelium was still vigorous in small patches and new sclerotia were being formed.

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[3] Lima bean agar: 1000 cc. water, 100 g. ground beans; soak 30 min., then boil 30 min.; express juice and restore to 1000 cc.; add 10 g. agar, cook in steamer, filter, autoclave at 5 lbs.

Hard Potato Agar[4] Growth was less rapid on this medium that that obtained on lima bean agar. A few definite sclerotia were formed but as a rule large irregular spongy masses formed on the surface of the medium as a crusted stroma. The surface of these remained spongy or granular; from the surface there constantly developed tufts of white mycelium which in turn became brown, rough and granular. The largest of these pseudo-sclerotia were 3 cm. long and .5 cm thick. They consisted of a loose plexus of mycelium the cells of which were enlaged like those of young sclerotia. (Figures 4 *A* and 5 *A*). Granular portions of these pseudo-sclerotia when broken out and transferred to various media always gave the characteristic development.

Cooked Bean Pods There was abundant development on cooked bean pods with formation of many rounded perfect sclerotia which were as a rule larger than the majority of those formed on *C. canadensis* in the field. The sclerotia were always external to the pod. Sections of the bean pods thus infected were made and it was found that the mycelium penetrated the cell walls and coiled about in the cells. The visible effect on the pod was simply that of shriveling. The mycelium grew vigorously for several months, penetrated to every part of the pod and seeds and there was also considerable aerial development.

On sterilized culms and leaves of *C. canadensis* and *Dactylis glomerata* the fungus grew vigorously and many rounded mature sclerotia were produced.

Bouillon There was a slow but considerable aerial growth on bouillon which spread over the surface of the medium. No sclerotia were formed.

Nutrient Gelatin An abundant growth was obtained on this medium, but only a few small sclerotia were developed.

Cooked Potato The growth spread over the surface of cooked potato as a thin but rather compact granular layer which was similar to the pseudo-sclerotia produced on the hard potato agar.

Ashby's Medium When this was poured on pure quartz sand and inoculated there was a steady growth of mycelium

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[4] Hard potato agar: 1000 cc. water, 200 grams of sliced potato tuber; cook in steamer 1 hr.; strain off clear liquid and restore to 1000 cc.; add 20 g. glucose and 30 grams agar; cook in steamer 1 hr., filter, autoclave.

which spread over the surface with considerable aerial development. The development of sclerotia was, however, feeble.

Diastatic Action To test the action of the fungus on starch the following method was used. To each of three tubes of hard potato agar and three tubes of lima bean agar, there was added one gram of finely powdered potato starch. The tubes were placed in an autoclave until the medium was liquid when the starch was thoroughly mixed by shaking and then poured into Petri dishes. A transfer of mycelium was made to each of the plates and an unusually vigorous growth of mycelium resulted. When this growth covered an area of about 4 cm. in diameter iodine in potassium iodide solution was poured into four of the plates, two of each medium, and allowed to stand several minutes. When this was rinsed the portion covered with the mycelium in all four plates showed white with only an occasional granule of blue, while the entire surface of the medium outside of the border of the fungus was a deep purple. Thus it is clear that the fungus can digest cooked starch in culture media. The other two plates were allowed to develop at length and on these, the fungus made an unusually vigorous growth. It rapidly spread over the entire surface of the medium and up the sides of the dish to the cover. Many small sized sclerotia and several unusually large ones were formed. These developments showed that the addition of starch to culture media increases the vigor of growth and the formation of sclerotia.

Pure Cultures in Soil The fungus was grown on ordinary marsh soil by the following method: masses of the top layers of peaty soil upon which infected plants of *C. canadensis* had been growing were placed intact, (with various grass roots, etc., included as in the soil) in test tubes and bottles which were plugged and sterilized in an autoclave at 15 pounds for twenty minutes. Transfers of mycelium from pure cultures to this soil resulted in a steady healthy growth of mycelium which penetrated the loose soil in all directions (See Figure 5 *B*). Many rounded sclerotia some of which were 5 m m. in diameter developed both at the surface and at various depths in the tubes of soil. Soil cultures of this description are now being used for experiments to test the infection of young seedlings.

Characteristics of the Fungus; Discussion and Comparisons

The fungus *Sclerotium rhizodes* in its relations to the perennial grasses studied exhibits a combination of characteristics not hitherto ascribed to any one fungus.

In the aerial parts of the host the behavior is somewhat similar to that of various smuts, especially as shown by the recent studies of Lutman (1910, p. 1204). He found for *Ustilago levis* on oats that in the growing plants the mycelium is most abundant at the nodes and in the growing points and that many leaves contained the fungus to their tips. The mycelium remains in or near the growing points but is intercellular.

McAlpine (1910, p. 10) has pointed out the tendency for the mycelium of *Ustilago nuda* on barley to persist in the underground portions of the plants and penetrate into new culms which were induced by repeated cutting of the old culms.

The seed fungus of *Lolium temulentum* as described by Freeman (1903, p. 14–16) shows quite a different behavior. Here the fungus seldom enters the roots and leaves, but lingers in the growing points and finally enters the nucellus layer of the seed from which it later infects the germinating embryo. There is, however, no destructive effect on the seed; in fact, Freeman notes (page 5) that infected seeds are larger and better developed, suggesting a symbiotic relationship which Hiltner's (1899, p. 835-837) work seems to show is due to the fixation of atmospheric nitrogen by the fungus.

Miss Ternetz (1907, p. 359) in her studies of various mycorrhiza finds the seeds of the macrosymbionts infected and raises the question as to the source of this infection. In *Andromeda polifolia* she observes mycelium in the rind of the older twigs which had reached this point by the coordinated growth of the plant and the mycelium. Thus she finds the mycelium wandering from roots into aerial parts, but she notes that it avoids chlorophyl bearing cells. Since she did not find mycelium in the flower stalks she believes that the source of the infection in the flowers is external, yet it seems probable from the positive evidence given above, that this infection of the fruit comes vegetatively from the roots.

As to its distribution within the host, *Phoma radicis Andromedae*, as described by Ternetz seems to be intermediate be-

tween strictly root inhabiting mycorrhiza and *Sclerotium rhizodes* on *Calamagrostis canadensis* as described.

Turning to the behavior of *Sclerotium rhizodes* in roots and underground stems, we find that its morphological characters and host relations are such that it might be considered as a mycorrhizal fungus. This term, however, has been so loosely applied to root inhabiting fungi that it has at present little specific significance. Following the rather limited observations of Kamienski (1881 and 1882), Frank (1885 and 1887), and Woronin (1885), the more extensive studies especially by Schlicht (1889), Janse (1896), and Stahl (1900) have shown that root inhabiting fungi are present in a large number of flowering plants. So called mycorrhiza have also been found in the tissues of certain cryptogams as has been shown especially by Atkinson (1893), Janse (1896), Nemec (1899), Lang (1899) and Campbell (1908).

It has been established that annuals, biennials, and perennials growing in all sorts of soil, belonging to a wide range of families and exhibiting autotropic as well as holosaprophytic modes of life, may possess root inhabiting fungi of endotropic or ectotropic character.

Schlicht (1899, p. 26) includes in his list of species possessing endotropic mycorrhiza the grasses *Holcus lanatus*, *Festuca ovina*, *Agrostis caninam*, *Aira caespitosa* and *Triodia decumbens*. To these Stahl (1900, p. 551, 552) adds the following grasses: *Sesleria coerulea*, *Agrostis alpina*, *Brachypodium pinnatum*, *Molinea coerulea*. It appears that with the exception of *Holcus lanatus* none of these grasses have been investigated as to the character and behavior of their mycorrhizal fungi. In fact but few of the large list of species possessing so-called mycorrhiza have been thus studied and it is by no means clear that there is a sharp distinction between symbiotic mycorrhiza and the parasitic and saprophytic forms.

Schlicht (1899, pp. 17-18) has described somewhat in detail the root fungus which he found in the grass *Holcus lanatus*. Here the fungus coated the exterior of the finer roots with a thin sparsely distributed felt of dark brown mycelium from which fibers penetrated into the inner cortex where they formed a sort of mantle about the central cylinder. He found knots of filaments, swollen portions of the mycelium, and in the older parts of the root irregular masses of disintegrating fungus. He

noted that the cell nucleus can exist in the same cells in which there is a plexus of filaments. Thus far his description and drawing (1899, Figure 12) shows almost identically the same conditions found by the writer in the roots of *C. canadensis*. The manner of penetration and the general character of the fungus seem identical in both cases. Schlicht, however, considered that the fungus in *Holcus lanatus* was limited almost entirely to the small roots. Although he noted the presence of the fungus in the older parts of the main roots he considered that here the mycelium went into a resting condition or died, or at any rate lost its mycorrhizal character. He found no evidence of parasitic behavior in the roots or of abnormal conditions and he argues at length for a symbiotic relationship. He did not discover any mycelium in the rhizome, bud or leaf as it exists in *C. canadensis*.

Schlicht collected his material about the year 1888 in the vicinity of Berlin, a region in which *Sclerotium rhizodes* had already been found on a number of grasses. It is also to be noted that in 1891 the fungus *Sclerotium rhizodes* was collected (Krieger, Fungi Saxonici No. 1399) on *Holcus lanatus* within 100 miles of Berlin. In view of the results here reported, it seems probable that the root fungus as described by Schlicht is *Sclerotium rhizodes*. The presence of the fungus on the leaves as it appears in Wisconsin on the various grasses might easily be overlooked, especially late in summer, unless there was a severe epidemic. Even when noticed on the leaves one would not be likely to associate it with a root inhabiting fungus.

Groom (1895) working on *Thismia Aseroe*, which is a holosaprophyte, finds a still more marked differentiation of the fungus in the various tissues. He does not observe any destructive effects due to the fungus. Conspicuous bladders and coils of mycelium are formed at the expense of certain host cells, but these he considers break down when mature thus returning to the cells certain food materials after which the cells resume their normal activity. According to this the vesicles here formed do not furnish food to the mycelium outside of the particular cells in which they are situated.

Magnus (1900) described the conditions which exist in the non-chlorophyl bearing plant *Neottia nidus avis*. In the cortex of the roots and the rhizome he finds an endotropic mycorrhizal fungus. In certain cells the fungus destroys the protoplast and

forms thick walled hyphae and bladder-like structures which he considers to be organs for storage of food. In the outer and inner layers of the zone of infection, however, he finds digesting cells in which the fungus is for the most part digested. Thus he finds that the fungus is locally parasitic in some cells and that in others it yields its substance to the host cells which continue to live.

Shibata (1902) finds much the same condition in Podocarpus, Alnus, and Psilotum. In the digesting cells of the last two named the wall substance of the fungus remains as densely staining clumps. In the cells destroyed by the fungus he notes the formation of vesicles which are filled with food.

Arzberger (1910) has recently investigated the root tubercles of *Ceanothus americanus*, *Elaeagnus argentea*, and *Myrica cerifera*. He finds in Ceanothus that hypertrophied cells and nuclei result from the infection, that the cell walls of host cells are dissolved and that later the cytoplasm and nuclei of these cells are absorbed. Soon the cell content of the fungus disintegrates, but not until the contents of the host cell are used up. In the root tubercles of the above named species he finds ultimately both the host cell and fungus die "as a result of their previous relationship," but he concludes that the material is in some way used by the adjoining healthy uninfected cells.

Regarding the vesicles or "sporanges" which are formed, Arzberger finds them to be terminal, spherical or pear shaped bodies. In Ceanothus and Myrica their contents break up into a few segments by a process which he considers analogous to spore formation in other fungi.

PHYSIOLOGICAL SIGNIFICANCE

In all of the cases described by these authors, with the exception of Schlicht, in some cells at least the fungus is wholly or in part destructive. They all find evidence, however, of a symbiotic relationship in the digesting cells or in the benefit which the surrounding cells may gain. The anatomical evidence for symbiosis in the case of endotropic mycorrhiza seems to rest on the observed ability of individual cells to recover from the invasion of the fungus or in the ability of adjoining cells to profit by the activities of the fungus though it may be destructive of certain individual cells.

The characteristics of these endotropic fungi within the cells during the early stages of infection and the formation of bladders as described by Magnus (1900, pp. 10-19), Shibata (1902, pp. 646, 657-660) and most especially by Groom (1895 pp. 333, 334 and 338-340) bear some noticeable resemblance to *Sclerotium rhizodes* although it is to be noted that the macrosymbionts referred to above range from autotropic to holosaprophytic plants.

The later developments of the *Sclerotium rhizodes* in the roots suggest a somewhat different relationship. I find no conclusive evidence that there are here digesting cells which overcome the fungus and resume a normal appearance after infection. A cell is never entered by the mycelium until it has ceased to divide and until it has reached nearly its maximum size. An immediate result of the invasion of such a cell is the accumulation of a large amount of densely staining fine granular cytoplasm as is shown in Figures 7 *C* and *G*. Soon coarse irregular shaped solid bodies appear which are like those described by Magnus and Groom and assumed by them to be the disintegrating fungus. Here, however, these bodies appear while the mycelium is intact and healthy. These clumps are plainly the products of the host cell; perhaps resulting from its stimulated activity. The healthy mycelium can often be found passing through empty cells in close proximity to cells showing these "clumps." The bladders are often of such size or number that they completely fill the cells leaving no trace of cell contents. There are furthermore fungus filaments which give direct connection between the mycelium and the bladders in different cells. When empty the thick heavy walls of the bladders remain for the most part rigid and intact.

These conditions lead one to the conclusion that in this case the intercalary bladders are formed at the expense of the host cells and that the food thus stored is used by the mycelium in other portions of the host tissue.

From the histological and cytological studies which the writer has made of the fungus, it appears that its relation to the individual cells of the roots and the rhizomes is one of mild parasitism and that the nearly mature cortical cells are invaded and the cytoplasm digested. As already shown the fungus is still more destructive in the leaves.

In certain areas of marsh meadows the Calamagrostis plants do not harbor the fungus. At least it does not appear on the leaves and thus far the study made of the roots does not show its presence there. The best development of the grass is in these fungus free areas.

It has been pointed out that a large number of culms are killed by the fungus especially in the early spring. The effect of this is marked in several areas that have exhibited general infection during the past four seasons. In areas where in 1908 there was a vigorous growth of Calamagrostis with considerable fungus appearing on the culms and in which the appearance of the fungus was still more marked in 1909 and 1910, there was in 1911 a marked thinning out of the grass. The observations in the field therefore agree with the conclusions based on the relations found in the roots: viz. that the fungus is perennial in the roots with injurious effect.

The original theory of Kamienski (1881) was extended by Frank to apply to many more cases of fungi in roots. Frank (1885, p. 33) maintained that the fungus conducts and prepares food for the host and especially that it assimilates humus compounds. He considered that even purely endotropic mycorrhizal fungi receive food from external sources and that the root contained, as it were, a trapped fungus, the host ultimately digesting the fungus (1892 p. 267).

Groom's view (1895, p. 354 and 356) differs in that he considers that the fungus in Thismia first absorbs food from the cells, that the fungus is not completely digested by the host cells, and that in the outer layers it "actually profits by the symbiosis" (1895, p. 356).

Stahl (1900) argues at length in favor of the view that both ectotropic and endotropic mycorrhiza furnish supplies of elaborated organic compounds from without. He attempts to show that the host plants in such cases are weak in photosynthesis and that they also manifest a decrease of ash content on account of this weak power of assimilation.

In the case of *Sclerotium rhizodes* in the roots of *C. canadensis* it is clear that the fungus filaments do pass out into the soil where there is reason to believe that they can thrive saprophytically. It is possible that these filaments may fur-

nish the endotropic portions with various mineral salts and organic compounds which may at first be drawn upon by the host cells thus accounting for the enlarged cells and the dense cytoplasm found in spur rootlets during the early stages of infection. Admitting the possibility of a benefit of this sort, it is certain that the ultimate effect of the fungus both on individual cells and on the entire plant is that of a parasite especially when conditions favor the development of the fungus on the leaves.

There have been numerous investigators who have noted parasitic behavior in various mycorrhizal fungi. Reess (1885) studied Elaphomyces on pine and believed that the fungus obtains food from the roots of the pine. Hartig (1886) opposed Frank's views of symbiosis and considered that the fungi in question were parasitic. Nadson (1908) found "mycorrhiza" penetrating roots and killing large numbers of oak seedlings. Boulet (1910) reports the presence of endotropic mycorrhiza on almond, apricot, peach, cherry, plum, apple, pear, etc., and states that the fungus lives as a parasite but still has a beneficial effect on the host except when it attacks the roots with unusual vigor.

It is such evidence as this that indicates the unsatisfactory state of our knowledge concerning the intimate physiological relations which exist when a fungus resides as is the case in endotropic mycorrhiza within living plant tissues. After all we judge parasitism and symbiosis by rather gross general results.

Possibly the same fungus may under different conditions manifest varying degrees of parasitism or of symbiotic relationship. A mycorrhizal fungus on an oak may be at one time symbiotic as Frank maintained and again the same fungus may be parasitic as Nadson reports.

On this point the behavior of *Sclerotium rhizodes* is especially interesting. Judged solely by its behavior in the roots it might be considered as entering into symbiotic relationship. At any rate it is not here apparently seriously parasitic. It may bring into the root various mineral and organic food substances which may in part be appropriated by the root tissues. There is no evidence, however, that the fungus is itself digested. In the leaves, however, it is vigorously parasitic. At the time when the culms have elongated there is no direct

connection between the mycelium in the leaves and that in the roots and it can not be considered that the vigorous development of the fungus in the leaves can supply the underground mycelium with food. The mycelium is perennial in the roots and probably in the soil, but is rather short-lived in the leaves. The vigor of the parasitic development on the leaves varies considerably with the season and with the species of host.

It is certain that the general effects of the fungus on the grasses here discussed is that of a parasite.

ECONOMIC SIGNIFICANCE

The occurence of this fungus on *C. canadensis* decreases the yield and quality of the hay obtained from marsh meadows. When the infection is as high as 47 per cent, as was the case in 1911 on the marsh meadow most carefully studied, this loss is considerable. The ability of the fungus to infect and injure such important grasses as *Phleum pratense* and *Poa pratensis* is also a matter of economic significance although the injury to these two grasses is not serious at present, so far as observed.

Cultivation of the soil and crop rotation naturally suggest themselves as a means of control for this fungus. This would be practicable in case the fungus becomes destructive in upland meadows. On nearly all the meadows where this fungus has been found tillage is now impossible and until improved drainage makes cultivation possible the fungus will necessarily run its course. The observations made thus far indicate that *Agrostis alba* is not attacked and if this be true the introduction of this grass into infected marsh meadows might be advisable. The susceptibility of *Calamagrostis canadensis* to attacks of this fungus makes the use of this species on semi-reclaimed marsh lands of doubtful value.

SUMMARY

The fungus *Sclerotium rhizodes* attacks the leaves of various grasses causing them to become dry, rigid and bent into characteristic crooks. Upon the leaves appear felts of mycelium from which sclerotia develop.

The development on the leaves is most vigorous during April and May when the death of many entire culms results.

At Madison, Wis., the fungus has been found on eleven different grasses: *Phalaris arundinacea, Calamagrostis neglecta, Poa pratensis, Panicularia nervata, Phleum pratense, Hordeum jubatum, Bromus ciliatus, Eatonia pennsylvanica, Agropyron caninum, Agrostis hyemalis,* and *Calamagrostis canadensis.*

It is especially injurious and destructive to *Calamagrostis canadensis* which serves as its principal host.

The fungus is vigorously parasitic in the leaves, less so in the buds and the stems, and but slightly so in the roots where it assumes some of the characters usually associated with mycorrhiza.

Fungus filaments extend out into the soil where they live more or less independently.

The mycelium is perennial in the soil and in the underground parts of the infected plants.

The infection of aerial parts occurs from the underground parts of the plant.

The mycelium is sterile so far as has been observed to date.

The production of spore bearing structures from sclerotia has never been observed.

The fungus is of considerable economic importance. It destroyed or dwarfed as many as 47 per cent of the plants of *Calamagrostis canadensis* on one marsh meadow near Madison in the season of 1911.

This fungus is quite generally distributed throughout Wisconsin. It has not been reported elsewhere in America; presumably it is quite widely distributed, however. Its parasitic attacks on the leaves of various grasses has long been known in Germany, Belgium and Scandinavia.

Further data are desired on the following points: additional host plants, geographical range of the fungus, economic importance, germination of the sclerotia, and infection of seedlings.

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EXPLANATION OF FIGURE 1

This photograph shows the habit of growth of *Calamagrostis canadensis* and the general effects of the fungus *Sclerotium rhizodes* as it appears early in the season. Three of the culms here shown were badly infeeted, the leaves were shriveled and rolled especially toward the tips, the charaeteristie erooks were present and the felt of myeelium ean be seen in the pieture at points marked *m*. Young selerotia were present at points marked *s*. The eulm whieh stands seeond from the terminal bud of the rhizome had escaped infeetion.

The investigations show that in such plants as this the fungus is present in the leaves, stems, buds, and roots and that the myeelium is also present in the soil and on the roots.

Explanation of Figure 2

A life sized picture of a rapidly growing culm of *Calamagrostis canadensis* which was vigorously attacked by the fungus. The appearance of the crooks and their method of formation are here well shown. The tips of the unfolding leaves were penetrated by the mycelium which held them together in a roll and hence as the leaves developed they arched upward. Often the growth tensions are such that a portion of the crook is twisted and folded as is the case here. Death of the entire culm soon results from such a vigorous development of the fungus.

Explanation of Figure 3

A. Upper portions of two older culms of *Calamagrostis canadensis* showing conditions somewhat different from the previous figures. In the left hand figure at point marked *m* is seen a partial lateral infection of a leaf. This is a common phenomenon. The right hand figure shows a complete series of crooks, five in number. The tips of the leaves are held at the points lettered. These figures illustrate the fact that every leaf of an infected culm possesses the fungus and that the mycelium gains entrance to the leaves when they are in the bud. While the tips of the leaves here shown are completely destroyed there is a less vigorous development of the fungus than is shown in Figure 2. This condition is seen during the middle of the season, especially during a rather dry period when few sclerotia are formed. Such culms may grow for some time but seldom produce flowers and seeds.

B. Portions of *C. canadensis* culms and leaves showing typical matured sclerotia as they are produced in the field under favorable conditions. Culms such as are shown at left hand side soon die from the attack of the fungus. (Natural size)

Explanation of Figure 4

A. A typical culture of *Sclerotium rhizodes* on hard potato agar showing abundant growth of mycelium and the crusted pseudo-sclerotia. Culture is ten months old.

B. Culture on lima bean agar nearly ten months old. The mycelium is less abundant than in *A* but the sclerotia are more perfect.

C. Development of the fungus on bean pods with a few well formed sclerotia and abundant mycelium.

EXPLANATION OF FIGURE 5

A. Two cultures, 15 months old, on hard potato agar. The mycelium is still quite vigorous and the large compound irregular shaped sclerotia are mature.

B. A pure culture of the fungus on peaty marsh soil upon which infected *Calamagrostis canadensis* had been growing. The soil with various grass roots included was sterilized and fragments of the mycelium from test tube cultures were placed on the soil. At the time the photograph was taken this culture was ten weeks old. The picture shows the mass of white mycelium that covered the surface while toward the bottom may be seen clusters of the mycelium which also spread quite generally through the soil mass. These soil cultures demonstrate that the fungus can live as a saprophyte in the soil.

EXPLANATION OF FIGURE 6

All drawings of this Figure are from *Calamagrostis canadensis.*

A. Part of a cross section through such an infected leaf roll as is shown at *m*, Figure 3 *A*. The drawing is from the outer leaf of the roll. The mesophyl of the leaf is entirely destroyed. (X 100).

B. Drawn from the center of a badly infected roll showing destruction and collapse of the tissues of the bundle. *A* and *B* show the conditions which prevail in the outer and inner portions of an infected roll of leaves. (X 140).

C. From the same cross section as *A*, but from the border of the infected area showing the mycelium advancing through the tissues. (X 100).

D. Distribution of the mycelium about the growing points of a terminal bud of a culm the expanded leaves of which showed infection such as seen in Figure 1. (X 100).

E. The bud from which *D* was drawn. Showing the distribution of the mycelium.

F. From a cross section of a stem near a node. This particular culm was two feet tall. Its leaves were infected. A node situated immediately below the surface of the ground with its bud was fixed during the first week in August. At that time the culm was mature. The bud was one which would develop during the next year. The mycelium was present in the outer layers of the culm as shown in the segment drawn. Drawings similar to this could be shown for the various nodes of infected culms. (X 100).

G. Portion of the bud mentioned above drawn in its relation to the stem *F* and from the same cross section. The mycelium is present in the tissues of the bud leaves as well as between the leaves where it remains over winter. (X 100).

Explanation of Figure 7

All from *Calamagrostis canadensis* unless otherwise stated.

A. Portion of a basal node showing the extent of penetration by the mycelium and the vesicles which are formed. (X 100).

B. Drawn from a longitudinal section through a basal node and its bud. *a* is a part of the node and *b* is from the outside leaf of the bud. The mycelium is here shown passing from stem to bud. Material was collected during the summer. (X 100).

C. From a longitudinal section of a fibrous root. Shows condition of cells soon after penetration by the mycelium; the nuclei are in various stages of degeneration and there is an accumulation of dense material in the cytoplasm. (X 140).

D. Cross section of an older root (the main root of Figure 8 *D*) showing mycelium and vesicles in empty cells. (X 100).

E. Cross section of a spur or lateral root. Shows the tendency of the fungus to develop in the innermost layer of the cortex. (X 140).

F. Mycelium in practically empty cortical root cells. The densely staining granules are the remains of the protoplasm of the host cell. Mycelium is intact. (X 140).

G. From a cross section of a lateral root of *Calamagrostis neglecta*. Mycelium is here seen on the surface of the root. Formation of vesicles, accumulation of dense protoplasm and abnormal nuclei are here shown. (X 140).

H. From root of *Poa pratensis*. Mycelium in the cortex. (X 100).

I. Early stages of vesicle formation. Nuclei and cytoplasm of host cells nearly disappeared. Later stages are shown in Figure 8, *F* and *G*. Figure 7, *C*, *E*, *F*, and *I*, and Figure 8, *F* and *G* present a series showing the stages in the development of the mycelium and vesicles, together with the accompanying disintegration and disappearance of the protoplasm of the host cell. (X 140).

J. A few cells of the mycelium grown on cooked potato showing the prevailing two-nucleated condition. (X 630).

EXPLANATION OF FIGURE 8

A. Drawing (X 140) of the mycelium as it develops on cooked potato. The oidium-like cells do not function as spores, but are similar to the cells in a young sclerotium.

B. Characteristic mycelium from the surface of a leaf of *C. canadensis* (X 140).

C. Drawing (X 140) of mycelium which developed from the cut end of an infected root.

D. A portion of an infected root of *C. canadensis* is here shown (X 10) with the enlarged spur rootlets and the mycelium as it exists in the soil and on the surface of the roots. The large bladder-like vesicles as they appear in the soil are here shown.

E. A small portion of a rootlet (X 100). The mycelium is here shown adhering to the surface of the rootlet and penetrating into the root tissues at two points.

F. Typical vesicles as they appear within cells of the cortex of roots of *C. canadensis* (X 175).

G. A single spherical vesicle within a cell of a root of *C. neglecta* (X 175).

H. Diagrammatic cross section of a leaf which is laterally infected. The dark portion represents the distribution of the mycelium. The tip of a second leaf is shown within the roll.

Figure 1. C. canadensis, showing the general symptoms of the Sclerotium disease.

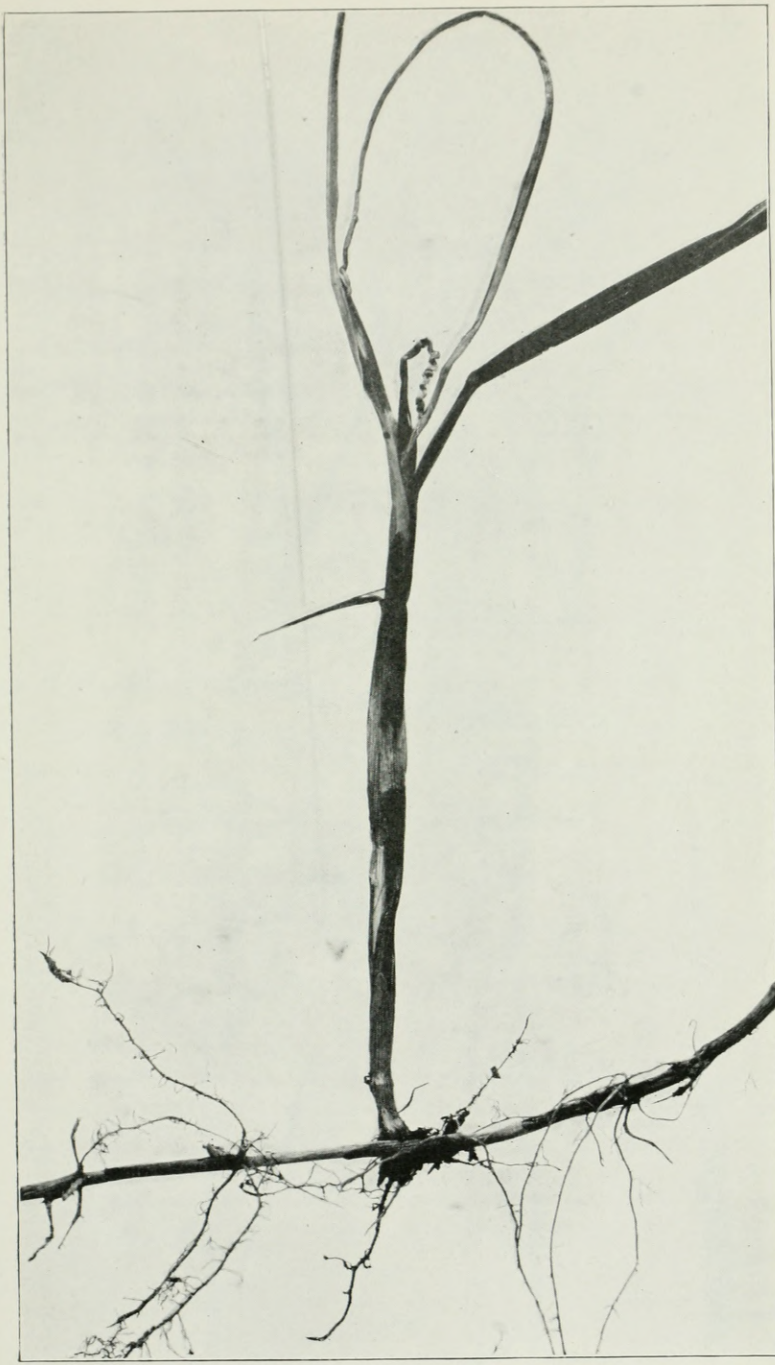


Figure 2. The characteristic "crooks" resulting from a vigorous attack of the Sclerotium

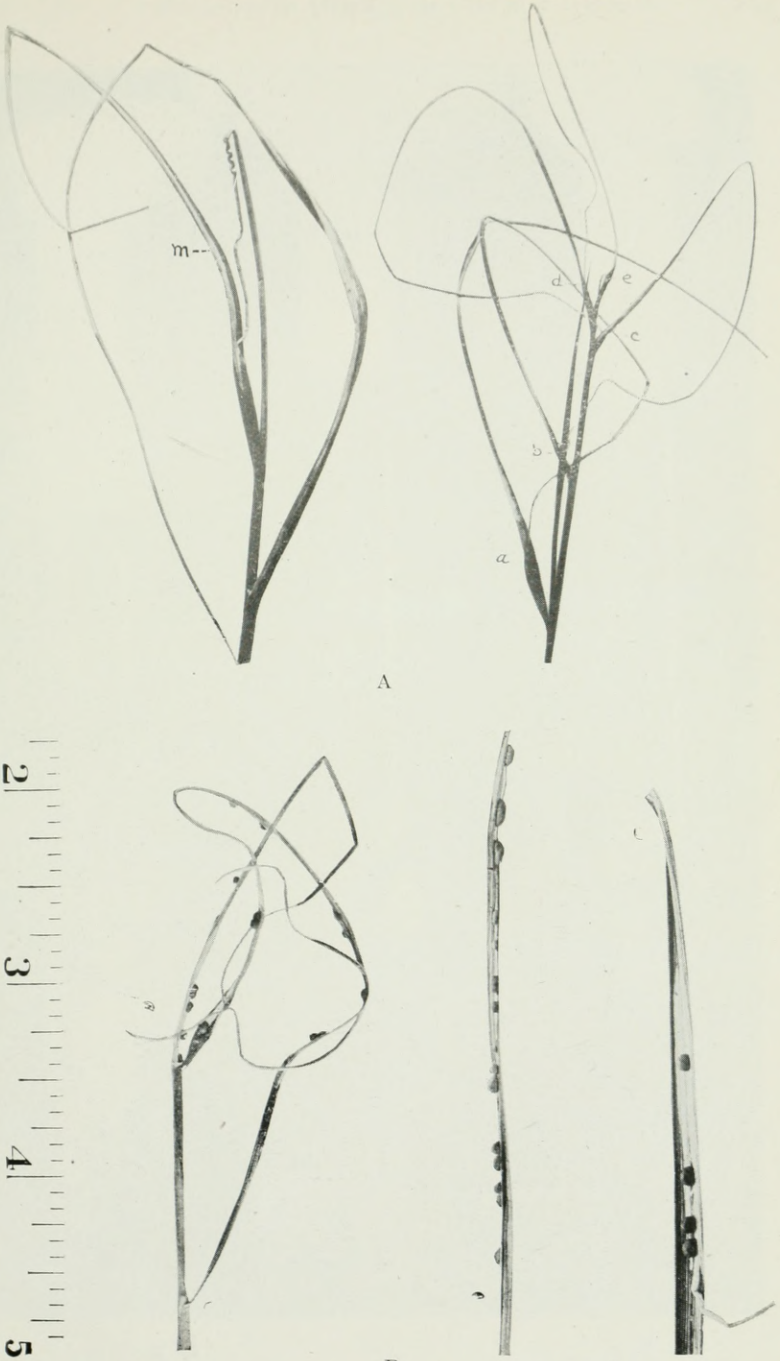


Figure 3. Illustrations of various leaf symptoms associated with the Sclerotium disease

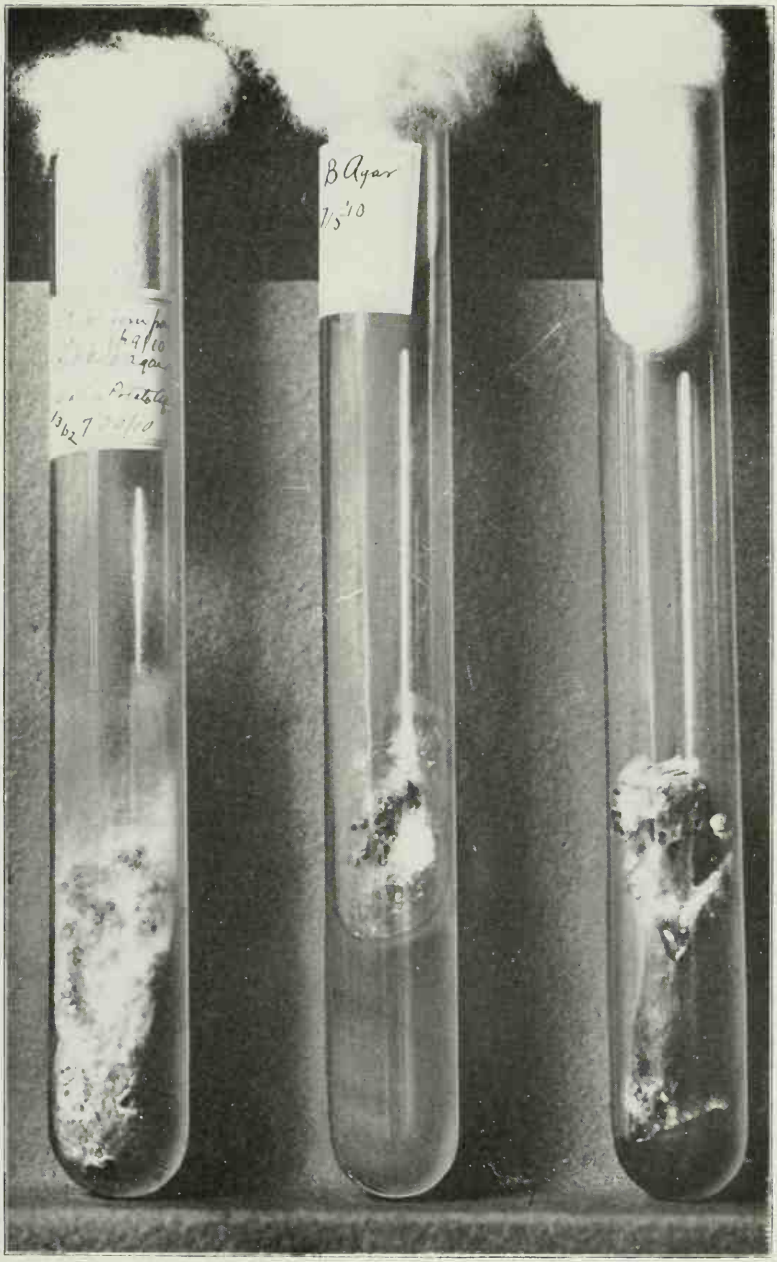
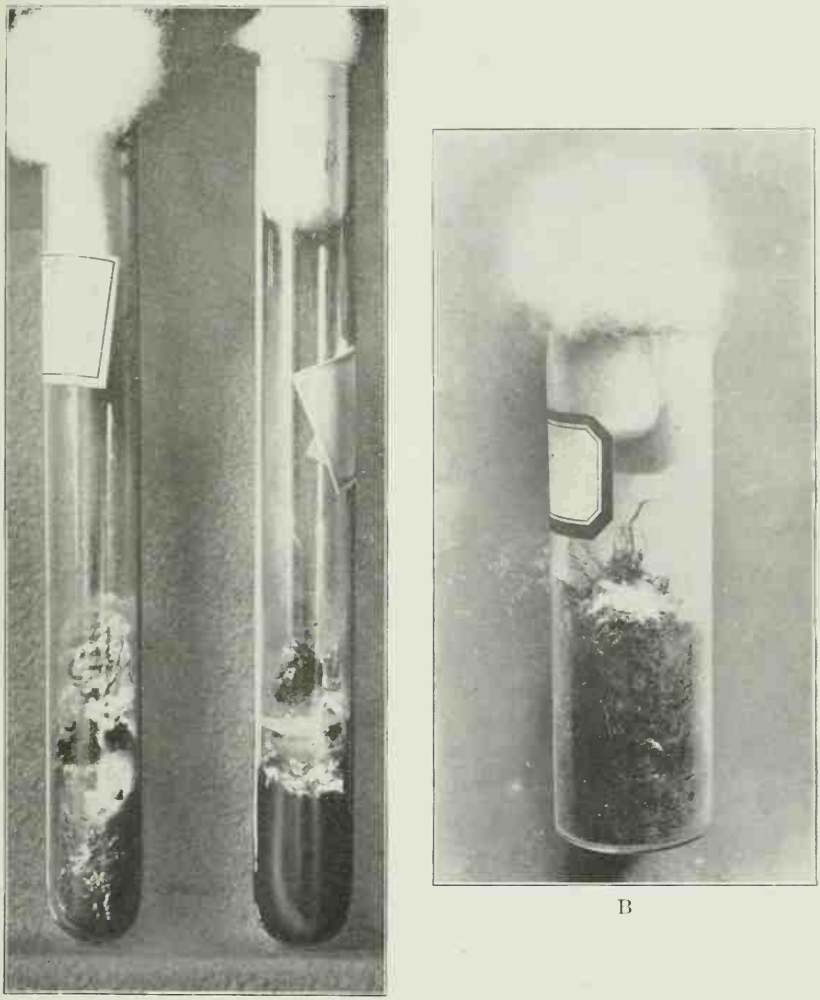


Figure 4. Pure cultures of Sclerotium rhizodes on various media



A B

Figure 5. Cultures of the Sclerotium; A showing the large sclerotia; B the mycelium in soil

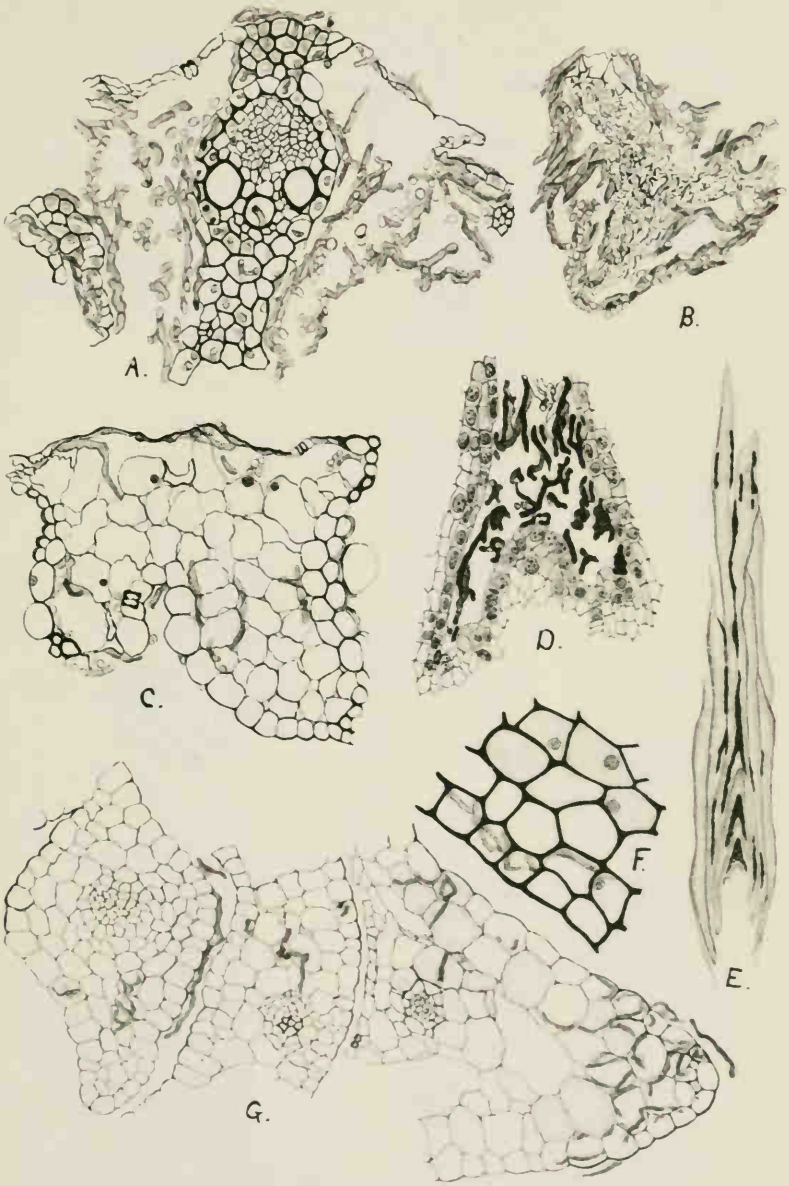


Figure 6. The mode of tissue invasion of grass leaves and stems by the Sclerotium mycelium

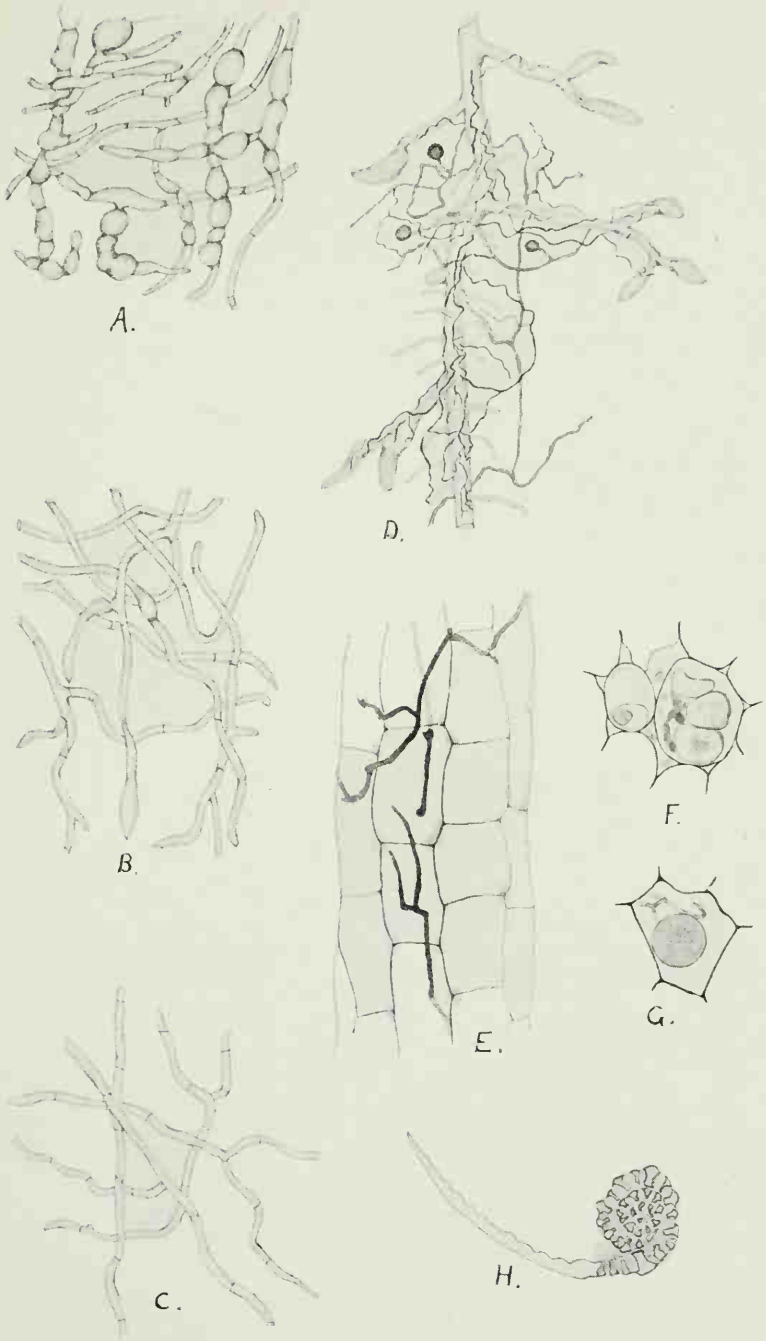


Figure 8. A–C, mycelium of the sclerotium; D–G, infected roots; H, infected leaf

Research Bulletin 31 March, 1914

The Control of Damping-Off Disease in Plant Beds

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CONTENTS

The Control of Damping-Off In Plant Beds[1]

JAMES JOHNSON

Introduction

The widespread occurrence and economic importance of soil organisms is at present becoming fully recognized. How to treat the soil in such a way as to destroy the undesirable soil organisms without injuring the productivity of the soil has become one of the great problems of the science of agriculture. The subject is complicated by the fact that, in addition to the harmful organisms, the soil contains a number of organisms which are desirable and frequently essential to the permanent productivity of soils. Most soils harbor fungi, insects, bacteria, and other lower forms of living organisms which are injurious to one plant or another. Many of these pests are obligate parasites and occur only where the host plant has been previously grown. In such cases crop rotation is, in most cases, a long but sure remedy. Some of the organisms, however, including the common damping-off fungi, are peculiar in that they are parasitic upon a wide range of hosts, besides being capable of existing saphrophytically upon the vegetable matter in the soil. Crop rotation is hence practically valueless as a remedy against these fungi.

The most noticeable occurrence of the damping-off disease is in plant beds and forcing houses, where large numbers of plants are grown closely together. The amount of injury arising from this intensive system of plant culture requires a rapid and efficient means for the control of the disease. The last two decades have witnessed the recommendation of a number of meth-

[1] The writer is greatly indebted to Prof. L. R. Jones of the Department of Plant Pathology of this Station for many helpful suggestions received during the progress of this study.

ods for the control of damping-off, many of which are reported in popular literature. The purpose of the experiments described in this paper has been largely to compare these methods. The work on this disease was at the outset primarily conducted for the purpose of controlling damping-off of tobacco seedlings. Other plants were used in some cases to facilitate the work, but the principles apply equally well in a general way to all plants grown in seed beds and subject to the disease. A large portion of the experiments have been conducted in the forcing house, and the results are applicable therefore not only to damping-off in plant beds but also, in a large measure, to the disease in forcing houses. Tobacco growing in Wisconsin is an industry which returns approximately $6,000,000 annually to the growers of the state. The damping off disease is of frequent occurence in the plant beds and causes a considerable loss in the destruction of seedlings. Indirectly, such losses result in delayed transplanting, transplanting of inferior plants, and sometimes in reduced acreage, making the control of the disease of considerable local importance. Experiments on the control of damping off by several investigators on various crops and soils have indicated some variations in results, possibly due to differences in soil. Sandy loam and clay loam soil containing considerable humus have been used in the experiments cited in this bulletin, and hence the results apply particularly to those types of soil. The studies have been mainly confined to damping-off by *Pythium debaryanum* (Hesse), though the same disease produced by Rhizoctonia has also been studied in this connection sufficiently well to warrant the assumption that from a practical standpoint the control methods apply equally as well in both cases. Before taking up the subject of control it is well to mention briefly some of the characteristics of the casual organisms.

THE CAUSAL ORGANISMS

Pythium debaryanum (Hesse) was first described and named by Hesse in 1874 (12) at which time he pointed out the destructiveness of this fungus as a parasite of seedlings. As early as 1881 De Bary (6) believed it generally present in garden soils of Europe and extended the list of host plants named by Hesse. Ward (32) in England, found it common in the garden soils of that country and studied its reproduction and relation to the

host tissue. More recently, the economic importance of this fungus has been emphasized in the United States by Atkinson (1) as causing the damping off of seedlings.

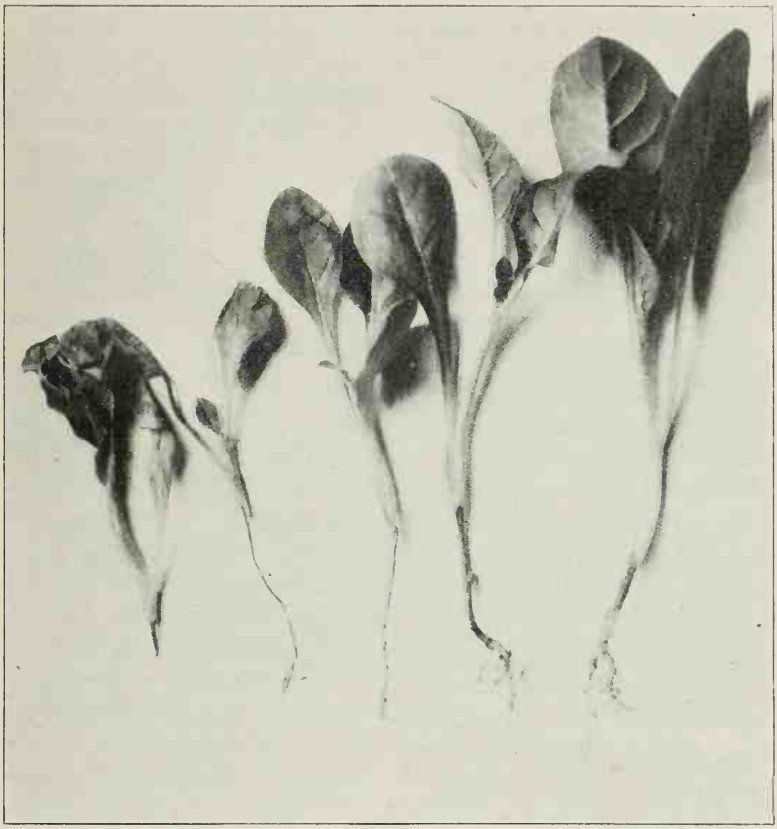

FIGURE I. TOBACCO PLANTS ATTACKED BY PYTHIUM DEBARYANUM.
Various stages of the disease are shown.

The attacks of this fungus are very largely confined to young plants. The fungus may, however, under exceptionally favorable weather conditions kill the embryo in the seed or grow upon old plants. The zone of first attack is practically always confined to the hypocotyl, either just at, or below the surface of the soil (Figure 1.) Under favorable conditions, however, the disease rapidly spreads up the stem of the plant and into the midrib of the leaves. The characteristic appearance of a seed

bed attacked by damping-off is a bending over and wilting of the diseased plants.

Pythium debaryanum is quite readily distinguished in diseased areas from most other damping-off fungi. The coarse, non-septate, highly granular, irregularly branching mycelium (Figure II,) can be easily observed on the interior and exterior of the host tissue when bits of the freshly infected tissue are teased apart and examined under the microscope. Ordinarily, the diameter

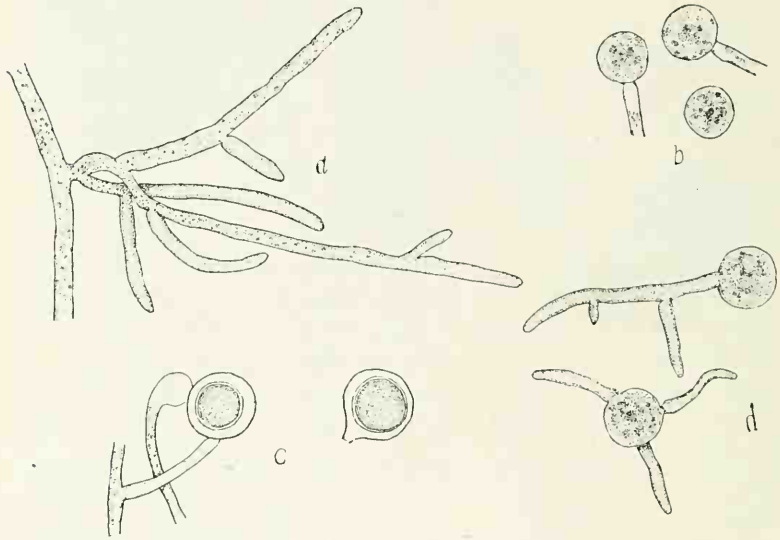

FIGURE II. MICROSCOPIC CHARACTERS OF PYTHIUM DEBARYANUM.

A, mycelium; b, conidia; c, oospores; d, germinating conidia.

of the hyphae will be found to vary considerably, the older hyphae usually being the larger. The very young portions of hyphae are frequently finely granular, but become coarsely granular with age, while the oldest hyphae are usually emptied of their contents. The mycelium penetrates the cells of the young and succulent tissues readily, seeming particularly adapted to passing through cell walls and obtaining nourishment from the interior of the cells, whereas in tissues damped off by Rhizoctonia the hyphae are more generally found between the cells. Where they pass through the cell wall the diameter is characteristically much reduced.

Reproductive bodies are not ordinarily found in damped off plants, though they may be produced quite frequently in certain

species of plants. For the study of reproductive bodies it is however, more convenient to obtain the fungus in pure culture upon various media. Thaxter's potato hard agar in petri dishes yields an abundance of conidia when inoculated with *Pythium debaryanum.* Oospores were found to be produced in great abundance upon sterile slices of green cucumber in petri dishes, or upon very young tobacco seedlings grown upon filter paper in petri dishes and inoculated with Pythium soon after germination. Zoospore producing bodies were not obtained upon any of various media tested for that purpose. The importance of the reproductive bodies in spreading the disease, or in carrying the fungus over unfavorable periods, seems comparitively unimportant, but this matter requires further study before anything definite can be said upon it.

Rhizoctonia was first described by De Condolle (7), in 1815, as a root destroying fungus. Since then it has been described as a root parasite upon a wide range of host plants in Europe. It was not until the early nineties that the fungus came to be recognized as a destructive parasite in field crops and plant beds in this country. Pammel (16) described Rhizoctonia as causing a root-rot of beets in 1891, and Atkinson (2) mentioned it as causing a damping-off of cotton in 1892 in Alabama and of various seedlings in New York (1) in 1895. Duggar and Stewart (8) have treated in some detail the occurrence of this fungus upon a large number of hosts in this country.

The genus Rhizoctonia includes several forms of sterile fungi which are recognized by certain characteristics of the mycelium and the manner of growth in pure culture. (Figure III.) The young branches are more or less narrowed at their point of union with the parent hypha and grow characteristically in a direction almost parallel to each other. A septum is also formed a short distance from the point of union with the parent hypha. The young hyphae are strongly vacuolated but this condition disappears in the older hyphae which are frequently yellowish in color. In pure culture on potato hard agar the growth is at first loose and white, but later becomes short, tufted, and brownish. Under the microscope these tufted growths are seen to consist of short cells, sharply constricted at the septa and irregularly branched. These shortened cells are capable at times of functioning as conidia. In older cultures brownish sclerotia are formed which, when

placed under favorable conditions, are capable of producing a new mycelial growth, and hence serve to carry the fungus through unfavorable periods. In certain forms true spores are said to be produced.

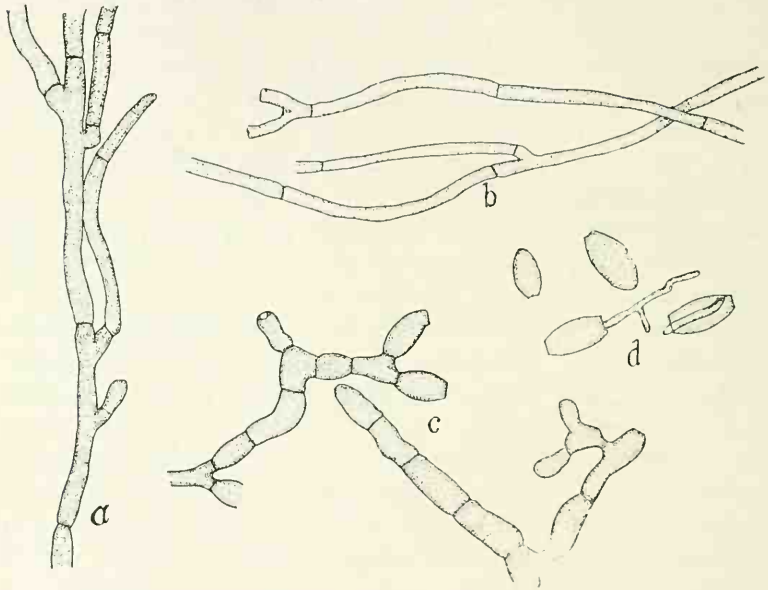

FIGURE III. MICROSCOPIC CHARACTERS OF RHIZOCTONIA.

A, young mycelium showing habit of branching; b, old, brown hyphae, usually empty; c, short tufted growth, characteristic in culture; d, cells resembling and functioning as conidia.

HOST PLANTS

Pythium debaryanum and Rhizoctonia sp. are now known to attack a wide range of plants[1] when favorable climatic condi-

[1] The following lists, though probably not complete, serve to illustrate the types of plants upon which these fungi are parasitic. *Pythium debaryanum* has been described as parasitic upon white clover *(Trifolcum repens)*, European millet *(Panicum miliaceum)*, false flax *(Camelina sativa)*, shepherds purse *(Capsella bursa pastoris)*, white pine *(Pinus strobus)*, beet *(Beta vulgaris)*, orchid *(Stanhopea saccata)*, egg plant *(Solanum melogena)*, spurry *(Spergula arvensis)*, corn, *(Zea mays)*, cress *(Lepidium sativum)*, pigweed *(Amaranthus retroflexis)*, white mustard *(Brassica alba)*, cucumber *(Cucumis sativus)* and certain species of Viscaria, Lobelia and Gillia.

Rhizoctonia has been described by Duggar (8) and others as parasitic in this country upon bean *(Phaseolus vulgaris)*, beet, *(Beta vulgaris)*, carrot, *(Daucus carota)*, celery *(Apium graveolens)*, cotton *(Gossypium herbaceum)*, lettuce *(Lactuca sativa)*, potato *(Solanum tuberosum)*, radish *(Raphanus sativus)*, rhubarb *(Rheum rhaponticum)*, ornamental as-

tions for their growth occur. Some plants are, however, very much more suspectible than others, owing to certain physiological or morphological factors. The amount of injury also depends markedly upon the manner of cultivation and the stage of development of the plants.

During my studies upon damping-off of seedlings I have observed *Pythium debaryanum* as occurring upon cress (*Lepidium sativum*), tobacco (*Nicotiana tabacum*), lettuce (*Lactuca sativa*), endive (*Cichorium endiva*) carnation cuttings (*Dianthus caryophyllus*), cucumber (*Cucumis sativus*), turnips (*Brassica rapa*), cabbage (*Brassica oleracea*), squash (*Cucurbita maxima*), cauliflower (*Brassica oleracea var. botrytis*), pumpkin (*Curcurbita pepo*), mangle-wurzel (*Beta vulgaris var macrorhiza*), balsam (*Impatiens balsamina*), kohlrabi (*Brassica oleracea var. caulorapa*), tomato (*Lycopersicum esculentum*), beans (*Phascolus vulgaris*) and geranium cuttings (*Pelargonium hortorum*).

Rhizoctonia has been observed to occur parasitically at Madison upon cress (*Lepidium sativum*), tobacco (*Nicotiana tabacum*), lettuce (*Lactuca sativa*), clover (*Trifolcum repens*), sunflower (*Helianthus annuus*), radish (*Raphanus sativa*), egg plant (*Solanum melogena*), pea (*Pisum sativum*), celery (*Apium graveolens*), tomato (*Lycopersicum esculentum*), violet (*Viola affinis*), beet (*Beta vulgaris*), geranium cuttings (*Pelargonium hortorum*) and sweet Willam (*Dianthus barbatus*).

HISTORICAL

The practice of heating soil by surface firing was probably practiced long before its actual sterilizing value was known. The benefits known to be derived were largely those of an improved physical condition of certain soils, the added fertilizer from the ashes of the burned material, and a reduction of weeds in the soil.

Atkinson (1) in 1895 pointed out several factors which influenced damping-off by Pythium in cutting-beds, suggested sev-

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paragus, (*Asparagus sprengeri*), China aster (*Callistephus hortensis*), carnation (*Dianthus caryophyllus*), sweet william (*Dianthus barbatus*), violet (*Viola odorata*), lambs quarters (*Chenopodium album*), tumble weed (*Amaranthus albus*), pigweed (*Amaranthus retroflexus*), white pine, cucumber, begonia, coleus, verbena, hydrangea, hardy candytuft, mammoth sage, phlox, pyrethum, snap dragon, raspberry, squash, clover, lucerne and peas.

eral cultural means for its control, and recommended steam sterilization in severe cases.

Stone (29) in 1900 emphasized the value of partial sterilization of forcing house soils, and its practical application for protection against the lettuce drop disease.

Selby (23) in 1906 recommended the use of 1-160 to 1-200 formalin (2-2½ pints to 50 gallons of water) for the control of bed rot of tobacco plants caused by Rhizoctonia. His experiments using this strength formalin gave partial control of the disease. Poor results, however, were obtained in some instances.

Jones (13) in 1906 working in Vermont, found that the use of 1-100 formalin reduced damping-off in pine seedlings from 90 per cent to 7½ per cent, and 1-200 formalin reduced the disease to 9 per cent.

Spaulding (26) in 1908 recommended the use of sulphuric acid (1 ounce to one gallon) against damping-off of coniferous seedlings, and obtained poor results with 1-100 formalin.

Gilbert (11) in 1909 using various methods of soil treatment for the control of root rot of tobacco *(Thielavia basicola),* recommended steam sterilization. He obtained poor results with 1-100 formalin in most cases.

Scherffius (24) in 1911 working in the Transvaal, used five different methods of soil treatment against damping-off in tobacco plant beds, viz., (1) open fire; (2) boiling water; (3) steaming; (4) roasting; (5) formalin. All factors considered, he obtained the best results with the open fire method. Roasting gave almost as good results as the first and was closely followed by steaming. The formalin treatment was rated as fourth, and boiling water gave results but little better than untreated plots.

Russell and Petherbridge (21), in 1912, used carbon bisulphide and toluol on greenhouse soils and reported marked increase in plant growth due to the effect on soil organisms, but did not state definitely the effect on the damping-off fungi.

Gifford (10) in 1912 compared steam sterilization and formalin treatment for the prevention of damping-off of coniferous seedlings by Fusarium in particular, and recommends the use of one per cent formalin for its control.

Various other statements are to be found in literature recommending the use of potassium sulphide, potassium permanga-

nate, lime, Bordeaux mixture, and potassium nitrate for the control of the damping-off diseases.

The status of the problem is such, following the work of the preceding investigators, that the subject appeared to need further study, especially along the following lines:—

1. The strength of formalin required for complete control of damping-off as with formalin 1-100 or weaker, previous investigators secured contradictory results, only partial control being obtained in most cases.

2. The relative value of other fungicides sometimes recommended for control.

3. The value and practicability of steam sterilization of soils in the field.

PREVENTIVES

Experimental Methods

The experimental data which is at hand upon the efficiency of various methods of soil sterilization against damping-off in plant beds is small and contradictory. This is due largely to the fact that the experiments have been carried on principally out of doors, where, owning to the influences of weather conditions, the data may in some instances be misleading. Furthermore, the control methods have been directed against the damping-off fungi as a whole which may again lead to complications from a scientific standpoint, though it may appear relatively unimportant from the standpoint of practice. Reinfection is also, of course, an important consideration and must be considered at all times where attempts are made to eliminate certain organisms from large exposed areas out of doors.

In the following experiments it has been attempted, as far as possible, to eliminate these factors, and to furnish conditions most favorable for the development of the disease. In a number of preliminary experiments begun in 1909 on forcing house benches, results were obtained which were indicative, but not entirely reliable because of changing atmospheric conditions and occasional reinfections. The results of these experiments will not, therefore, be given in detail. They served to show, however, that sprinkling the plants with dilute solutions of potassium sulphide, formalin, copper sulphate, ferrous sul-

phate, and ammonia at strengths below that toxic to plants was inefficient in checking the disease after it had once started.

Preliminary experiments in the fall of 1910 indicated that a satisfactory temperature and humidity for damping-off could be maintained in a small damp chamber, and that the possibility of reinfections through the air was practically nil. A large damp chamber 18 feet long and 3.5 feet wide was then constructed on one of the forcing house benches. (Figure IV.) The top was made of hot-bed sash placed about 2.5 feet above the bottom of the bench. The sides of the chamber were also partly glass, allowing sufficient light to enter from the sides so as not to cause the seedlings to grow spindly. The unit area used for soil treatment in this chamber consisted of a forcing house flat which measured 21 inches by 16 inches and 3 inches in depth, holding 28 to 30 pounds of soil. This chamber holds 23 flats of this size.

In earlier experiments the control was aimed at both Pythium and Rhizoctonia in the same soil, and no inoculations were made as all the soil used was found to contain these fungi naturally. It was observed that under certain conditions, particularly of temperature, Rhizoctonia would be the main casual organism of damping-off, while under other conditions Pythium seemed to predominate. It was also thought possible that a control measure might effectively restrain one organism and not the other. This complicated matters to such an extent that in the later experiments the soil and flats were all sterilized, and inoculations made in pure culture in the following manner:

The flats were filled with a soil composed of three parts of compost, two parts of garden loam, and one part of sand. They were then set one on top of the other, with one-fourth inch space between each, and a large, tight box inverted over them. Steam was run under this box from the forcing house heating system for one and one-half hours, which was sufficient to sterilize the soil so far as the damping-off fungi were concerned. By this method a temperature of practically 100 degrees Centigrade was maintained for one hour. The soil and flats, while still warm, were placed in the damp chamber, which had been previously flushed out with water and sprayed with a strong solution of copper sulphate.

About six days previous to this, pure cultures of Pythium and Rhizoctonia were started on corn meal. After the flats had

cooled sufficiently, the corn meal cultures were spread in small pieces over the surfaces of the soil. The flats were then covered with plate glass which had been fumigated with formaldehyde gas over night. After four or five days the fungus had grown profusely into the soil from the corn meal. The soil was now worked up in order to obtain as even a distribution of the fungus through it as possible. The flats were then ready for treatment with the fungicides.

In the experiments described "sterile" checks are flats which were left uninoculated, and "untreated" checks are those which were inoculated with the disease, but not treated with any fungicide. The checks received the same amount of water as the treated flats. The amount of liquid fungicide used on all flats was 2500 cubic centimeters which was sufficient to saturate the soil to the bottom. After treatment the flats were again covered for 48 hours with the glass plates, which reduced evaporation to a minimum, and gave the volatile fungicides, especially, time to act. The covers were then removed and placed vertically between the flats preventing contamination from one flat to another. After four to six days the flats were sufficiently dried out to work the soil again, after which the seed was sown. The temperature of the chamber varied from 23 to 30 degrees Centigrade.

In the earlier experiments tobacco plants were used, but owing to the length of time required for germination and growth, garden cress *(Lepidium sativum)* was substituted in the later trials. This plant serves admirably for this purpose because of the readiness with which it damps off and the short time required for its germination and growth. Cress is to be looked upon as a sensitive indicator of the presence of any living damping-off fungus and as a measure of the efficiency of the fungicide under conditions favorable to the fungus.

EXPERIMENTS WITH FORMALIN

The trials made in treating the soil with formalin at various strengths were carried on in connection with the other treatments for comparative efficiency. On account of the importance of the formalin treatment, however, the flats treated with this chemical, with their checks, have been grouped into Table I. Formalin (40 per cent formaldehyde) of the best purity was

used in the experiments. The experiment was carried on in four different series. In Series I and II, the casual organisms were both Pythium and Rhizoctonia, with tobacco seedlings used as host plants. In Series III and IV Pythium only was present in the soil, and cress was used as the host plant. In Series III, only the per cent of plants killed above ground was taken into consideration, while in Series IV the per cent of plants killed both above and below ground was considered. The formalin strengths used are given in parts by volume.

TABLE I. EFFECT OF FORMALIN SOIL TREATMENT ON DAMPING-OFF

SERIES	PERCENT OF PLANTS DISEASED						
	Untreated soil	Strength of Formalin Used					Sterile soil
		1-200	1-150	1-100	1-75	1-50	
I	50	10	10	.....	.....	.....	0
	90	.....	90	.....	.....	.....	0
	75	.....	80	.....	.....	.....	.....
	.....	.....	75	.....	.....	.....	.....
II	80	.....	.....	10	.....	.....	0
	.....	.....	.....	20	.....	.....	0
	.....	.....	.....	20	.....	.....	.....
	.....	.....	.....	80	.....	.....	.....
	.....	.....	.....	10	.....	.....	.....
	.....	.....	.....	40	.....	.....	.....
III*	15	75	.....	65	.....	0	0
	20	.....	.....	.....	.....	0	0
IV	85	.....	95	65	35	0	0
	90	.....	.....	75	40	0	0

*Apparently low per cent of disease in untreated soil is due to fact that the large per cent of plants killed below the surface were not included in this estimate.

It will be seen from this data that formalin treatment at the strength recommended by various authors, i. e. 1-100 to 1-200 is practically no better than no treatment for damping off under the conditions of these experiments. Series II shows that 1-100 formalin will not control Rhizoctonia, as microscopical examination showed this fungus to be the main cause of the damping-off in this case. The wide range of results in Series II is due largely to the flats being affected by different temperatures, a factor which was better controlled in the later experiments. In Series III and IV the flats treated contained only Pythium, which was not destroyed by 1-75 formalin and lesser strengths, but was completely controlled by 1-50 formalin (Figure V). It is possible that a strength somewhat less than used to control Py-

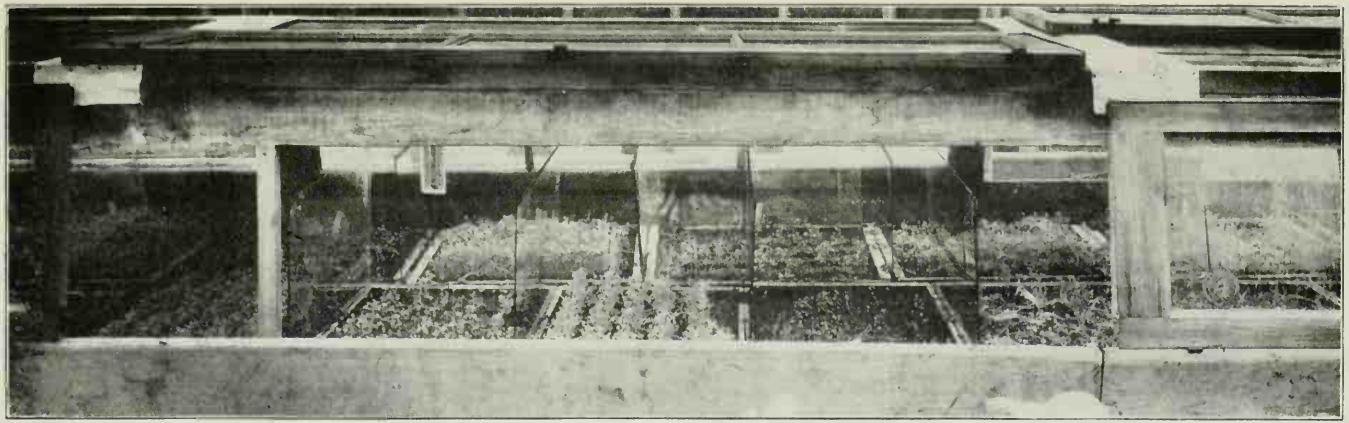

FIGURE IV. DAMP CHAMBER USED IN CONTROL EXPERIMENTS.
The flats are separated by glass partitions to avoid infection.

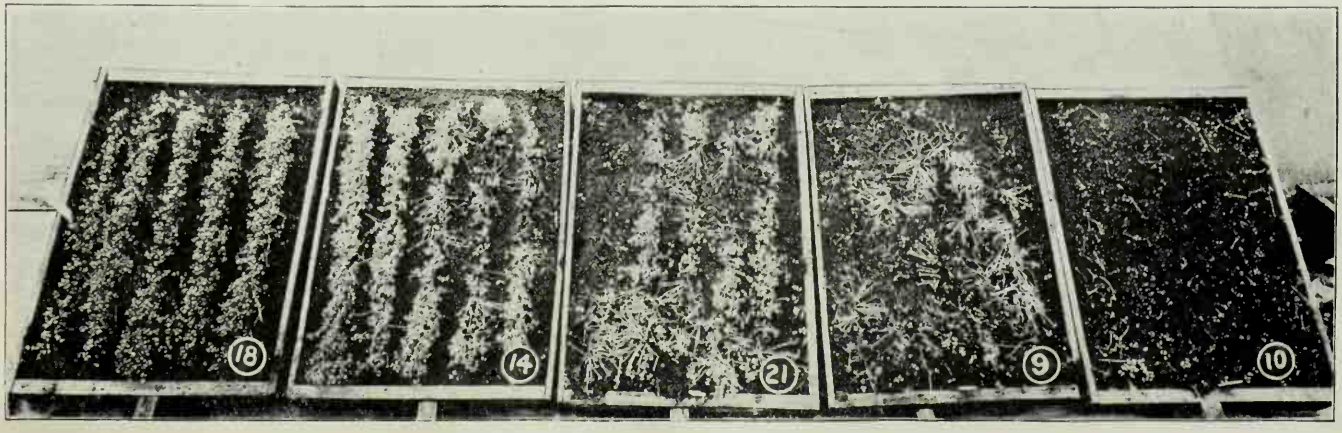

FIGURE V. INFLUENCE OF FORMALIN SOIL TREATMENT ON DAMPING-OFF.
Growth of cress on Pythium infected soil treated with formalin of various strengths: (18) 1–50 formalin; (14) 1–75 formalin; (21) 1–100 formalin; (9) 1–150; (10) no treatment. Strengths below 1–50 failed to completely control the disease.

thium will destroy the mycelium of the sterile fungus, Rhizoctonia, but that is immaterial from a practical standpoint, as a strength sufficient to destroy both fungi must be used in order to be certain of results. A strength of 1-50 formalin may be slightly in excess of what is actually required to kill Pythium, but it it a common practice in using fungicides to work with strengths somewhat beyond that which is actually required, provided no injury to the host occurs. Selby (23) recommends the use of 2-2½ pints of formalin to 50 gallons of water, (1-160 to 1-200,) one gallon of the solution per square foot of seed bed, applying it at intervals of a few hours, as most soils will not take up this amount of water at one time.

According to the writer's observations, two quarts of the 1-50 solution applied to each square foot is sufficient, especially if the bed is covered with some material to hold in the fumes. This is on the principle that a strong solution acting for a shorter time is more efficient than a weaker one acting for a longer time. The stronger formalin will also penetrate downward into the soil further, and the soil can be worked sooner than when twice the amount of solution is added. The practice should then be to use four quarts of formalin per 50 gallons (one barrel) of water, applying at the rate of two quarts per square foot, or approximately one barrel to one rod of seed bed six feet in width.

An interesting observation of the effect of formaldehyde upon the fungus has arisen in this connection. If we separate the amount of injury caused by Pythium on cress seedlings into the percentage of disease above ground and percentage of disease below ground, we find that the percentage of seeds killed before germination in the untreated checks is very high. Table II illustrates the effect of formalin of different strengths upon the relative rate of development of the fungus.

TABLE II. INFLUENCE OF FORMALIN ON DAMPING-OFF FUNGUS

Treatment	Strength	Percent disease below surface	Percent disease above surface
Untreated check		80	10
Formalin	1—150	0	95
Formalin	1—100	0	75
Formalin	1—75	0	40
Formalin	1—50	0	0
Steam sterile		0	0

According to this data, 1-150 formalin effectively prevents the fungus from attacking the cress seed in the soil. This has also been observed to be the case in 1-200 formalin. On the soil treated with 1-150 formalin, the plants damped off rapidly, however, soon after they appeared above ground. The percentage of damping-off up to a certain time decreased with the increase of strength of formalin, the weaker strengths of formalin (1-75 to 1-200) having a checking influence upon the fungus which disappears in time. This may explain the fact that relatively good results have been obtained with the lower strength of formalin. If the climatic conditions favorable for the development of the fungus come on a time before the checking influence of the formalin has passed off, good results may be obtained, but if these favorable conditions appear some time later, the treatment with the formalin at the lower strengths may be practically valueless. This checking influence of formalin below 1-75 will usually pass off in from 10 to 15 days after treatment, and is consequently of little value, especially to those plants requiring longer periods in the plant beds. There can be but little doubt that 1-50 formalin kills the fungus as the treated soil remains apparently sterile for weeks, providing infection from the outside does not occur. The partial control obtained by Selby and Gifford, using formalin at strengths of 1-100 and below, can be explained by this checking influence of the fungicide together with unfavorable weather conditions for the parasite after the formalin had left the soil.

MISCELLANEOUS SOIL TREATMENTS

During the course of the experiments with formalin, several other fungicides were tried for the prevention of damping-off. The strength of the fungicides used, especially that of the non-volatile ones, is limited by the toxicity to the plants. Several preliminary experiments were carried on in order to determine the percentage strength of the fungicide which could be used without too much injury to the plants. These preliminary experiments indicated to some extent the uselessness of the application of many of these substances, but further trials were continued in connection with the formalin controls

and some data obtained, which is grouped in the following table.

TABLE III. PER CENT OF DISEASED PLANTS IN MISCELLANEOUS SOIL TREATMENTS

Treatment	Per cent by weight	Experiment 1.			Experiment 2.		
		Treated	Untreated check	Sterile check	Treated	Untreated check	Sterile check
Potassium sulphide	0.8	15	20	0	.....	.....	.....
Potassium sulphide	1.	.....	.....	.....	95	85	0
Lime sulphur†	2*	20	20	0	70	85	0
Copper sulphate	0.8	15	15	0	.....	.....	.....
Copper sulphate	1.	.....	.....	.....	5	90	0
Copper acetate	0.12	18	20	0	.....	.....	.....
Copper acetate	0.2	.....	.....	.....	35	85	0
Copper nitrate	0 8	20	20	0	.....	.....	.....
Bordeaux mixture‡	1.	10	20	0	25	85	0
Sulphuric acid*	0 4*	20	20	0	.....	.....	.....
Sulphuric acid	0.5*	.....	.....	.....	65	85	0
Potassium permanganate	1.	.....	.....	.....	92	85	0
Potassium nitrate	0.5	10	20	0	.....	.....	.....
Mercuric chloride	0.12	60	20	0	.....	.....	.....
Hot water	.....	25	20	0	85	85	0

*Per cent by volume.
†Sherwin Williams Lime Sulphur Solution, sp gr. 1.2491.
‡5—5—50 formula.
§Sp. gr. 1.84.

A comparison of the percentage of disease in treated and untreated soil in Table III shows little or no benefit from the application of these substances as fungicides to the soil in amounts which will permit of seed germination and plant growth. These figures do not, however, indicate the true state of affairs in all cases. Certain of the chemical agents appeared to produce some inhibitory action upon the development of the fungus for a short time after the application, but this action apparently disappeared too soon to be of any value. This inhibitory action on the fungus was most conspicuous as effecting the percentage of seedlings killed below the surface of the ground. In the case, for instance, of treatment with 2% lime sulphur fully 75 per cent of the plants appeared above ground before damping-off, as compared with only 5 to 10 per cent in the case of untreated soil. Treatment of a steam sterile soil with lime sulphur (2 per cent) showed retarded germination and growth of cress, but a perfect stand. Similar inhibitory action was also observed to occur in the potassium sulphide, copper sulphate, and mercuric chloride treatments. The inhibitory action of these fungicides upon

fungus development was correlated with an inhibition of plant growth, and consequently little hope for their value as soil fungicides is maintained. The essentials of a good soil fungicide appear to be that it must first possess strong fungicidal action and secondly that it must be volatile in order that it may be freed from soil before the seed is sown.

Covering the soil with screenings of sphagnum moss gave indications of checking the disease to some extent, and is worthy of trial on a small scale. Sand covers gave poor results in some cases and are of doubtful value for checking the disease. Steam sterile surface soil two inches in depth, allowed to remain on the surface soil from 6 to 7 days before the sowing of the seed, appeared to be of no value. A three inch layer of sterile surface soil, however, showed very little disease except that which was later determined to have arisen from outside infection. Under practical conditions a three to four inch layer of sterile surface soil should in most cases effectively prevent damping-off, at least during the period when the seedlings are most susceptible. The use of such substances as lime, ammonia, toluol and carbon bisulphide have also given negative results in the control of damping-off so far as they have been tried in these experiments.

STERILIZATION BY HEAT

Several methods have been devised by various experimenters for the sterilization of soil by heat. The writer found that forcing house flats such as those used in the experiments could be cheaply and efficiently sterilized under an inverted box into which streaming steam was run for one and one-half hours from the forcing house heating system. Fourteen flats were sterilized at a time, or an equivalent of 28 to 30 square feet of soil. This method, though impracticable out of doors, is a good one for a small greenhouse where seedlings and cuttings are started in flats.

The methods used for sterilizing soil by heat, which lend themselves to out door seed bed conditions, fall under three heads, (1) steaming, (2) surface firing, (3) roasting.

The most practical method yet devised for the sterilization of seed beds by steam is the "inverted pan method". This method was first used by Shamel for sterilizing nematode infested soils

in Florida. A galvanized iron pan, six feet by ten feet and six inches deep is inverted over the area to be sterilized after it has been prepard for seeding. As the edges of the pan are sharp they can be pressed into the soil an inch or more, thus forming a tight compartment under the pan into which the steam is run 30—60 minutes from a boiler at a pressure of 80—150 pounds. The time of steaming depends largely upon the type of soil and its moisture content and compactness. Loose sandy, moist but

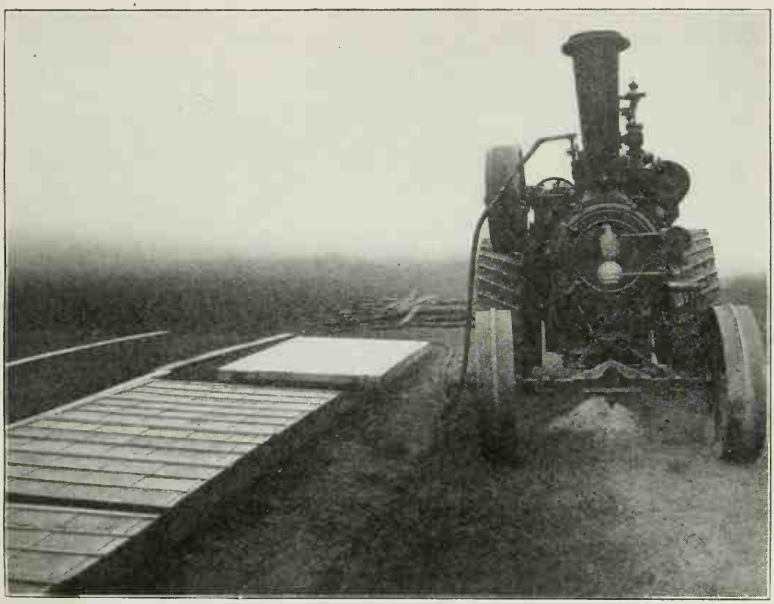

FIGURE VI. STERILIZING SEED BEDS BY THE "INVERTED PAN" METHOD.

not wet soils, are more easily and rapidly steamed than heavy and wet soils. The pan used at this station (Figure VI) in tobacco seed-bed experiments was made 8 inches in depth, which is probably more desirable in some cases. The pan could be made 12 feet in length without loosing any efficiency where large boilers are used. The handles should preferably be placed on the sides instead of on the ends so that the pan could be moved from one section of the bed to another without the operators walking on the sterilized soil. The weight of such a pan is approximately 400 pounds. A one inch steam hose should be used to connect it with the boiler. A traction engine such as is used for threshing is most convenient to furnish the steam.

Other methods of steaming are the "sub-surface pipe" system and the "steam rake" method. The former consists of an underground system of 1½ inch perforated pipes, 18 inches apart, running lengthwise of the bed, 6 to 8 inches below the surface. The perforations, about ¼ inch in size and 6 inches apart, should be on the under side of the pipe. Steam is run into these pipes at a pressure of 80 to 100 pounds for a period of about 1 to 2 hours. The method is practicable only when beds are located for several years in the same place. The steam rake or harrow method consists in forcing steam into the soil through hollow perforated pipes resembling the "spikes" of a harrow. The results are the same, but the method is usually regarded as less efficient than those previously mentioned.

Surface firing has been quite popular in the past, but it is giving way to other methods, owing largely to the scarcity of combustible material in many places, and to the labor connected with the process. The method consists simply in producing a hot fire for an hour or more over the section of the bed to be sterilized after it has been fertilized and fitted for seeding. Any combustible material may be used such as brush, straw, or dry wood.

Roasting or "pan-firing" requires the removal of part of the soil from the seed bed into the pan and returning it after being sterilized. The pan may be simply a large piece of sheet iron, resting upon supports, underneath which is a fire. This pan should be set on the bed and the soil to a depth of 6 inches on one side shoveled upon it and roasted for an hour, being careful not to let it become so dry that the vegetable matter is burned out. Then a like area on the other side of the bed is sterilized and in the meantime the soil underneath the pan has been sufficiently heated, and the pan may be moved to another section of the seed-bed and the operation repeated.

EXPERIMENTS UNDER FIELD CONDITIONS

In the spring of 1912 the "inverted pan" method, surface firing, and formalin treatment (1–100 solution and two quarts per square foot) were compared on tobacco beds. Steam was run into the inverted pan for various lengths of time ranging from 25 to 45 minutes, at a pressure varying from 100 pounds down to 25 pounds in some cases at the end of the application.

As no damping-off occurred in the unsterilized plots, the comparative value of the different periods of heating could not be determined, and the experiment will not be given in detail. Some interesting results were obtained, however, upon the effect on weeds and growth of the plants. The weed seeds were all killed at as low an exposure as 25 minutes, starting at 80 pounds and running down to 25 pounds pressure. (Figure IX.) The plants in the unsterilized plots were not one-half as large as those in the sterilized plots one month after sowing the seed.

FIGURE VII. STEAMING NOT ONLY PREVENTS DISEASE BUT ALSO KILLS WEEDS.

A, Soil steamed. B, Soil not steamed.

(Figure VIII.) The cost of one weeding of the untreated plots was equal to the cost of steaming an equal area. This together with an assurance against bed-rot and other pests, and an increased growth in the plants, made the process an economical one and well worthy of use on all tobacco farms. The sterilizing of tobacco seed beds by the "inverted pan method" can be most cheaply done by a few neighboring farmers in cooperation, or better still by a person who owns a steam traction engine who will do the sterilizing by contract at so much per rod of seed bed.

The surface fired plot was second best, being much better than the untreated plots, and no weeds were present. The formalin treated plots came third, the principal effect being a considerable reduction in the number of weeds, although this effect

is not usually claimed for it. Considerable stimulation in the growth of the plants was also noticed.

The practical value of steaming tobacco beds was further demonstrated in the spring of 1913, when the writer steamed approximately ten thousand square feet of soil for different growers in Rock County, Wisconsin. The results were so markedly successful in destroying weeds and producing earlier and better plants, that these and other growers who saw the demonstrations are preparing to steam all their seed beds hereafter. The results of field experiments have shown on the whole that it probably does not pay to steam for damping-off alone, since its occurrence is comparatively infrequent. The other important results of soil steaming however, such as increasing the growth and uniformity of the plants, killing the weed seeds and injurious insects which may be in the soil, and preventing infection with other diseases make it an economical, if not a necessary process.

SECONDARY EFFECTS OF SOIL TREATMENTS

The treatment of soils by heat and liquid fungicides to destroy the undesirable organisms results in a number of secondary effects which have been the subject of some study in recent years. These effects are primarily perceptible upon the plants grown in the treated soils. It has been found that the physical, chemical, and biological nature of the soil is altered by heating.

The following tabulation will illustrate these effects upon the germination and growth of seedlings as observed from flats treated by heat and by various chemicals used at the strengths mentioned. The unit area used for treatment was that of a forcing house flat holding from 28–30 pounds of soil. Twenty-five hundred cubic centimeters of the solution was applied to each flat in every case.

As we are interested in the secondary effect of sterilization by heat and formalin principally, we will consider these facts briefly. The first effect that can be noticed in a heat sterilized soil is a change of physical condition which results in a more rapid drying out of the surface layer of soil. According to Lyon and Bizzell (15,) this appears to be due to a loosening of soil, which aids more rapid percolation downward. This effect cannot be said to be particularly disadvantageous except when

the seedlings are very young and need moisture at the surface, when it is readily overcome by two or three additional waterings.

TABLE IV. EFFECTS OF SOIL TREATMENTS ON PLANTS

Treatment	Per cent by weight	Germination	Growth after 15 days
Steaming 100° C. 1½ hrs.	.....	Retarded	Stimulated
Formalin, 1-50 (sowing after 3 days)	.....	Retarded	Stimulated
Formalin, 1-50 (sowing after 7 days)	.....	No effect	Stimulated
Copper sulphate	1	Retarded	Inhibited
Copper acetate	0.2	No effect	Slight inhibition
Copper nitrate	0.8	No effect	Inhibited
Ammonium copper carbonate	0.12	Markedly retarded	Inhibited
Bordeaux mixture	1.	Slight retardation	Slight inhibition
Potassium sulphide	1.	Marked retardation	No effect
Lime sulphur sp. gr. 1.2491	1.*	Retarded	No effect
Sulphuric acid c. p.	0.5*	No effect	Slight inhibition
Potassium permanganate	1.	Slight retardation	No effect
Potassium nitrate	0.5	No effect	No effect
Ferrous sulphate	1.	No effect	No effect
Hot water	.....	No effect	No effect
Mercuric chloride	.12	Retarded	Inhibited
Surface firing	.....	Retarded	Stimulated

* Percent by volume.

Gifford (10), however, reports that in the case of coniferous seedlings the loss of moisture in the upper layers of soil as a result of steam sterilization is a disadvantage of sufficient importance to prevent its general adoption against damping-off.

Pfeiffer and Franke (19), on steaming soil for three hours at a pressure of one atmosphere, found that the heated soil produced a crop of greater dry weight and higher nitrogen content than the unheated.

Schulze (25) noticed that the sterilization produced injurious effects upon plant growth in the early stages of development, but that as the plants grew older, they grew more vigorously in the sterilized soils.

Pickering (18) found that heating moist soil to temperatures from 60 degrees to 150 degrees Centigrade, retarded germination of seeds, and in some cases decreased the total number of seeds germinating. He also obtained increased growth of plants in heated soil, which was attributed to an increase in soluble nitrogen.

Koch and Linken (14) heated sandy soil with steam for two hours at two atmospheres pressure, and found that the soluble plant food was increased. They also found that the immediate

results of heating were injurious, but that it was unimportant and that the later yield was improved.

Russell and Hutchinson (20) heated soils to 98 degrees C. and found that an increased production of ammonia followed for at least 150 days. This increase in ammonification is attributed to an increase in the number of ammonifying bacteria in the soil as a result of the destruction of the larger organisms, especially protozoa, which destroy the ammonifying bacteria, and are hence injurious to the productiveness of soils.

Lyon and Bizzell (15) steamed different soils under a pressure of two atmospheres for periods of two and four hours and found a marked injurious effect resulting, as shown by the growth of plants in the soil. The time required for the soils to recover from the treatment was usually in the same order as their relative productiveness. These experiments indicate that the injurious effect varies with the amount of steaming and the type of soil. These results are not quite applicable to partial soil sterilization against damping-off since the amount of steaming is much less and the recovery consequently more rapid in the ordinary soil treatment.

Russell and Petherbridge (21), using carbon bisulphide, toluol, and steam sterilization, report a retardation in germination, but a greatly increased yield as a result of sterilization of greenhouse soils.

These results, as a whole, are in accord with the writer's observations of the effect of steam treated soil upon seedlings of cress and tobacco. The very marked increase in growth of tobacco plants on heated soil makes it appear that this practice should be generally adopted by tobacco growers. It is recommended particularly to those growers who have yearly difficulty in obtaining an early stand of vigorous plants. The heating of soil does not appear to affect all soils similarly. According to Stone (30), soils low in organic matter may be injured by heating, and this fact should be considered before preparing to heat certain soils. In the writers experience with nearly a score of Wisconsin soils, heating has never resulted injuriously.

The effect of formalin sterilization upon soils has not been the subject of as much experimentation, and the real nature of the effect has not, to the writer's knowledge, been determined. If the seed is sown before most of the fumes of formaldehyde have

left the ground, germination is retarded. If the soil is allowed to dry out for a week, however, this effect disappears. It is interesting to note, also, an increased vigor and growth of seedlings grown upon formalin sterilized soil. This effect with the strengths of formalin used in these experiments occurs usually sooner, but not to as great an extent as in the case of steam sterilization. This increase is again possibly due to an effect upon the chemical nature of the soil or, more probably, to the effect upon its bacteriological content. It has also been supposed that the plants may take up small amounts of formaldehyde directly, resulting in a stimulation of growth. The amount of weeds was also much reduced in formalin treated soil, an effect also noted by Clinton (3) in treating soil for root-rot of tobacco.

Cultural Remedies

Although prevention by such measures as have been described is the only certain protection against damping-off, much can be done in a cultural way towards obtaining a relatively small loss from the disease.

Infected soils. As an increased amount of the causal organism in the soil will naturally be present in a soil where damping-off has previously occurred, later seedlings in the same soil will be more likely to damp off when the weather conditions become favorable to the fungus. It is, therefore, a wise precaution to avoid sowing seed in the same beds where the disease has previously occured, unless the soil is sterilized by heat or formalin. It has been found possible by repeated sowings of cress in diseased soil to fill it so completely with the causal organisms that practically every seed in the later sowings is killed before it germinates. A change of seed bed is, therefore, desirable and necessary in case of bad infestations. New ground, especially fall plowed, old blue grass sod has shown itself in many cases to contain very little, if any, of the damping-off fungi, besides being relatively free from weed seeds, thus making it a good seed bed if the soil is otherwise of the right nature.

Thick sowing. Although the damping-off fungi are capable of attacking plants standing singly under especially favorable weather conditions, no method of culture is more conducive to damping-off in the seed beds than thick sowing of seed, with the resultant crowding of plants. The following table illus-

trates the relation between the amount of seed sown and the percentage area damped off. The experiments were carried on with tobacco in flats in the greenhouse where favorable conditions for damping-off were maintained.

TABLE NO. V. EFFECT OF THICK SOWING ON PERCENTAGE OF DISEASED PLANTS

FLAT NO.	WEIGHT OF SEED SOWN.		Plants diseased.
	Per flat.	Per 100 sq. ft.	
	Grams.	Ounces.	Per cent.
1	0.1	0.16	0
2	0.2	0.33	0
3	0.3	0.49	8
4	0.4	0.66	15
5	0.5	0.83	35
6	0.6	0.99	75
7	0.7	1.16	80
8	0.8	1.33	80
9	0.9	1.49	92
10	1.0	1.60	96

The first signs of the disease occured in flat No. 10 very soon after the plants had obtained their first two leaves. The disease started last in flat No. 3, but not until the plants were of considerable size and were crowded. This record illustrates quite markedly that the amount of damping-off varies directly with the thickness of sowing. (Figure X). This is due to several factors, the first of which is probably the increased humidity of the atmosphere around the base of plants when crowded, as a result of lessened air circulation and increased shading. Crowding also affords easier transfer of the fungus from one plant to another and is instrumental in producing more rapidly growing and succulent stems, which are more susceptible to attack. Thick sowing is especially likely to occur in sowing small seed. One ounce of tobacco seed with a germinating capacity of 90 to 95 per cent should be made to cover 5 to 6 rods of seed bed six feet in width, whereas in practice it is frequently sown on two rods.

Soil Types. Certain types of soil are particularly favorable for the damping-off fungi, and should therefore be avoided. Such are the soils which contain a high percentage of undecomposed vegetable matter, and those which are poorly drained. In an experiment using mixtures of varying percentages of manure,

FIGURE VIII. STEAMING INCREASES VIGOR AND UNIFORMITY OF PLANTS.

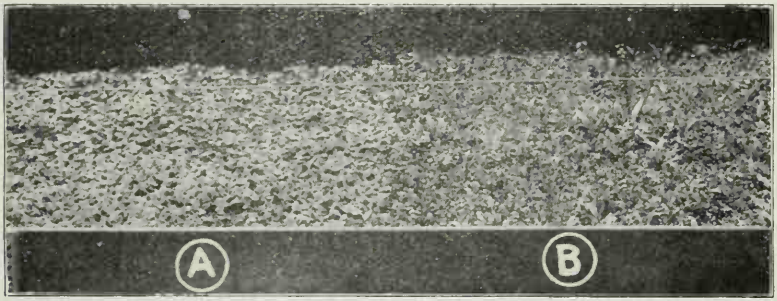

FIGURE IX. STEAMING ELIMINATES THE COST OF WEEDING.
a, steam sterilized; b, no treatment.

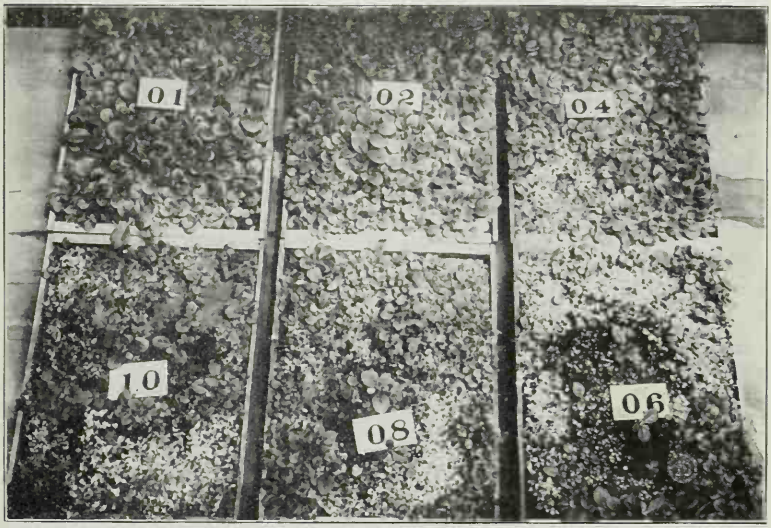

FIGURE X. THICKNESS OF SOWING FAVORS DAMPING OFF.
Figures on boxes show amount of seed in grams, sown in each flat.

compost, garden loam and sand, sterilized and inoculated with Pythium, the most rapid spread and prolific growth of the fungus was obtained in a mixture of 50 per cent manure and 50 per cent garden loam, and the least growth in garden loam and pure sand. As the fungus lives on the organic matter of the soil in the absence of the host plant, it is reasonable to suppose that the fungus content of soils rich in organic matter is greater than that in soils poor in organic matter. This is one reason why a layer of pure sand on the surface of the bed should afford a considerable protection against damping-off. Yet experiments show that the application of a layer of sand does not prevent damping-off. This may be partially explained as follows: The damping-off fungi attack a plant just at or above the surface of the soil probably because the fungus is aerobic. Therefore in a perfectly aerated soil the disease would be more likely to occur further down on the hypocotyl. A layer of sand or moss, however, still allows considerable aeration down to the surface of the soil proper and hence permits the fungous development and consequent disease below the artificially applied layer. Some advantage in sand and moss, however, is undoubtedly supplied by its mulching qualities, on account of which the application of less water to the beds is required. A lower average percentage of moisture in the surface soil is thus maintained.

Soil Moisture. The following experiment was planned for the purpose of determining the relation of the development of Pythium in the soil to the moisture content of the soil. Dry sand was graded into various sizes by means of 20, 40, 80, and 100 mesh sieves. Ten grams of each grade was put into each of six test tubes. To each tube was then added enough dilute nutrient solution to make up to the calculated percentages of moisture given in Table VI. The tubes were then sterilized and inoculated with Pythium. After 4 days when considerable growth had been made in most of the tubes the following results were noted on the 20 mesh sand. The same general relations as regards the surface and sub-surface growth existed in the other grades of sand.

This data indicates that the fungus remains below the surface in a well drained soil when the moisture content is low. When the moisture content of the soil is high, however, or when it is saturated, as frequently occurs in poorly drained seed beds,

the fungous growth is forced to the surface. This is presumably on account of the lack of oxygen, since the fungus is aerobic. At the surface of a saturated or nearly saturated soil it finds ideal conditions for development, both as regards oxygen and moisture, and if plants are present which are susceptible to the disease, damping-off is almost certain to occur. In well drained soils, however, the fungous development is most active some small distance beneath the surface, where there is both sufficient moisture and oxygen. This zone may be, and frequently is, below the base of the hypocotyl, in which case the plants may escape attack from the disease.

TABLE VI. INFLUENCE OF SOIL MOISTURE ON GROWTH OF PYTHIUM.

Number	Moisture	Amount of surface growth	Amount of sub-surface growth
	Per cent		
1	0	None	None
2	10	None	Slight
3	15	None	Slight
4	20	Slight	Marked
5	25	Fair	Marked
6	30*	Very marked	None

(*) Sand saturated

Effect of Transplanting Diseased Plants. In the spring of 1908 tobacco seedlings were transplanted to the field from a bed badly infested with damping off. Many plants were set out which had a trace of the disease upon the stem, though the badly diseased plants were discarded. Soon after transplanting several plants died in the field, and examination showed them to have damped-off, undoubtedly from the disease transferred from the plant bed. Later in the season when the plants were about half grown, several gave the appearance of being stunted and yellow, as if from malnutrition. Examination showed in all cases a full or partial girdling of the stem below the surface of the soil. (FigureXI). At the time of the maturity of the crop about two per cent of the plants, apparently normal in their growth, were broken down by a heavy wind storm. Practically all these plants showed a break just below the surface of the ground, where the stem was wholly or partially girdled, to all appearances due to a continuation of the disease started in the plant

bed. In 1909 healthy and infected plants were set out in the field in separate rows, the infected plants, with few exceptions, made a normal growth, and examination when they reached maturity showed no injury or only scars where the disease had apparently continued, but later healed over. The recovery of the infected plants was attributed to the relatively dry season.

FIGURE XI. GIRDLING OF TOBACCO STALK DUE TO DAMPING OFF DISEASE.

A type of field injury which results in the affected plants being stunted in their growth and readily broken down by wind storms.

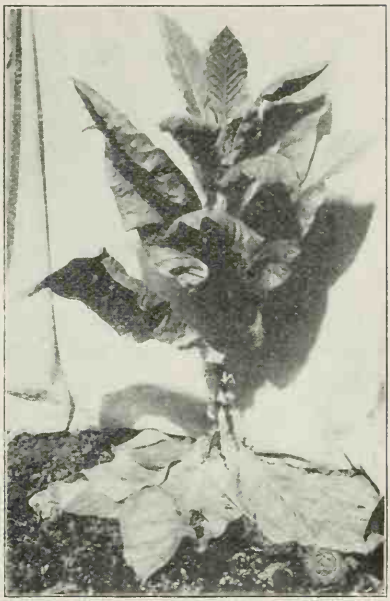

FIGURE XII. INJURY FROM PYTHIUM DE BARYANUM IN THE FIELD.

The disease frequently extends a considerable distance up the stalk and passes into the leaves causing them to droop and die.

The season of 1912 was again very wet, and a large amount of of this field injury due to damping-off took place. In a plot not over one-half acre in extent, over two hundred plants were counted which were diseased. The injuries on the whole, however, extended further up on the stalk than in the previous years. Ordinarily, the stalk was blackened a distance of 18 to 36 inches above the surface of the ground, producing a condition of the nature of "blackleg". The disease also frequently entered the leaves, causing them to droop and turn yellow from lack of food. (Figure XII). Selby (22) observed a similar trouble in Ohio tobacco and considered it to be associated with damping-off due

to Rhizoctonia. A similar disease is also reported from Java, being due to *Phytophthora nicotinianae* (Breda de Haan), and a "blackleg" of tobacco in Japan has been reported as due to *Bacillus nicotinianae* by Uyeda (31). Clinton (4) has observed a "canker disease" in Connecticut which is without doubt identical to this, and he suggests that it may be a bacterial disease.

At this station *Pythium debaryanum* was repeatedly observed on diseased plants at the margin of the diseased areas and taken in pure culture therefrom. *Pythium debaryanum* in this case appears to possess an extraordinary degree of parasitism, being usually known as attacking only succulent seedlings. These observations indicate that it is poor practice to transplant seedlings with any signs of the damping-off disease upon their stems. The persons who pull the plants from the plant beds for transplanting should therefore be required to discard all plants showing browned or blackend spots on the stem. The possibility of direct infection in the field seems most remote.

CHECKING THE DISEASE

The control measures considered up to this time, that is, those of prevention and culture, are means which must be taken before the seed is sown, and cannot be applied thereafter. More often the practical plant grower does not consider these factors and desires a remedy after the disease has begun to do considerable injury in the plant beds. This is an impossible task providing the weather conditions remain favorable to the disease; hence the importance of foresight in the use of preventive and cultural methods. However if the weather conditions are not continually favorable to damping-off, much can be done towards checking the disease in plant beds by a proper regulation of the temperature and moisture conditions in hot beds and cold frames.

Temperature Relations. Providing other conditions are favorable, damping-off is aided by relatively high temperatures of the soil and surrounding atmosphere. The optimum temperature for growth of Pythium is about 33 degrees C. (92 degrees F.) while that of Rhizoctonia lies at about 25 degrees C. (77 degrees F.). The growth of either below 16 degrees C. (61 degrees F.) is very slow. When damping-off has started among the plants

the grower should endeavor to keep the temperature as far below the optimum as possible. Presupposing the presence of both fungi as actively causing the damping-off, this will be almost an impossible task owing to the wide range of temperature which they cover. However, when damping-off occurs and the temperature of the glass or canvas covered frame is above 70 degrees F., it is important to remove the covers to reduce the temperature as much as possible.

Moisture Relations. As was pointed out before, a relatively high percentage of moisture in the soil favors damping-off. This is also true of the humidity of the atmosphere surrounding the plants. The best aerial growth of the fungi is only obtained in an atmosphere saturated with moisture. Where aerial growth of the fungi occurs, the spread of the disease from plant to plant is very much more rapid than when it has to make its way through the moist soil or through parts of plants in contact with each other, because of the lessened resistance offered by passage through the air. The humidity of the atmosphere of covered plant beds is usually much higher than that outside. Good ventilation, provided by partly or entirely removing the covers, will quickly reduce this humidity and dry out the surface soil more rapidly, hence effectively decreasing or entirely checking the spread of the disease.

Infected areas. Damping-off frequently starts at one point in the seed bed and spreads from this point as a center of infection. Where only a few of these centers of infection occur, and the weather promises to be favorable for the disease for some time, much can be done towards checking the trouble by removing the infected plants and soil from the bed, together with the surrounding area somewhat beyond the last signs of disease. In case of the damping-off of valuable plants where only a few are grown, it is best to prick out the healthy plants and transplant them separately into other soil. Spraying the plants with potassium sulphide, Bordeaux mixture, formalin, ammonia, mercuric chloride, lime or sulphur in an effort to check the disease after it had once started gave negative results.

SUMMARY

Damping-off of seedlings in plant beds in Wisconsin is commonly caused by one of two fungi, *Pythium debaryanum* or Rhizoctonia.

These fungi are capable of attacking a large variety of different plants, as well as of living upon the dead organic matter of the soil, and are hence extremely persistent when once present in the soil.

The disease is favored particularly by certain weather conditions, such as excessive moisture and high temperatures, and very little can be done to check the disease when such conditions prevail. Therefore this necessitates the adoption of methods which kill the fungi in order to prevent the disease.

The preventive methods are of such a nature that they must be applied before the sowing of the seed, since the methods used to kill the fungi would also kill the seed.

A number of chemical agents have been tested as fungicides against damping-off, but of these formalin alone has proven of any value under conditions favorable to damping-off.

Treatment of the soil with formalin at strengths of one part formalin to one hundred parts of water and lesser strengths, as frequently recommended, does not kill the fungus. Although it may hold the disease in check for some time, it will allow it to develop later if weather conditions permit. The value of formalin at these strengths is therefore dependent, in a large measure, upon the time of the appearance of weather conditions favorable for damping-off.

Treating the soil with 1-50 formalin at the rate of two quarts per square foot of soil, will kill the fungi which cause damping-off, and will hence effectively prevent damping-off under the most favorable weather conditions for fungous growth. Formalin soil treatment is also somewhat beneficial in stimulating the plant growth and in killing some weed seeds. The chief objections are the cost of the formalin, the time required for it to act, and the time required for the soil to dry out.

Sterilization of the soil by heat has proven the most satisfactory method of preventing damping-off from all standpoints, excepting that under certain conditions it may be more expensive than the formalin treatment. Steam sterilization by

the "inverted pan" method is especially recommended where a steam traction engine is on the farm or can be obtained in the neighborhood.

Aside from preventing damping-off, there are several beneficial secondary effects of soil sterilization by heat. These are principally the killing of all weed seeds and insect pests of the soil, and greatly increased size and vigor of plant grown on such soil.

As a cultural control of damping-off, growers should avoid infected, poorly drained soils and thick sowing of seed.

The only means of checking the disease after it has occured in the plant beds is to remove the covers in order to reduce the temperature and the moisture of the soil and of the air immediately above the plants.

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Black Rot, Shed Burn, and Stem Rot of Tobacco

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CONTENTS

Black Rot, Shed Burn, and Stem Rot of Tobacco

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I BLACK ROT OF TOBACCO [1]

Introduction

During the fermentation of tobacco there frequently occurs a decay of the leaf which is known among packers as black rot. The disease[2] is said by packers to occur particularly in the northern cigar tobacco sections of the United States, including especially the states of Wisconsin, Connecticut, Ohio, and Pennsylvania.

The importance of this disease to the tobacco industry is illustrated by the fact that the loss in Wisconsin alone, in 1892, was estimated at more than a million dollars. The disease rarely occurs to such a marked degree, but conservative packers estimate the loss to the industry as a whole in the United States, in some years at several hundred thousand dollars.

The tobacco packers have long been seeking the cause of this trouble as well as suggestions for its control. The writer has had an opportunity to observe this disease for several years, believing that some definite microorganism must be associated with the disease. In the winter of 1912 an opportunity for a close study of this trouble was made possible by the discovery of the disease, during the course of its development, in a packing

[1] The writer is indebted to L. R. Jones, plant pathologist, of this Station, for many suggestions in preparing the manuscript for publication, and to Mr. George E. Gary, of the P. Lorillard Tobacco Company, for valuable observations on the disease under practical conditions.

[2] The term "disease" is extended in this bulletin to include maladies occurring upon the tissues of non-living matter.

case of tobacco. The slow progress of the studies in the past has been due largely to the peculiar conditions under which the trouble occurs. In practice the disease is not usually discovered until it has run its course, since there is little or no evidence from the outside as to what is happening in the interior of a box of fermenting tobacco. The disease, occurring as it does during the fermentative process, has been considered by some as an advanced stage of fermentation. Though this is not the case, the close association with the fermentative process and its complexities, together with the several other factors which may influence the disease, such as infection, moisture, temperature, aeration, pressure, and character of leaf, tend to make it very difficult to state definitely as to the possible occurrence of the disease and the amount of damage to be expected.

It would be desirable before discussing this disease to take up in some detail the practical observations and theories of a number of packers, the methods of tobacco culture and fermentation, and especially the facts and theories regarding tobacco fermentation, since these matters are all pertinent to the question of cause and control. For the sake of brevity, however, the methods of tobacco fermentation and the processes immediately preceding it will only be briefly mentioned. In the northern tobacco growing sections of the United States the crop is usually harvested during the latter part of August and first of September. The plants are ordinarily cut off at the base and hung up in sheds where they go through the curing process, which is a gradual drying out of the leaves, and a changing from the normal green color to the chocolate brown. During this process the leaves lose fully from 70 to 80 per cent of their moisture, which passes off into the atmosphere, the leaves becoming thin and dry at the end of about two months. After curing, the leaves require moistening before they can be taken down and handled without mechanical injury. This is accomplished by a day or two of relatively moist weather or fog, after which the tobacco is said to come into "case", and is taken down, stripped from the stalks, and packed into bundles weighing approximately fifty pounds. The relative amount of moisture the leaves are allowed to take up in casing is important and will be considered later.

The crop is usually delivered in bundles to the packers, by whom it is graded. The leaves of equal quality and length are tied into small "hands". The hands of the same grade are

packed under pressure into boxes or cases of about 300 or 400 pounds each, with the butts out and the tops overlapping in the center. The boxes are then stored away for the fermenting or "sweating" process, at temperatures which vary greatly with the time of the year, and the methods practiced by the packer. In case no attempt is made to regulate the temperature in the store room the process is called a natural sweat. In case the temperature is maintained above the normal by artificial heat, it is spoken of as a forced sweat. A more recently introduced process is known as bulk sweating. In this process the hands are packed regularly into stacks usually of about 2000 pounds or more, instead of being packed into boxes. The fermentation goes on more rapidly and actively in this process. After the temperature has reached about 100 or 120 degrees F. the piles are taken up and repacked in a similar way. This may be repeated three or four times, or the hands may be packed into boxes after the first bulking, depending upon the judgment of the packer. The natural sweat is most commonly practiced in the northern tobacco sections, and it is in this method that black rot usually occurs.

Signs of the Disease

Black rot, as the name implies, is recognized by the dark color taken on by the rotted areas in place of the normal chocolate brown. The disease as it occurs during its progress is characterized by being relatively moist, though there is no such decomposition and slime as is found in so-called wet rots. The leaf maintains its form until mechanically disturbed. The black rot as packers know it is quite conspicuously a dry rot, and the affected leaves when handled break up into a powder, the cells having lost all their cohesion. When black-rotted "hands" are shaken the injured portions drop out very readily. (Figures 1 and 2). Experienced packers easily recognize black rot as soon as a diseased bulk is opened, by the characteristic odor which differs from that of normally fermenting leaves. The odor is not obnoxious and would not be recognized as peculiar by the ordinary observer. In some instances the rot usually occurs only in the innermost portions of the bulk of tobacco, and especially at the tip end of the leaves where they were overlapped in packing. Where the disease develops only in small areas throughout the case it suggests small, local favorable conditions for its de-

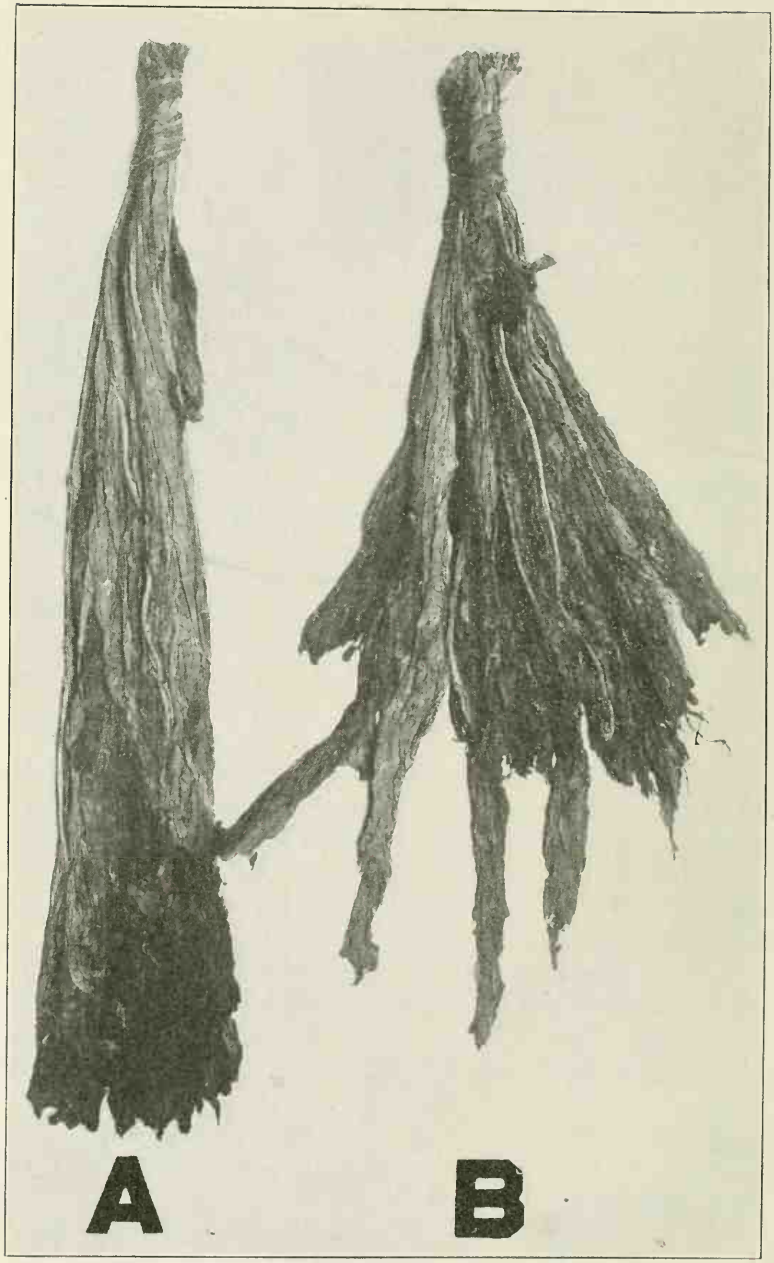

FIG. 1.—BLACK ROT UNDER PRACTICAL CONDITIONS

A Hand of tobacco, lower end diseased

B A similar hand shaken out, showing extent of injury

velopment and it is in such small patches that it usually occurs. In bad cases much or all of the inner pack may be decayed, leaving but a shell of sound leaves which, owing to the unfavorable conditions for the disease found there, are not injured. Where

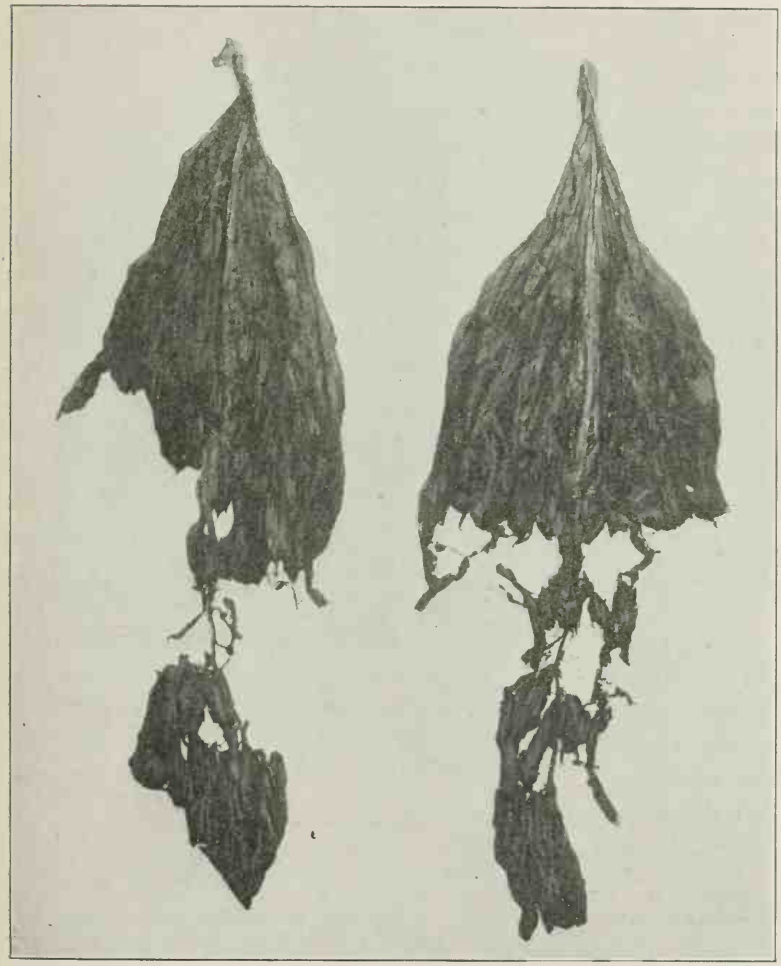

FIG. 2.—A SEVERE CASE OF BLACK ROT

These tobacco leaves show the location and extent of the injury.

the rot has been allowed to run unchecked to "maturity" there usually appear small areas of "black rot soot," filling the small air spaces which sometimes exist between the leaves. The line of demarcation between rotted and sound areas is usually quite

sharp, and the sound portions are still of value if large enough to warrant their separation. The disease may also occur in the bundles if they are allowed to start fermenting before being packed into cases.

THE CAUSAL ORGANISM

Isolation.—Small pieces of freshly diseased tissue were teased apart on a slide and examined under a microscope. On the margin of the bits of decaying leaf there frequently could be seen small pieces of fungous hyphae, which were with difficulty distinguished from pieces of cell tissue. Pieces of diseased tissue were then plated out on potato hard agar in petri dishes and incubated at a temperature of 35 degrees Centigrade. After from 12 to 24 hours there occurred large colonies of bacterial growths, which were followed in from 48 to 60 hours by a marked development of fungal growths particularly of a species which was later determined as *Sterigmatocystis nigra, V. T.* Pure cultures were immediately made of this fungus, and other forms which developed conspicuously on the plates including two species of *Penicillium* and two or three forms of bacteria. Samples of black rot were then obtained from three different warehouses and from several different cases in one warehouse. The rotted portions were carefully removed with sterile forceps to petri dishes and carried to the laboratory where they were plated out separately. Samples of black rot were also obtained from the 1909 and 1910 crops of tobacco and in all cases *S. nigra* was found present in great abundance. On some of the 1912 crop which had rotted the spores of *S. nigra* were present in very great abundance on the diseased tissue.[3]

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[3] Loew, O., Physiological studies of Connecticut leaf tobacco. U. S. Dept. of Agr. Rpt. 65: 7–57. 1900. See particularly 48, footnote reporting *Sterigmatocystis nigra* observed by E. A. Bessey on a specimen of black rot. In correspondence Dr. Bessey stated that he had no proof that this fungus caused black rot aside from finding it on diseased tissue.

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Rapaics, R. A., Dohány kormos rothádasa. Magyor Dohanyujog 30, 2–4 1913. Rev. in Mo. Bul. Agr. Intel. and Plant Dis. 4. 659 1913. *Sterigmatocystis nigra* reported as occurring upon tobacco in Europe. This is said to be the first European report of the occurrence of this fungus on decaying tobacco.

Description.—The decay frequently occurs without the sporulating stage appearing upon the diseased tissues, however. An analysis of the air of several different rooms in a Madison warehouse where black rot was occurring was made by exposing plates of potato hard agar for one and two minutes, and incubating at

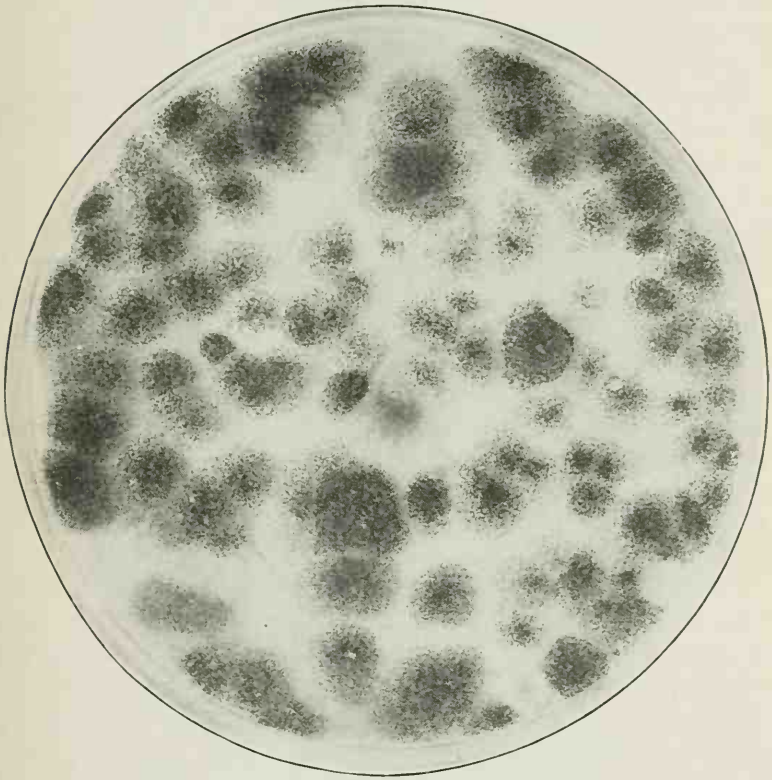

FIG. 3.—*STERIGMATOCYSTIS NIGRA* ON POTATO AGAR
The plate was exposed for one minute in an infected warehouse.

35 degrees C. The characteristic development of bacterial colonies, followed by *Sterigmatocystis* colonies, appeared. In a room where diseased tobacco was being separated from sound tobacco an agar plate exposed for one minute developed approximately 75 colonies of *Sterigmatocystis*. (Figure 3.) Air analyses were made in the same way of two other warehouses in Madison and four in Edgerton, where black rot was not known to occur at the time. *Sterigmatocystis* colonies were obtained in

all the exposures but in smaller numbers than in the first case mentioned.

Inoculation experiments.—Because of the abundant and general occurrence of *Sterigmatocystis*, it seemed likely that this organism, rather than any of the others obtained in culture, was the cause of black rot. Experiments in inoculation were now begun using pure cultures of *Sterigmatocystis nigra*. Tobacco leaves were spread out, piling one on top of the other, and from this pile, circles three inches in diameter were cut, avoiding as much as possible the midrib of the leaf. Thirty-five grams of leaves cut in this manner were packed tightly in four-inch petri dishes and sterilized in the autoclave at 245 degrees F. for 30 minutes. Under sterile conditions these leaves were separated and sprayed with 10 cc. of water containing *Sterigmatocystis* spores from an atomizer which had previously been sterilized. Checks were made by spraying with sterile water only. The plates were then subjected to pressure and finally sealed with paraffin and incubated at 35 degrees C. After five days the white threads of mycelium could be seen in portions of the inoculated plates and after eight or ten days the slender conidiophores of *Sterigmatocystis* stood out conspicuously in large masses around the margin of the leaves as shown in Figure 4. After 15 days, some of the dishes were opened and the texture found to be destroyed, whereas the checks remained as sound as the day they were packed. When allowed to dry out, the similarity to the natural rot was at once evident and was pronounced typical rot by several packers. It was thought possible that the sterilization of the leaves might enable the fungus to develop more readily upon them, but this was disproved by inoculating unsterilized leaves, and obtaining black rot. Fully 75 inoculations of tobacco have been made, and in practically every case where sufficient moisture and a proper temperature were present, black rot occurred. Inoculations of leaves in the same way with cultures of the other fungi and bacteria obtained from black rot tobacco failed to produce after two trials any signs of the disease.

Some study has been made of the causal organism, microscopically and in pure culture, in order to determine the species correctly.[4] This fungus has been the subject of much micro-physi-

[4] The organism was also determined by R. Thaxter of Harvard University as *Sterigmatocystis nigra*. V. T.

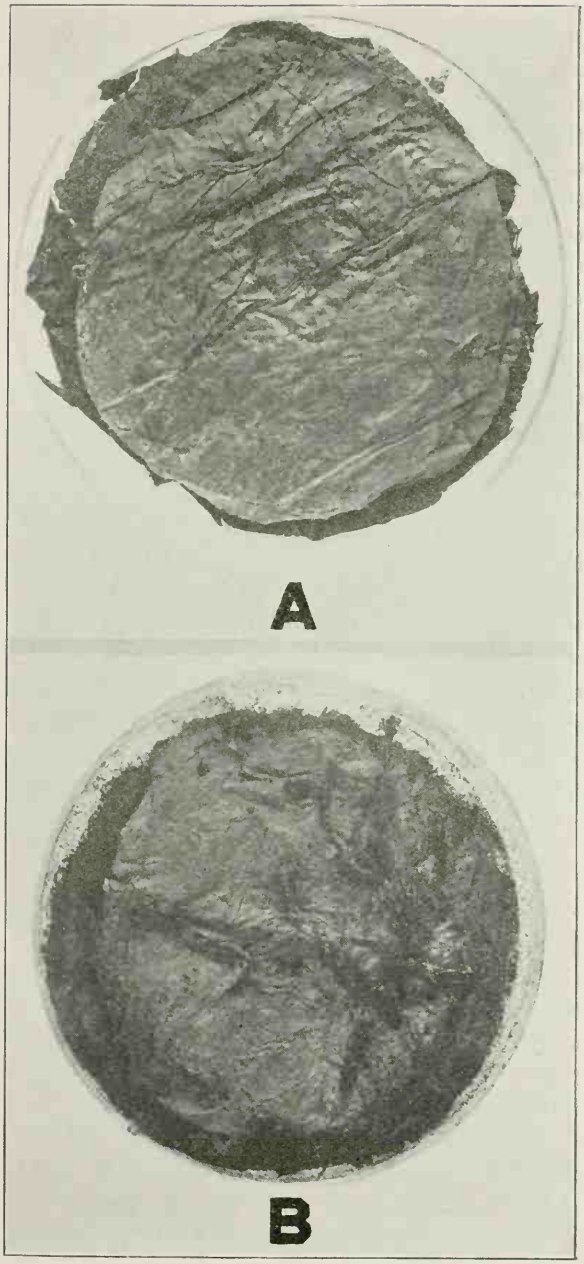

FIG. 4.—ARTIFICIAL INOCULATION

A Check
B Inoculated with *Sterigmatocystis nigra*

ologic experimentation in the hands of many investigators. The fungus appears to vary considerably in measurements with the various substrata upon which it has been grown, and consequently too much stress cannot be laid upon the measurement of the organism. The following description of the fungus taken from black rot and grown upon hard potato agar is submitted. The mycelium is a hyaline, septate, and branched thread from 3 to 10 μ in diameter. The conidiophores are hyaline, erect, and continuous, ending in a globose vesicle from 30 to 40 μ in diameter, just below which there usually is a slight constriction. The conidiophores measure from 240 to 400 μ in length and from 10 to 13 μ in width. The sterigmata are fusoid, and measure from 10 to 12 by about 3 or 4 μ; conidia are blackish brown, globular, smooth or warty, and about 3 or 4 μ in diameter.

Sterigmatocystis nigra has long been known to play an important part in various industries,[5] in both beneficial and injurious ways, and it is not at all surprising to find it developing in fermenting tobacco. It is said to be instrumental in the manufacture of gallic acid from tannin, and in the manufacture of opium, besides being responsible for the cork disease on cork oak trees. The fungus possesses the power of saccharifying starch and in inducing oxalic acid fermentation. A large number of enzymes have been isolated, especially proteolytic enzymes, and pectinase, the latter of which may account for the complete destruction of the texture of the tobacco leaf in black rot.

FACTORS INFLUENCING THE DISEASE

In the fermentation of tobacco there is great variation in conditions, brought about by a number of abnormal and artificial factors. The results of fermentation are consequently quite variable and whether a certain case becomes damaged by black rot or not will depend upon the presence or absence of one or more factors which influence the disease. The greater the number of influencing factors, the more complexities arise in the study of the disease. The four primary factors in relation to the disease are (1) infection with *Sterigmatocystis nigra*, (2) the percentage of moisture in the leaf, (3) the temperature of the leaves, and (4) the air supply. In addition to this there are several secondary factors which influence these primary factors, such as amount of pressure on leaves, character of leaf, time of packing, temperature and humidity of storage rooms, amount of

[5] Lafar, F., Handbook of Technical Mycology, 2, 11, 322. 1904.

infection, and degree of fermentation, which are important to consider in practice. The four primary factors will be considered separately, since the control measures must be based upon them. In influencing the disease these factors are so very closely interrelated and dependent upon each other that it is difficult to deal with one without considering the effects of the other. This point can be illustrated by supposing the tobacco packed with a percentage of moisture too low to allow *Sterigmatocystis* to develop. As fermentation proceeds tobacco loses from 10 to 15 per cent of its weight.[6] Three-fourths of the loss is water and the chemical activity resulting may liberate sufficient moisture in local portions to allow the fungus to grow. The rapidly developing fungus, however, may soon exhaust its oxygen supply and check its own development. A marked rise or fall in temperature, or a succeeding diminution of moisture, may bring about the same result. In general, favorable and unfavorable conditions may arise intermittently and in close sequence where such a number of primary and secondary factors are involved, rendering the occurrence and percentage of damage from the disease very problematical.

Infection.—The possibilities of infection of tobacco with *Sterigmatocystis nigra* under ordinary conditions is very great. The fungus is found to occur almost universally and has been isolated from soil and various kinds of fodder, as well as tobacco. Black rot sometimes occurs in bundles before they are taken to the warehouses for packing, which indicates that infection may take place on the farm. In tobacco packing houses where the disease has once occurred, however, the spores are very much more abundant, more infection takes place, and more black rot is likely to occur.

Moisture.—The percentage of moisture in tobacco at the time of packing varies considerably. There is some skepticism among practical men as to the influence of moisture on black rot, owing to the fact that comparatively wet tobacco is sometimes unaffected by black rot. As will be pointed out later, this is perhaps primarily a temperature relation influenced by moisture. In order to obtain some definite data on the matter of moisture relations, the following experiment was carried out.

Thirty-five rolls of tobacco leaf weighing about 100 grams each were made by cutting leaves into strips two and one-half inches

[6] Jenkins, E. H., Shrinkage during fermentation. Conn. Agr. Expt. Sta. Rpt. 1896 : 285.

in width. These rolls were raised to different percentages of moisture and infected by spraying with water and *Sterigmatocystis* spores from an atomizer. After lying loose in a large moist chamber for a day in order to let the moisture permeate the leaves uniformly, the strips are rolled up and weighed quickly to a hundredth of a gram. The roll was immediately put into an ordinary glass tumbler which was inverted into a half-petri dish. Melted paraffin was then poured into the space between the tumbler and the rim of the petri dish forming a very satisfactory sealed chamber. (Figure 5.) These chambers were incubated at from 32 to 35 degrees C. for approximately two months. The rolls were then taken out and weighed quickly, after which they were placed for three days in a drying oven at 90 degrees C., and weighed again for dry weight. The loss due to destruction of dry matter by black rot lowered the dry weight of the rotted samples in such a way as to render it impossible to get the actual percentage of moisture in each sample. The loss of weight during incubation as shown by weighing before drying was correlated with the percentage of disease. In the rolls where no rot occurred there was only an average loss of 0.36 per cent, whereas in the considerably rotted rolls the loss of weight ran up to 1.57 per cent, and in those badly rotted up to 6.4 per cent of the total weight. These losses represent largely the percentage of dry matter destroyed by the black-rot organism and to a small extent that due to fermentation. The loss of weight during incubation was added to the dry weights and the average dry weight of the rolls determined, which was used as a basis of arriving at the percentage of moisture in each roll. The results of this experiment are summarized in Table I.

TABLE I

RELATION OF AMOUNT OF BLACK ROT TO MOISTURE PERCENTAGE IN LEAF 32–35 DEGREES C.

Moisture, per cent	Tests	Amount of Black Rot		
		None	Considerable rot	Badly rotted
26–32	11	11	0	0
33–39	13	5	8	0
40–46	11	0	6	5

The data show quite conclusively the increase in black rot with the increase in percentage of moisture in the leaf. Though

no black rot occurred in these experiments when there was less than 32 per cent of moisture present, this does not mean that black rot will never occur at lower percentages of moisture. At higher temperatures and correspondingly more active fermentation, it is possible that black rot may occur even with 4 or 5 per cent less moisture. Sufficient moisture should of course be maintained in the leaf to facilitate handling without mechanical injury and to allow fermentation to take place. According to Whitney and Means[7] this should be about 23 or 24 per cent in Florida leaf, and, according to the same authorities, the presence

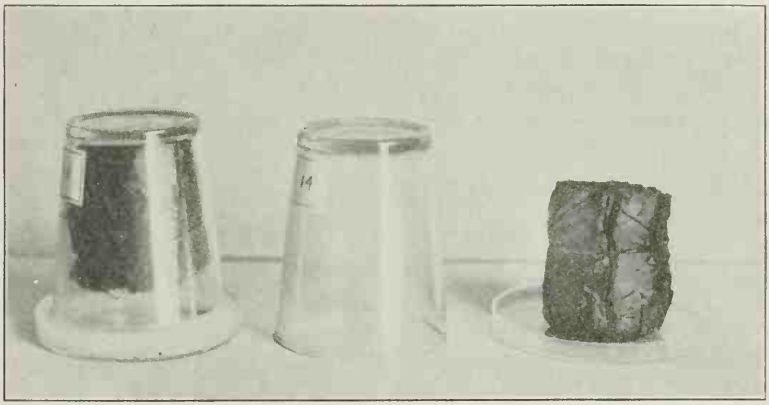

FIG. 5.—BLACK ROT MOISTURE DETERMINATIONS

This illustration shows a simple and satisfactory sealed chamber used in moisture determinations.

of 26 per cent moisture is likely to induce danger during fermentation. There is undoubtedly a considerable difference in the percentage of moisture at different points in the same case, which may account for the frequent small local areas of black rot which are to be found under warehouse conditions.

Temperature.—Relatively high temperature is a predisposing factor for black rot. The fermentation of tobacco is accompanied by a rise of temperature. Black rot is a disease occurring during fermentation and, as far as is known does not occur on tobacco except during the fermentative process. This at once leads to the conclusion that the growth of the fungus *Sterigmatocystis nigra* is favored by relatively high temperatures. In or-

[7] Whitney, M. and Means, T. H., Temperature changes in fermenting piles of cigar-leaf tobacco. U. S. Dept. Agr. Rpt. 60 : 7-28. 1899.

der to determine the relation to temperature conditions, equal weights of pieces of tobacco leaves were packed in each of six petri dishes after having been inoculated and brought to an equal percentage of moisture, favorable for the black rot. They were then sealed iwth paraffin and two placed at each of the following temperatures, from 18 to 20 degrees C., from 31 to 33 degrees C., and from 44 to 46 degrees C. Black rot occurred immediately at from 31 to 33 degrees C. after 10 days, but did not occur at all in the higher or lower temperatures at the end of 28 days.

In order to determine further the relation of growth of *Sterigmatocystis nigra* to temperature, fourteen tubes of potato hard agar were inoculated with spores of the fungus from a pure culture and placed at seven different constant temperatures ranging from 10 to 46 degrees C. The growths of the fungus at the same temperature were remarkably equal, and were estimated in percentage of surface area of agar slant covered at the end of the 24, 48, 72, and 96 hours as shown in Table II.

TABLE II

RELATIVE GROWTH IN PERCENTAGE OF *Sterigmatocystis nigra* AT VARIOUS TEMPERATURES

Temperature, C.	10–12°	15–16°	21–23°	28–29°	31–33°	40–42°	44–46°
24-hour culture							
I	0	0	3	40	50	30	0
II	0	0	3	40	50	30	0
48-hour culture							
I	0	0	30	95	100	90	0
II	0	0	30	95	100	90	0
72-hour culture							
I	0	0	60	100	.....	100	0
II	0	0	60	100	.....	100	0
96-hour culture							
I	0	0	80	.....	.....	.....	.....
II	0	0	80	.....	.....	.....	.....

According to this table the most rapid growth of *Sterigmatocystis nigra* took place at a temperature between 31 and 33 and at from 40 to 42 degrees C. the growth was being checked by heat. Unfortunately, no constant temperature chamber at temperatures between 34 and 40 degrees was at hand, but the table indicates that the optimum temperature must lie somewhere between 34 and 40 degrees according to this data or approximately 37 or 38 degrees C.—98–100 degrees F. According to some authors[8]

[8] Lafar, F., Handbook of Technical Mycology. ed. 2, II, 322. 1904.

the optimum temperature for *Sterigmatocystis nigra* is approximately 40 degrees C. and the minimum 7 degrees C.

From this table can be drawn the conclusion that black rot will only occur between temperatures of about 20 to 44 degrees C.—68 to 111 degrees F.—when all other factors are favorable. It is very probable that the limits of temperature in which black rot occurs are much more restricted than this, since it is favored by active fermentation which cannot be said to occur until a temperature of at least 30 degrees C.—86 degrees F.— is reached. The temperature of fermenting tobacco may run up above 44 degrees C.—111 degrees F.—under some conditions, especially in bulk fermentation, and even in case fermentation, by either natural or forced sweat, in which instance the development of black rot is impossible.

In order to ferment tobacco properly it is impossible to avoid the temperatures which are most favorable to black rot, but in this connection it is very important to consider the length of time the tobacco remains at this temperature. If we compare the height of temperature and the comparative rate of rise of temperature in natural, forced, and bulk fermentation in practice, we find the following to be true. In natural sweat, the temperature rises ordinarily to from 30 to 40 degrees C.—86 to 104 degrees F.—in a period of one to two months, depending upon the outside temperature. In the forced sweat a similar temperature is ordinarily reached in from one to three weeks, depending upon how much heat is applied from the outside. In bulk fermentation, however, a temperature of from 40 to 60 degrees C.—104 to 140 degrees F.—may be reached in from 3 to 6 days.[9]

In natural fermentation, then, the tobacco leaves remain at a temperature favorable for black rot throughout the entire time of active fermentation, and if sufficient moisture is present in the leaf, damage is almost certain to occur. Forced fermentation is little better unless it raises the temperature above the maximum—about 111 degrees F.—in less than 5 or 6 days, as laboratory experiments have shown that considerable damage

[9] For more detailed information along this line the reader is referred to the following publications:

Whitney, M. and Means, T. H., Temperature changes in fermenting piles of cigar leaf tobacco. U. S. Dept. Agr. Rpt. 60. 7–28. 1899.

Jenkins, E. H., The fermentation of the tobacco crop. Conn. Expt. Sta. Rpt. 1899, 291 to 297.

McNess, G. T. and Massey, G. B., Tobacco investigations in Ohio. U. S. Dept. Agr., Bur. of Soils, Bul. 29: 7–38 1905.

occurs in 5 or 6 days with all conditions favorable. In bulk fermentation the tobacco passes through the dangerous temperatures rapidly, usually in 4 to 5 days, and the bulk then is repacked before the rot starts, in which process considerable moisture is lost, diminishing to a large extent the possibility of black rot in the next bulk.

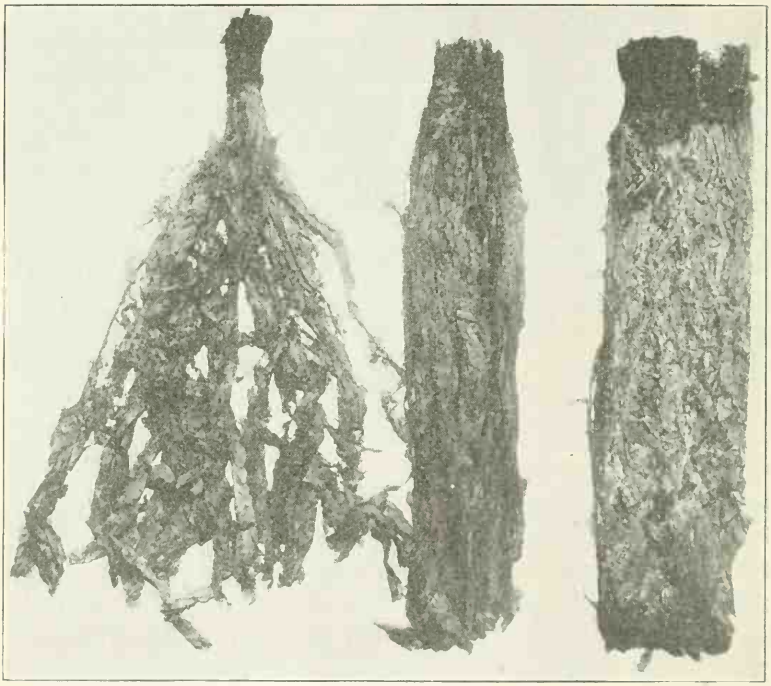

FIG. 6.—BLACK ROT PRODUCED BY ARTIFICIAL INOCULATION WITH *STERIGMATOCYSTIS NIGRA*

These hands of tobacco were inoculated and placed under conditions favorable to the development of the fungus. It completely destroyed them.

Oxygen.—The amount of oxygen present has unquestionably a bearing on the extent to which black rot occurs. In the interior of a tightly compressed case of tobacco the amount of air present must be quite small, and readily exhausted by any growing organism before air can diffuse in from the exterior to take its place. More frequently in cases affected by black rot the disease occurs in small local areas, which suggests that it has been checked by some factor. This may frequently be due to a change of temperature or a reduction of moisture, but it is also possible that it is sometimes due to an exhaustion of the air supply, which

may prevent the growth of the fungus until the factor of temperature or moisture has changed sufficiently to prevent its resumption. Owing to the apparently very unfavorable conditions of air supply under which it grew it was at first suspected that the fungus causing black rot must be anaerobic. Attempts were then made to grow the fungus in the absence of oxygen, using in turn the method of pyrogallol-potassium hydrate, the hydrogen replacement, and the paraffin oil covering. In all cases growths of *Sterigmatocystis* were obtained, but much slower and less vigorous than those in air. Owing to the partial development of certain other known aerobic forms of fungi under the same conditions, it was concluded that the oxygen was not removed sufficiently or rapidly enough to prevent growth. The experiments, however, indicated that the fungus was capable of growing slowly in the presence of a much reduced oxygen supply such as possibly occurs in the interior of a bulk of fermenting tobacco. The absence of oxygen is probably especially common in the tobaccos packed relatively wet, and may help to explain why wet tobacco sometimes will not black rot, although this can usually be explained by the fact that relatively wet tobacco ferments more actively and hence develops a temperature unfavorable for black rot more quickly and maintains it longer than relatively dry tobacco. No importance, however, can be given to the matter of oxygen relations in the consideration of control measures, owing to the manifest impossibility of controlling it in practice.

Suggestions for Control

The most certain method of controlling the disease would be the destruction of the fungus either by killing the spores or by preventing their germination. There is little hope of doing this after the tobacco has once become infected, without possibly injuring the quality. It is possible, however, that satisfactory methods of sanitation and fumigation may be developed which will serve to hold the disease under control. The sorting and packing rooms of a warehouse where black rot has once occurred is a constant source of infection. The destruction of the spores of *Sterigmatocystis* in these places would no doubt aid in a large measure to prevent a serious recurrence of the disease. This would not be a complete safeguard, however, because, as has been pointed out infection may take place before the tobacco

reaches the warehouse. A thorough cleaning of the entire warehouse, removing and burning all the refuse, thoroughly sweeping and possibly washing the floors and walls, followed by painting or whitewashing are secondary means which should be resorted to. The possibility of formaldehyde fumigation of the warehouse is worthy of consideration by packers who are willing to practice strenuous methods in attempts to check this disease.

The most essential point to consider in preventing black rot are the matters of percentage of moisture in the leaf at the time of packing and the temperature of fermentation. The growers should use all means possible to have the crop properly cured before freezing weather in the fall, and should not let the tobacco come into too high case before packing in bundles. When the packers obtain relatively moist tobacco, there are several courses open to them to reduce this moisture before packing.

1. If only a few crops are in "high case"—have a high percentage of moisture—they may be allowed to remain in the bundle until the latter part of the packing season, when they will have lost considerable of this moisture.

2. Bulk fermentation, for reasons already stated, may be substituted for case fermentation with those crops which contain a dangerously high percentage of moisture.

3. One "bulking" may be made before packing in cases, the extra handling removing considerable moisture. Where warranted, two bulkings previous to casing may be made.

4. The tobacco may be packed into cases and transferred immediately to a room temperature of from 40 to 42 degrees C. to insure the tobacco rising above a temperature of 44 or 45 degrees C.—from 110 to 113 F. in three or four days. Unless the temperature in the case remains above about 110 or 113 degrees F. for some time this forcing may be more injurious than beneficial, and a higher room temperature in some instances may be necessary.

5. Pack the tobacco directly into cases, and store it in rooms where the cases will not start active fermentation until late spring or early summer, when the tobacco should have dried out sufficiently to prevent rot from occurring during fermentation. This method probably has the disadvantage in being more likely to bring on the trouble known as "must" or "white mold".

6. It is possible that a drying machine or "evener" may be used to regulate the moisture content of all the tobacco passed through it to a percentage that would be just sufficient to let it

ferment properly, but insufficient to allow damage to result. The tobacco should pass through the "evener" after the sorting process and immediately preceding packing.

II SHED BURN AND STEM ROT OF TOBACCO

Introduction

Shed burn is a term applied to a decay of tobacco which occurs on the leaf tissue during the curing process. A similar decay on the midrib of the leaf during the same process is known as stem rot. These troubles are of frequent and general occurence throughout the tobacco districts of the country and especially in sections where the natural curing process is practiced. Shed burn and stem rot are the cause of a very considerable loss to the tobacco growers when conditions are favorable for the development of the diseases. Shed burn, pole burn or pole sweat as it is variously called, is usually recognized in mild cases by the development of small dark spots upon the leaf and a resulting loss of elasticity of the affected parts. In severe cases these spots may run together, destroy the texture of the entire leaf, and render it valueless for wrapper purposes.

Stem rot is usually characterized by the development of whitish or pinkish molds upon the midrib of the leaf, which may cause decay to such an extent that the leaf drops from the stalk or the leaf tissue separates from the midrib. These shed diseases are known to appear under practical conditions as a result of relatively high humidity and temperature in the curing shed due to similar climatic conditions, poor ventilation, or too close hanging of the plants in the shed.

According to Sturgis[10] shed burn is a bacterial decay following a partial destruction of the leaf tissue by fungi. Examination of shed burned leaves in Connecticut, in 1891, showed the presence of two forms of bacteria, one belonging to the genus *Bacterium*, and the other being a *Micrococcus*. A fungus belonging to the genus *Cladiosporium* was invariably found associated with the decay and apparently always preceded the bacterial decay. In the same report Sturgis ascribes the stem rot disease to *Botry-*

[10] Sturgis, W. C., Preliminary report on the so-called "pole burn" of tobacco. Conn. Agr. Expt. Sta. Rpt. 1891: 168–173 and 184–185.

tis longibrachiata and suggests sanitation and fumigation with sulphur for control, together with regulation of the temperature and humidity of the shed.

A short time later Behrens[11], working in Germany, attributed the decay of curing tobacco to two closely related fungi, *Sclerotinia libertiana* Fckl. and *Botrytis cinerea* P. According to his observations when these fungi attack the midrib of the leaf the effect is a stem rot and when they attack the leaf tissue the shed burn disease is produced.

Sturgis[12], continuing his observations in 1899, found a species of *Alternaria* ordinarily present, and believed this organism was the cause of the primary decay of the particular specimens of shed burn examined in that year. Plating out species of diseased tissues brought out several species of bacteria and other fungi, which occurred in such small numbers, however, as to preclude the possibility of their being instrumental in the destruction of tissue. Decay of curing tobacco has also been attributed by others to a species of *Pleospora, Botrytis vulgaris,* Fr. and to two different species of *Mucor.*

In the fall of 1910, observations on the shed diseases of tobacco were begun in Wisconsin, in order to determine in a preliminary way the causal organisms in this state. These observations were continued in the autumns of 1911 and 1912, in a general way, for the purpose of corroboration.

INVESTIGATIONS

Search was first made for specimens of diseased leaves in various sheds during the curing season, and in this way several samples of stem rot were obtained from different tobacco sheds in different localities, and a few specimens of shed burn were also collected. The specimens were taken to the laboratory and the causal organisms generally determined directly, if they were abundant and sporulating. In some cases, however, the pieces of diseased tissue were plated out on potato hard agar, and the fungi obtained in pure culture from which their morphology was more carefully studied. Some inoculation experiments were also made by placing short pieces of green and thoroughly cleaned tobacco midribs in test tubes on a moist plug of cotton,

[11] Behrens, Dr. J., Trockene und nasse Fäule des Tabaks. "Der Dackbrand." Ztschr. für Pflanzenkrank. 3 : 82.

[12] Sturgis, W. C., Further notes on the pole burn of tobacco. Conn. Agr. Expt. Sta., Rpt. 1899 : 265–269.

and inoculating with pure cultures of the fungi isolated. Although infection was slow in all cases, and did not take place until the cells of the leaf had apparently become weakened from starvation, the tests served to show that the fungi obtained were capable of producing stem rot.

Another method of obtaining the causal organisms was to place a number of mature tobacco leaves, taken directly from the field, in a tight, sterile chamber where the developments of mi-

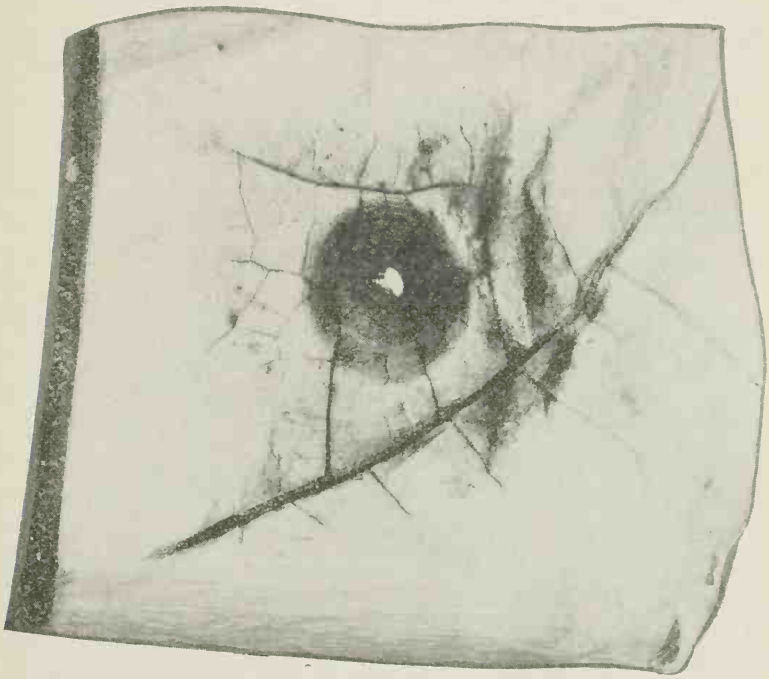

FIG. 7.—SHED BURN OF TOBACCO

The early growth of *Fusarium* upon the tissue of a tobacco leaf. Color of leaf removed with alcohol to contrast diseased area.

cro-organisms could be watched daily and the source of infection from the curing shed was eliminated. In three or four days the beginning of fungus growths could usually be observed, especially on the wounded portions of the midrib. As the leaf tissues began to change color in spots, indicating the death of the cells, it almost immediately decayed and became covered with mycelial threads of various fungi. The midrib proper, being the last to change color, usually resisted the attacks of fungi for the longest time but finally became completely overrun with them and frequently rotted down to a slimy, putrid mass. Pure cult-

ures were made of decaying tissues from these chambers in the same way as those taken from the sheds.

By these methods the writer has collected fourteen different forms of fungi from decaying tobacco during the last three years, many of which, however, occurred only rarely, and only three or four of which can be said to be of general occurrence under ordinary field conditions. A species of *Fusarium* was found of the most frequent and widespread occurrence on decaying tobacco. Occurring quite frequently, but not to so great an extent, was *Tricothecium roseum* and *Penicillium brevicaule,* and in a few instances a species of *Alternaria* and one of *Botrytis.* In the small curing chamber, *Fusarium* and species of *Penicillium* and *Mucor* seemed to predominate, and were later followed by bacterial decay.

CONCLUSIONS

Sturgis has previously suggested that the initial decay in shed burn may be due to one or more of various fungi. The results of Behrens observations together with those obtained here point toward the conclusion that no particular organism can be universally ascribed as the cause of this disease. Moreover, it is to be expected that dead or dying plant tissues, such as occur in curing tobacco, will become substrata for various saprophytic organisms when conditions favorable for their development occur. Furthermore, it seems most reasonable to suppose that such organisms as sometimes exhibit considerable parasitic action should be the first, and the ones most likely to produce the decay of curing tobacco. For this reason we may expect that the purely saprophytic fungi should occur more rarely, if at all. The occurrence of species of *Fusarium, Botrytis, Sclerotinia,* and *Alternaria* seems to indicate that these organisms exhibit some parasitism upon the slowly dying leaves.

These observations indicate that the causal organisms of shed burn and stem rot vary in different sections of the country, and that it is in all probability a matter of the organisms with which the leaves happen to become infected, either from the field or from infectious material in the curing shed. The organism actually producing the decay may also vary with the temperature and humidity as well as with the stage of curing, and consequently we may find a considerable variance in the different sheds and in different years in the same locality. Sturgis' con-

clusion that the fungi are not the principal organisms of decay, but that the bacteria which follow are mainly instrumental in causing decay has not yet been definitely verified, but the difference here is probably a matter of the comparative amount of decay. When exceptionally favorable conditions for the development of microorganisms occur, as when the leaf is saturated with uncombined water, the development of bacterial decay is marked, but such conditions are comparatively rare in tobacco sheds. The ordinary shed burn, as far as my observations go in Wisconsin, seems to be due entirely to fungi.

Sturgis attributes stem rot in Connecticut to *Botrytis longibrachiata* alone, while Behrens attributes stem rot in Germany to a *Sclerotinia* and a *Botrytis,* which also cause the shed burn disease. In Wisconsin, *Fusarium* particularly seems to cause the decay known as stem rot and shed burn equally well, and for this reason it appears that the two diseases may be caused by the same organism and that they differ mainly in the point and time of decay.

The control of these diseases by fumigation of the shed before hanging the tobacco seems impracticable because of frequent infection of the leaves in the field as shown by the decay of leaves when hung in sterile chambers. The practice of sanitation, especially the removal of refuse from the sheds where the diseases have once occurred, will no doubt aid in diminishing the amount of infection. For the present, the growers must look forward to the proper construction of curing-sheds with the object of controlling the humidity by proper ventilation and shutting out the moisture during damp weather. The regulation of temperature and relative humidity by fires in the shed when there is danger of the disease, or the entire substitution of the use of artificial heat for the natural curing method now in use in Wisconsin are methods of control which could be beneficially resorted to in this state.[13]

Summary

BLACK ROT

Black rot is a disease of leaf tobacco occurring during the fermentation process, especially in the northern cigar tobacco producing sections.

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[13] For practical discussion of control see Johnson, J., The control of diseases and insects of tobacco. Wis. Agr. Expt. Sta. Bul. 237 : 7–34, 1914.

The loss from this disease is sometimes very great and consequently a consideration of control measures is important. The controls recommended are based on the determination of the causal organism of the disease, and the factors which influence its development.

The rot has been shown to be due to the development of the fungus *Sterigmatocystis nigra* V. T. upon the leaves in the packing, causing them to blacken and decay in spots.

In order for black rot to occur, tobacco must first become infected with *Sterigmatocystis nigra* after which a moisture content of 26 per cent or more, a temperature of from 30 to 44 degrees C.—86–111°F.—and proper aeration must be present.

The amount of damage occurring in fermentation will vary principally with the actual amount of moisture in the leaves at the time they go actively into fermentation, the length of time a favorable temperature is maintained, and the amount of infection present.

The control of the black rot disease under warehouse conditions must depend on the regulation of the moisture percentage in the leaf, and the temperature of fermentation. Where the disease has previously occurred sanitary methods should be used, and in extreme cases fumigation with formaldehyde may be of much value.

SHED BURN AND STEM ROT

The shed burn and stem rot of tobacco in Wisconsin may be due to one or more of several different saprophytic fungi. A species of *Fusarium* has been found to be most commonly the cause of these diseases in this state.

The difference between shed burn and stem rot appears to be primarily one of location of disease rather than a difference in the causal organisms. If the fungi attack the midrib, the resulting decay is called stem rot. If the leaf tissue is attacked the decay is called shed burn.

The prevention of the infection of tobacco with the spores of these diseases by fumigation of the curing sheds with sulphur, as suggested by Sturgis, seems impracticable owing to the large amount of infection from the field. In controlling the disease the growers must rely upon regulation of the temperature and humidity of the curing shed by proper ventilation or the application of artificial heat.

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Germination and Infection with the Fungus of the Late Blight of Potato

(*Phytophthora infestans*)

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CONTENTS

Germination and Infection with the Fungus of the Late Blight of Potato (Phytophthora infestans)[1]

I. E. MELHUS

INTRODUCTION

Spore germination and infection are the preliminary stages in the destruction of host tissue by most fungous parasites. It is well established that these early stages are dependent, in a large measure, upon environmental conditions and the interrelations of host and fungus. How and to what extent they react on the spread of any disease is not well understood and affords a fertile field for further contributions to plant pathology.

A specific example illustrating these general statements is offered in the case of the potato disease caused by *Phytophthora infestans*. It has been recorded many times[2] that the advent of favorable weather conditions coupled with the presence of the fungus and abundant host tissue, invariably leads to an epidemic. The control of this disease, or any other which develops in a similar manner, depends chiefly upon the elimination or destruction of these early stages. In other words, the fundamental principle underlying disease control, whether it be by rotation, sanitation, or spraying, involves the prevention of spore germination and infection. Hence the desirability of detailed information concerning

[1] This publication is based on investigations made in the laboratory of Plant Pathology at the University of Wisconsin, in collaboration with the Office of Truck Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. I wish to express my indebtedness first to the University of Wisconsin and second to the U. S. Department of Agriculture, for providing financial support to make this investigation possible. My sincere thanks are due to Professor L. R. Jones for many helpful suggestions during the progress of this study and the preparation of the manuscript, and also to Professor R. A. Harper, formerly of the University of Wisconsin.

[2] Ward, H. M. Diseases in plants, pp. 149-151. 1901.
Lutman, B. F. Twenty years' spraying for potato diseases. Vt. Agr. Expt. Sta. Bul. 159: 225-247. 1912.

not only the morphology of germination and infection, but also the retarding and accelerating external influences, becomes apparent. Much as *Phytophthora infestans* has been studied since its first appearance in Europe in 1842, our knowledge of the early stages of its life history has been extended little since the writings of Schacht (1856) and De Bary (1863). At that time the important morphological facts were carefully pictured and correctly described; but other important problems relating to external influences or environmental conditions were merely suggested, not solved.

The purpose of this publication is to record and discuss data and observations collected during the last three years on these early stages of the development of *Phytophthora infestans*. The subject matter falls chiefly under three heads: (1) the effect of external influences on spore germination; (2) the toxic action of certain chemicals and spray mixtures on germination at optimum temperature conditions; (3) infection and its relation to environmental conditions. These will be discussed in the order named.

THE EFFECT OF EXTERNAL INFLUENCES ON SPORE GERMINATION

In a previous paper on Cystopus (Melhus, 1911) it has been shown that the methods used by the earlier students of the Peronosporaceae, while adequate for learning the morphological facts connected with spore germination, were too crude and inaccurate to be followed in a more extended study dealing with the effect of environmental influences. This fact led me first to work out a method of germinating the asexual spores in abundance, at will. Following this, such questions as the minimum, optimum, and maximum temperatures for germination were determined. Other problems relating to germination were taken up subsequently, such as germination out-of-doors, time requirement, percentage germinating, effect of temperature on period of motility, and germination of zoöspores. The studies then undertaken with Cystopus have been extended to Phytophthora.

Method

Essentially the same method as that used in the experiments with Cystopus just referred to has been followed in these later studies and hence it needs little explanation except as to source of spore material and treatment of the cultures.

Viable spores were obtained from two sources, pure cultures on potato blocks, and infected potato foliage. From the potato-block cultures the best spore material was available from the fifth to the ninth day after inoculation. On the foliage the best spores developed two or three days after the first visible evidence of infection. The spores were removed from the pure cultures with a sterile needle and immediately stirred into a drop of water on the microscope slide. Spores were removed from the infected foliage (1) by carefully scraping them from the leaf with a thin-edged scalpel and (2) by placing a small drop of water on the portion of the leaf bearing the spores and then, with the dropper, immediately collecting it again. The latter method yielded more spores and was the one usually employed. Spores were never taken from leaf tissue that had begun to soften, as it often does when infected leaf or stem tissue is placed in a very moist atmosphere. The best spore material was obtained when newly infected plants were placed in an atmosphere sufficiently dry to prevent the tissues from thus softening materially.

Clean slides were used, each bearing a drop of tap water which was later impregnated with spores. These slides or cultures, as they will be called in this bulletin, were now placed in a damp chamber, made by placing three layers of moist filter paper in the bottom of a six-inch petri dish and subjected to known temperature conditions.[3] The number

[3] For temperatures below 14° C. an ice box was used with an ice capacity of 400 pounds. In order to know the continuous temperature during any and all of the tests, a Richard's self-registering thermometer was kept in the ice box. With proper care it was possible to maintain a fairly uniform temperature for twenty-four hours—the variation not exceeding a degree. In this manner it was readily possible to obtain temperatures as desired between 8 and 14° C., but for temperatures lower than 8° C., e. g., from 2 to 6° C., another device had to be used. A caloric cooker of 3-gallon capacity was filled with water of the required temperature and placed in the ice box. In this way it was again possible to maintain temperatures ranging from 2 to 6° C. for a period of from eight to ten hours and for a longer time by adding a few pieces of ice every three hours. Temperatures between 0 and 1° C. were obtained by placing cultures on large blocks of ice in the ice box. For temperatures between 14 and 21° C., another caloric cooker was used in the way described above, except that it was kept in a room having a comparatively even temperature. For temperatures between 24 and 31° C., an electric incubator was used.

of tests made on the same date varied from one to nine, the usual number being two. Tests were always made at two or more temperatures at the same time. The duration of the various experiments varied considerably, ranging from 45 minutes to 48 hours, depending upon the object of the experiment. Examinations of the cultures were made at intervals of from one to six hours in some cases; in others no examination was made until the experiment was terminated.

EXPERIMENTAL DATA ON SPORE GERMINATION OF PHYTOPHTHORA

As is well known, the conidia of *Phytophthora infestans* may germinate either by the liberation of zoöspores or by the production of a germ tube without the intervention of zoöspores. When the conidium becomes a sporangium and liberates zoöspores, germination may be said to be *indirect* and when the conidium produces a germ tube instead, germination may be spoken of as *direct*. This distinction will be made throughout this paper.

Following the method just outlined, a study has been made of the relation of temperature to conidial germination of both types, and also of the behavior of the zoöspores after their discharge from the sporangium. Other external influences such as light, dry air, oxygen, and density of solution have been considered in turn.

THE INFLUENCE OF TEMPERATURES BETWEEN 0 AND 30° C. ON GERMINATION

Having previously learned that relatively low temperatures exert an important influence upon the germination of conidia of *Cystopus candidus*, it seemed desirable to determine whether or not a similar correlation of temperature and germination exists with *Phytophthora infestans*. To this end many tests were made at various temperatures between 0 and 30° C., as indicated in Table I.

From one to nine cultures were made at a time, the usual number being two. This is not apparent in every case in the data given in Table I, because of the grouping together of like tests in the table. The duration of the tests varied

from 3 to 48 hours. The percentage of germination is in terms of the number of cultures showing germination. All observations were discontinued when there appeared to be

TABLE I.—GERMINATION OF PHYTOPHTHORA INFESTANS AT VARIOUS TEMPERATURES BETWEEN 0 AND 30° C

0–1° C.																	
Cultures *....	3	3	3	4	3	6	6	2	11	2	2	2					47 a
Hours.....	5	9	24	25	3.5	4	10	3	2	6	1.5	48					18 b
Results.....	—	—	—	—	—	—	—	—	—	—	—	—					0 c
2–3° C.																	
Cultures.....	3	3	3	3	3	3											18 a
Hours.....	3	4	24	5	6	10											8.7 b
Results.....	—	—	—	—	+	+											33 c
5–6° C.																	
Cultures.....	8	9	6	6	9	3											41 a
Hours.....	6	2.5	5.5	3	2.5	3											3.8 b
Results.....	+	+	+	+	+	+											100 c
8–9° C.																	
Cultures.....	3	3	3	4	6	6	3										28 a
Hours.....	2	4	2	5	2.5	2.5	3										3 b
Results.....	+	+	+	+	+	+	+										100 c
10–11°C.																	
Cultures.....	5	16	39	13	3	8	12	14	3	2	2						117 a
Hours.....	1.8	3	2	1	4	5	2.3	1.5	1.2	2.2	.8						2.2 b
Results.....	+	+	+	+	+	+	+	+	+	+	+						100 c
12–13°C.																	
Cultures.....	12	9	11	4	4	2	5										47 a
Hours.....	2	1.5	1	2.5	3	5	.8										1.8 b
Results.....	+	+	+	+	+	+	+										100 c
14–15°C.																	
Cultures.....	3	20	9	2	1	2	2	4	2	2	5	4					56 a
Hours.....	4	2	2.5	3	2	2.5	3	4.5	3	5	1	1.5					2.8 b
Results.....	+	+	+	—	—	—	—	+	+	+	+	+					80 c
16–17° C.																	
Cultures.....	1	2	2	2	2	2	2	5	4								22 a
Hours.....	2	2.5	3	4.5	4.5	3	5	1	1.5								3 b
Results.....	—	—	—	+	—	+	+	+	+								68 c
20–21° C.																	
Cultures.....	3	5	7	4	12	2	5	2	4	2	2	2	2	2	1	3	58 a
Hours.....	15	3	2	2.2	3	2	21	1	1.5	8.3	4.5	2.8	5.3	1.5	3.5	22	6.2 b
Results.....	+	+	—	—	—	+	+	+	—	—	—	+	—	+	—	+	38 c
24–25° C.																	
Cultures.....	3	3	2	2	2	3	4	5	3								27 a
Hours.....	3	2	2.2	3	2	2	3	21	22								6.7 b
Results.....	—	—	—	—	+	—	—	+	+								37 c
29–30° C.																	
Cultures.....	6	6	6	6	6	6	6	3									45 a
Hours.....	24	24	20	24	24	24	24	30									24.3 b
Results.....	*	*	—	—	—	—	—	—									0 c

* The number of slides each carrying a drop of water charged with viable spores.

The minus sign (—) indicates that no germination took place, and the plus sign (+) that germination did occur.

a, indicates the total number of cultures made; b, the average time in hours; and c, the percentage of the total number of cultures showing germination.

* Very sparing germination.

no further evidence of germination. In most cases this was not a long period. Table I includes the results of 156 different tests, involving 568 cultures. No germination was secured at 0–1° C. showing that this temperature is below

the minimum. The results at 2–3° C. were variable and plainly very near the minimum. All cultures between 5 and 13° C. germinated in less than six hours, the time being longer at 5° than at 13. Above 13° the percentage of germination gradually decreased as the temperature increased. No indirect germination was observed at 24–25° C., suggesting that 24° is very near the maximum for indirect germination. At 29–30° C. only very scanty direct germination occurred in two cultures; in one culture three spores germinated at this temperature and in the other only two, plainly indicating that the maximum temperature for direct germination was not far from 30° C.

A graphic representation of the influence of temperature on germination is shown in Figure 1. Curve A was constructed from the data shown in Table I. It expresses the approximate relationship of germination and temperature. It will be seen that all of the cultures at temperatures between 6 and 13° C. gave germination but the percentage gradually decreased as the temperature changed from 13 to 21° C. At this point Curve A shows a less rapid decline which may be due either to an insufficient number of tests at temperatures between 13 and 20° C. or to the possible capacity of the conidia to germinate directly when the temperature becomes too high for zoöspore-formation. It will be shown that this latter is the correct explanation. The curve resumes regularity at 25° C. and reaches zero at 30° C.

It has already been pointed out that the time required for germination varies with the temperature, and this relation is shown in Curve B, Figure 1. In this curve is shown the average time required at the various temperatures. It should be said, however, that when the tests upon which Curve B is based were made no attempt was made to determine in every case the shortest time required for germination. This was quite impossible without constantly watching each culture. Frequent examinations were made, however, and the curve is based on a large number of tests. This permits an approximation probably not far from correct. The average time was determined by dividing the number of different experiments by the total time. The relation of time to temperature is evident from Curve B. The average time required in twenty experiments at 13° C., was 1.8 hours.

Above 13° the time gradually increased as shown in Curve B. Using time as the basis, the optimum probably lies between 10 and 13° C. There is a slight advantage in favor of the higher of these limits, since the average time was shortest at temperatures between 12 and 13° C.

FIG. 1.—RELATION OF TEMPERATURE TO GERMINATION

Curve A represents the number of cultures in which germination occurred at temperatures between 0 and 30° C. The ordinate represents percentage germination and the abscissa temperature. Curve B shows the variation in time required for germination between 2 and 25° C. The ordinate here represents time.

EXTENT OF GERMINATION AS DETERMINED BY COUNT

In Table I is shown the percentage of the total number of cultures held at a given temperature that did or did not show germination. It is plain that this is not as specific and direct as might be desired. It was therefore thought advisable to learn the percentage of germination in each culture by counting the spores in a given field. This was done and the results are shown in Table II.

The counts were all made with a Leitz No. 4 eyepiece and $\frac{1}{4}$objective with the tube length adjusted to give a magnification of about 124 diameters. Three different fields were counted in each culture and the sum taken as representing the prevailing condition. Three things were sought in the counts made: (1) the number of spores that germinated

indirectly, as shown by the empty sporangia, (2) the number that germinated directly, (3) the number that did not germinate at all.

TABLE II.—DIRECT AND INDIRECT GERMINATION OF CONIDIA OF PHYTOPHTHORA INFESTANS*

Cultures	6	6	6	6	6	6	6	3	6	6	6	6	6	6		To-tals	Per cent
Hours	24	24	24	16	20	16	24	20	24	24	19	23	28	18			
5–6° C.																	
Direct	0	0	0		0		0		0	0						0	0
Indirect	144	82	226		96		30		78	70						726	55
Not germinating	98	152	4		52		21		176	93						596	45
10–13° C.																	
Direct	4	8	3	10	1	3	1	2	1	1	0	0	4	3		41	0.8
Indirect	199	214	178	224	506	208	196	446	496	541	582	189	26	60		4065	80
Not germinating	89	42	65	155	46	20	91	82	119	127	60	24	16	40		976	19
17–18° C.																	
Direct	2	24	3	5	5	67	2	10	26							144	13
Indirect	26	22	25	6	60	12	48	32	72							303	27
Not germinating	48	86	259	22	36	27	0	54	126							658	59
20–21° C.																	
Direct	14	31	15	39	90	62	13	40	46	24	18	34	82	36	40	584	18
Indirect	24	22	84	0	9	25	5	6	60	64	2	15	48	47	69	480	14
Not germinating	122	62	119	172	84	248	23	61	210	289	105	215	214	76	217	2217	67
22–23° C.																	
Direct	36	39	10	8	5	20	85									203	25
Indirect	3	2	3	0	2	0	2									12	1.4
Not germinating	62	176	204	6	43	89	9									589	73
26–27° C.																	
Direct	34	40	5	8	0	0	0	48								135	14
Indirect	0	0	0	0	0	0	0	0								0	0
Not germinating	50	181	165	29	39	143	89	97								793	85
28–30° C.																	
Direct	3	0	2	1	0	0	0	0	0	0						6	0.2
Indirect	0	0	0	0	0	0	0	0	0	0						0	
Not germinating	224	152	241	362	146	286	296	144	406	202						2459	99

* The figures indicate the behavior in each culture as determined by counting the number of spores in a given area.

In this series of experiments seven temperatures were used between 5 and 30° C. Forty-two cultures were subjected to temperatures of 5–6° C. on seven different dates and three fields were counted per culture, a total of 126 different counts. The cultures at the other temperatures were treated likewise. Table II, shows the results of these tests, and at the foot of each column the total counts and percentages of germination are recorded.

It is evident from these data that 5–6° C. is too low for the highest percentage of germination and that temperatures above 20° C. are too high. This is clearly shown in Figure 2, Curve A, and confirms the results shown in Table I. That

temperatures between 10 and 13° are the most favorable is again shown in this set of data involving observations of many thousand spores. It must be emphasized that Curves A in Figures 1 and 2 have the same general outline, and this fact indicates, in my judgment, that the minimum, optimum, and maximum have been quite accurately fixed. Curve B in Figure 2 is worthy of note in that it has its beginning

FIG. 2.—TEMPERATURE IN RELATION TO DIRECT VS. INDIRECT GERMINATION

Curve A shows the percentage of indirect germination that took place at various temperatures between 0 and 27° C. Curve B shows the percentage of direct germination between 6 and 30° C.

very near the point considered the optimum for indirect germination and reached its highest point at 23° C., a temperature very near the maximum for indirect germination. As shown in Curve B, the maximum for direct germination is at or very near 30° C.

The proportion of spores that germinated even at the optimum temperature, ranged from 13 to 99 per cent. That variation should take place is only to be expected considering the extreme susceptibility of the spores to other external influences besides temperature. Some of these will be discussed in turn later in this paper.

These data show clearly that (1) an average of only 80 per cent of the spores of Phytophthora germinate, but the proportion may vary at the optimum temperature from 13 to 99 per cent; (2) the maximum for indirect germination lies between 23 and 26° C.; (3) direct germination begins at or near the optimum for indirect germination, gradually increases up to or nearly to the maximum for indirect germination, and approaches zero at 30° C.

THE EFFECT OF CONSTANT AND INTERMITTENT TEMPERATURE ON GERMINATION

With the fact established that low temperature materially favors germination, the question arose as to the effect of constant and intermittent temperatures on the germination of the spores of *Phytophthora infestans*. It was thought probable that the favorable effect of the low temperature was due to the temperature change.

Extra care was exercised in the selection of conidia for the study of this question. They were taken from pure cultures on potato plugs which were growing vigorously and producing spores in abundance. The water in which conidia were placed was raised to the temperature at which the cultures were to be incubated. At the same time another lot of conidia was placed in water at 20° C., held at this temperature for five minutes, and then incubated at a lower temperature, as shown in each case in the table. The time required for germination was used as a basis of determining the beneficial effect. In order to help establish the time at which germination first began, several extra cultures were carried in each experiment to be used in establishing the shortest time. By so doing, it was not necessary to disturb all of the cultures under investigation.

It will be noticed in Table III, that germination took place in shorter time in the cultures at constant temperature than in those held for five minutes at 20° C. and then placed at the lower temperature. Thus the time required in the seven different experiments averages 56 minutes when constant temperatures were used and 60 minutes at the intermittent temperatures. The spores in two of the tests at the intermittent temperatures failed to germinate. The difference in

time is so slight that it may well be due to variation in the spore material or inaccurate observation, but the two negative results strengthen the evidence in favor of the constant temperature condition. At any rate, it seems safe to conclude that a short period at high temperature has no beneficial effect.

It was next thought desirable to learn whether a change from low to high temperatures was more favorable to germination, and whether subjecting spores to a low temperature for a few minutes would be sufficient to cause germination. The tests recorded in Table III show that subjecting spores to low temperatures for short periods and then to high is not conducive to germination.

TABLE III.—CONIDIA OF PHYTOPHTHORA INFESTANS SUBJECTED TO INTERMITTENT TEMPERATURES

Date	Cultures	Cultures at intermittent temperatures					Cultures at constant temperatures		
		Early conditions of experiment		Later conditions of experiment					
		Temp.	Time	Temp.	Time	Germination	Temp.	Time	Germination
		°	Min.	°	Min.	*	°	Min.	*
Feb.									
7	2	20	5	9	60	+	9	60	+
7	2	20	5	9	60	+	9	45	+
8	2	20	5	13	60	+	13	50	+
8	2	20	5	13	65	+	13	60	+
10	6	20	5	10	65	+	10	60	+
9	5	20	5	12	50	—	12	60	+
14	3	20	5	14	60	—	14	60	+
Dec.									
14	3	12	30	25	120	—	12	60	+
16	3	12	30	25	120	—	11	120	+
21	2	12	30	25	120	—	12	80	+
Feb.									
7	2	1	30	9	90	+	9	120	+
8	2	1	60	13	80	+	13	60	+
Sept.									
11	2	1	60	10	990	+	10	120	+
13	2	1	300	10	1380	—	9	120	+
17	2	1	90	10	96	—	10	60	+

* The plus sign (+) indicates germination and the minus sign (—) no germination.

Ten other cultures were first subjected to 1° C. for a period varying from 30 minutes to five hours and then restored to temperatures more favorable—between 9 and 13° C. Germination resulted in 6 of the 10 cultures. There was no indication that this low temperature for the periods indicated in the table was in any way strikingly favorable.

THE INFLUENCE OF TEMPERATURE ON THE PERIOD OF MOTILITY OF THE ZOÖSPORES

In connection with the studies of conidial germination, opportunity was afforded for observing the behavior of the zoöspores. According to De Bary (1861), Farlow (1875), Ward (1887), and others, their motility is supposed to vary from a few minutes to two hours. It was noticed that in some cases, in fact in a great majority of the cultures held at 13° C., the optimum temperature for indirect germination, zoöspores continued motile longer than is recorded by earlier students of this problem. It was noticed too that the presence of foreign matter in the culture and the time required for the formation and liberation of the zoöspores from the sporangium, also exerted an influence on the period of motility.

TABLE IV.—INFLUENCE OF TEMPERATURE ON DURATION OF MOTILITY OF ZOOSPORES OF PHYTOPHTHORA INFESTANS

Cultures	Duration of motility at different temperatures, C.			
	5–6°	11–12°	20–21°	24–25°
	Hrs.	Hrs.	Min.	Min.
3	14	3	60	25
4	24	10	90	10
8	19	8	30	30
9	21	5	30	10
4	22	6	45	20
Average	20	6.4	51	19

Table IV shows the results of a series of experiments in which the conidia were carefully selected as to viability and placed in distilled water to insure absence of foreign matter. Unless 90 per cent or more of the conidia germinated, the material was not used. Every effort was made to insure favorable conditions for normal periods of motility. Zoöspores produced at the optimum temperature for germination were taken from the original cultures and stippled in small drops on clean slides and subjected to one of the four different temperatures shown in Table IV. Several preliminary tests, not recorded here, were made to obtain a general idea of the period of motility. Table IV shows the results of five different tests, and averages for the periods,

to which the reader is referred, are placed at the foot of each column.

It is significant that the period of motility decreases as the temperature increases, thus showing an inverse ratio. Loss of motility, which is a preliminary stage in the germination of the zoöspore, takes place most rapidly, not at the temperature most favorable for indirect conidial germina-

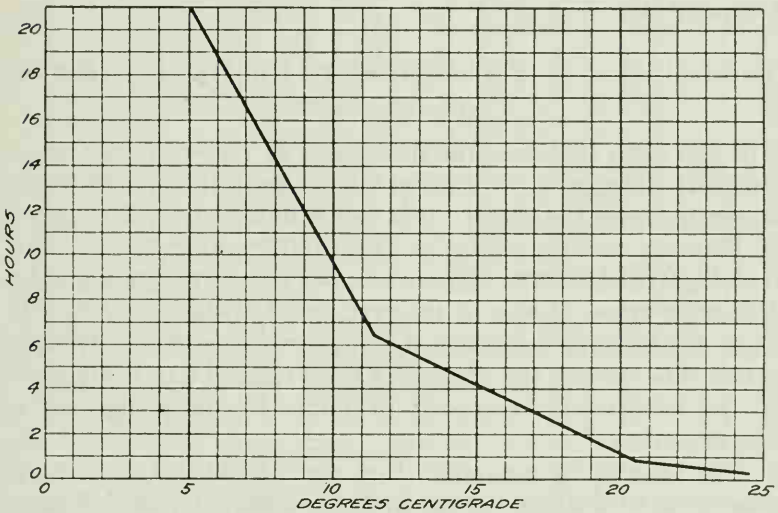

FIG. 3.—EFFECT OF TEMPERATURE ON DURATION OF ZOÖSPORE MOTILITY

tion, but very near the optimum for direct conidial germination, namely, about 25° C.

The relation of temperature to the period of motility is expressed in terms of a curve in Figure 3. Although only a few points have been determined, the curve seems to suggest quite definitely the response of the spore. It is safe to assume that it would approach zero if the temperature were increased above 25°, probably reaching it at some point in the neighborhood of 30° C., the maximum for direct conidial germination.

This curve is of interest from still another point of view. It is well established that temperature influences the rate of respiration within certain limits and that the latter reacts on the rate of growth. Growth in the case of the zoöspore

leads first to the deposition of a cell wall about the spore and later to the development of a germ tube. In view of these facts, the curve shown in Figure 3 may represent not only the period of motility, but also the relation of the rate of respiration as influenced by temperature expressed in terms of duration of motility of the zoöspores. The period of motility within the limits given depends, in other words, on the rate of respiration, which in turn is determined by the temperature.

THE RELATION OF TEMPERATURE TO THE GERMINATION OF THE ZOOSPORES

It has been shown in the discussion of Table IV that temperature influences the rate of loss of motility and this has suggested that the growth of a cell wall about the zoöspores is likewise facilitated by a favorable temperature. The development after the zoöspore comes to rest by pushing out the germ tube, is also of interest and the data in Table V show the further influence of temperature on this. In this set of experiments the zoöspores were treated like those used in the experiments recorded in Table IV, only held for a longer period.

The number of zoöspores that did or did not germinate was counted on equal areas in three different parts of each culture and the results are recorded in Table V. Likewise, three typical germ tubes per culture were measured and the average was taken as the prevailing condition. The cultures at 5–6° and 11–12° C. were held 20 and 6 hours longer, respectively, to allow the zoöspores to lose their motility. The cultures at the three other temperatures received no extra time allowance to compensate for the period of motility because of its brevity. This probably had not a very great effect on the results.

By referring to the averages in Table V, it will be seen that not all the zoöspores germinate even after they are liberated from the sporangium. According to the data in Table V, the greatest number germinated at 11–12° C. As has been described by earlier workers, those that failed to germinate enlarged, became hyaline, and finally disintegrated. This was observed more often at the temperatures above 20° C. than at those below. The bursting of the

zoöspores placed at the higher temperatures may have been due to the sudden transition from low to high temperature, the increased osmotic activity at the high temperature being too great for the naked mass of cytoplasm. This fact makes it quite probable that many of the spores which disintegrated

TABLE V.—EFFECT OF TEMPERATURE ON THE GERMINATION OF THE ZOÖSPORES OF PHYTOPHTHORA INFESTANS

Cultures	5	5	3	5	3	3	6	Average
Hours	32	26	29	32	28	28	34	
5–6° C.								
Number	102	42	38	207	60	33		
Growth μ	67	44	99	89	76	41.6		70
Germination, per cent	43	80	71	89	80	45		68
11–12° C.								
Number	131	159	57	79	43	24	48	
Growth μ	195	195	201	121	185	147	196	177
Germination, per cent	69	97	100	72	100	92	96	89+
14–15° C.								
Number	122	47		67	107			
Growth μ	195	208		122	190			179
Germination, per cent	66	92		90	90			84.5
20–21° C.								
Number	39	63	48	127	71	49		
Growth μ	198	224	272	147	222	192	204	208
Germination, per cent	85	90	88	40	96	82		80+
23–24° C.								
Number		50	33		42	60		
Growth μ		233	281		234	201		239
Germination, per cent		48	85		95	82		77.5

at the higher temperature were missed in the final count, and if such is the case, the percentages germinating at 20–21° and 23–24° C. would be less than given in the table. Even neglecting this probable error, and accepting the accounts and showing the exact effect on the zoöspores of the various temperatures, it is still evident that the lower temperature, 11–12° C. is more favorable for the early stages of zoöspore germination than the higher temperatures.

The effect of temperature is most marked on the rate of growth of the germ tubes. This is graphically shown in Figure 4, the growth being most rapid at 23–24° C., a point probably not far from the optimum.

THE EFFECT OF LOW ATMOSPHERIC MOISTURE ON THE CONIDIA OF PHYTOPHTHORA

Widely different statements occur in the literature as to the conditions and time necessary for the spores of Phy-

tophthora to be killed when subjected to a non-saturated atmosphere. In view of this fact, experiments were undertaken to investigate this subject and the results are recorded in Table VI.

Well-infected leaves with abundant spores were picked from the infected host growing in the same greenhouse, and placed in the laboratory to dry. Some of the spores were

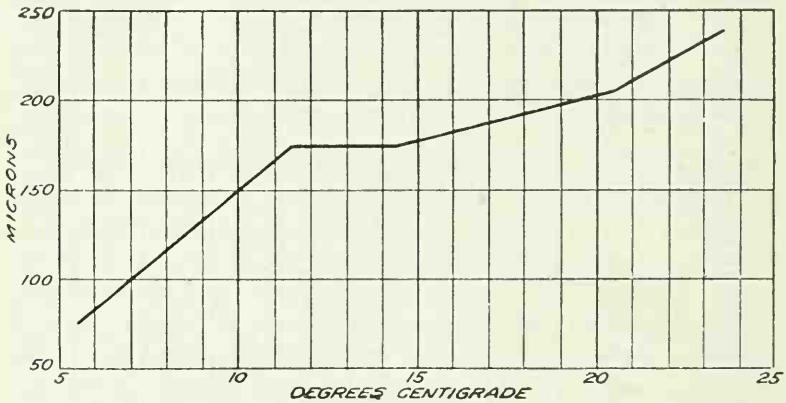

FIG. 4.—EFFECT OF TEMPERATURE ON RATE OF GERM TUBE GROWTH OF ZOÖSPORES AFTER THEY HAVE LOST THEIR MOTILITY

taken from the fresh leaves and, by trial, shown to have normal vitality. Germination tests were again made after the leaf tissues were dry and dead, when in all cases the spores failed to germinate. In cases of rapid drying, six hours was sufficient to kill all spores.

LIGHT IN RELATION TO GERMINATION

It has been reported by De Bary (1863), Farlow (1876), and others that light checks the germination of spores of various Oomycetes. It has been shown in another paper, (Melhus 1911), that if the temperature is kept sufficiently low, Cystopus spores will germinate regardless of the amount of light, be it diffuse or direct. It was thought desirable to study similarly the spores of Phytophthora.

Cultures were prepared, and placed either in the light or in the dark. Light was excluded simply by placing the cultures in the ice box. The influence of light was studied by placing

cultures in a large glass moist chamber dish, ten inches in diameter and four inches high, in which cracked ice had previously been placed. The slides were laid on a small rack

TABLE VI.—EFFECT OF DRY AIR ON THE VIABILITY OF THE CONIDIA OF PHYTOPHTHORA INFESTANS

Date	Cultures	Age of spores	Spores dried	Incubation			
				Time	Temperature	Germination	
						Direct	Indirect
				Hours	Degrees		
Mar. 11	2	2	4 da.	24	12	0	0
Mar. 8	2	2	1 da.	4	12	0	0
Mar. 11	2	3	4 da.	25	12	0	0
June 3	2	4	3 da.	22	12	0	0
June 3	3	4	3 da.	22	12	0	0
Sept. 23	4	1	20 hrs.[1]	24	12	0	0
Sept. 23	4	1	20 hrs.[2]	24	11	0	0
Apr. 1	4	1	6 hrs.[3]	24	13	0	0

[1] Dried in laboratory 20 hours at 22–25° C.
[2] Artificially dried in a desiccator.
[3] Dried at 25° C. for six hours on leaves until they were dry and dead.

just above the ice with a small thermometer. This dish was then placed in the desired light relations, either in the greenhouse or outdoors. The direct sunlight naturally tended to raise the temperature within the dish, and the ice had to be renewed frequently during each trial. Under these conditions, it was impossible to keep constant temperatures, but this is not necessary to prove that light does not influence germination. The extremes in each trial are given in Table VII,

TABLE VII.—EFFECT OF LIGHT ON THE GERMINATION OF THE SPORES OF PHYTOPHTHORA INFESTANS

Date	Cultures	Cultures in light			Cultures in dark		
		Hours	Temperature	Result	Hours	Temperature	Result
			Degrees C.			Degrees C.	
Jan. 31	2	8	12–16	+	3	12	+
Feb. 1	6	6	14–16	+	4	13	+
May 2	6	10	15–21	+	3	10–13	+
May 6	3	4	12–18	+	2	12	+
June 4	3	7	10–14	+	3	13	+
June 6	3	5	8–13	+	3	13	+
Feb. 4	2	3	10–15	+	3	12	+

the variation being from 4 to 6 degrees, which, of course influenced the time required for germination. It has already been shown that indirect germination is retarded by temperatures above the optimum.

METHOD OF GERMINATION IN THE FIELD

It was next thought desirable to learn something about the type and prevalence of spore-germination under field conditions where the plants are exposed to natural infection. The best time to study this fungus is when the potato foliage is wet with either rain or dew. This means that observations must be made after a rain or in the morning when the dew is present. The latter time was chosen as most favorable. Forty-two such examinations were made on six different dates, from September 7, to October 12, between 7 and 8:30 A. M. The dew drops were taken from both sides of the leaf, from shaded and unshaded leaves, from places where there was little moisture, and from others where there was considerable. In no case was anything but direct germination found, and this type was present in every collection. The number of zoöspores in the drops examined varied markedly, depending, it is believed, upon the amount of sporulation on the two previous days. It was noticed that when the amount of spore material was limited, the number of zoöspores present was materially decreased. It should be noticed that the minimum temperature on the days when collections were made, ranged from 10 to 13 degrees C., temperatures very favorable for the direct germination of Phytophthora conidia, according to my laboratory studies.

TOXIC ACTION OF LEAF JUICES

It is well known that potato leaves and stems soften in the late stages of Phytophthora infection. It was noticed that very scanty germination was obtained when spores were taken from such tissues. Tests were made to learn whether this might not be due to unfavorable secretions present in the decayed potato tissue. A series of four different experiments, three cultures each, was made, testing germination in such juices from infected leaf tissue, with controls in water. No indirect and only scanty direct germination took place in the leaf juice, whereas the controls germinated abundantly. The reason, then, for the low percentage of germination when the spores are taken from decaying tissues, is found not in the spores, but in the presence of leaf juices. It is very pos-

sible that they contain acids, or other decomposition products, that have an inhibiting effect on germination.

Effect of Frost

It is generally known that the spores of Phytophthora are thin walled, and readily killed by frost, but whether they are more resistant than the foliage, is not known. This question was tested by taking spores from the leaves killed by the first "killing frost" in the fall of 1911. Six different tests of five cultures each were made, but in no case did the spores germinate, indicating that a chill sufficient to kill the potato foliage, also kills the spores.

Effect of Sugar on Germination

With many fungi, sugar has a tendency to stimulate growth. Whether such is the case with *Phytophthora infestans*, is shown by the studies that follow. A series of dextrose solutions was made up, ranging from one to twenty per cent. Conidia of Phytophthora were placed in these various dilutions, in the same way as already described when using water.

It was found that zoöspore germination took place readily in a 1-, 4-, or 8-per cent solution, but more sparingly in a 16-, and never in a 20-per cent solution. The zoöspores that were produced in the 16-per cent solution, were more or less distorted, and abnormally shaped, and remained motile only a very short time. This is in accordance with the behavior of certain motile algae in similar solutions. Only direct germination took place in the majority of cases in the 16-per cent solution.

It was of interest to see if germination was more abundant in a 5-per cent dextrose solution than in water. The length of the germ tubes at the end of 24 hours was also noted in both cases. In the dextrose solution 1,361 spores germinated as compared with 751 in water. The average length of the germtube was 200 μ in the solution and 197 in water.

The difference in the growth during the first twenty-four hours was not so great. Whether it would have become greater after a longer time was not determined, but it is probable. It is obvious from these studies, that both direct

and indirect germination can take place in dextrose solutions ranging from 1 to 16 per cent, and that no germination results in a 20-per cent solution. It is also clear that a 5-per cent solution does not facilitate indirect germination, but rather tends to inhibit it, if the amount of germination in water is taken as a standard.

OXYGEN AS A STIMULUS TO GERMINATION

It is claimed by Ward (1887), Klebahn (1909), and others that oxygen markedly stimulates germination. In order to test this with Phytophthora, conidia were placed in hydrogen peroxide of various strengths. Merck's brand, which contains approximately 4 per cent, was so diluted as to give the following percentages: 2, 1, 0.5, 0.125, 0.0625, and 0.0312. Viable spores were placed in a drop of each of these solutions on slides, and subjected to temperatures of from 10 to 13 degrees C. for 24 hours, with controls in water. The controls showed abundant germination after 2 hours, and so did the spores in 0.0312-per cent hydrogen peroxide. A few of the spores in the 0.0625-per cent solution also showed slight germination but none occured in the stronger solutions, even after 24 hours. The following day this experiment was repeated, and germination again took place in the weakest two solutions. Only direct germination developed in the 0.0625-per cent solution. This experiment was repeated again with a similar outcome, as shown in Table VIII, where the results of the three trials with the weakest solutions are brought together.

TABLE VIII.—EFFECT OF 0.0312-PER CENT SOLUTION OF HYDROGEN PEROXIDE ON THE GERMINATION OF THE CONIDIA OF PHYTOPHTHORA INFESTANS

	May 2	May 3	May 20
Number of cultures	6	6	6
Time, hours	24	24	24
Spores in solution			
Germination of conidia			
Direct	0	0	2
Indirect	115	59	78
Conidia not germinated	8	96	82
Average length of germ tubes μ	131	179	164
Spores in water			
Germination of conidia			
Direct	0	0	0
Indirect	46	114	119
Conidia not germinated	2	49	22
Average length of germ tubes μ	196	201	206

It will be noticed from these tests that not only did a higher percentage of the spores germinate in water than in the hydrogen-peroxide solution, but also that the germination of the zoöspores and the further growth of their germ tubes was retarded by the hydrogen peroxide. Oxygen in a nascent state, according to these results, is apparently not a stimulant, but rather a toxic agent.

In several cases the hydrogen-peroxide solution was replaced by water after 24 hours, but this did not revive the spores. Tests were also made, using a 0.0156-per cent solution. Here the amount of germination was the same as in water, the oxygen present being insufficient to produce a noticeable effect. Whether a still weaker solution would act as a stimulant is a question. The data in hand, however, do not suggest that such is the case.

Later, spores were placed in water containing practically no free oxygen. For this trial, distilled water was boiled for two hours, cooled rapidly to 13° C., charged with spores, and cultures placed in an oxygen-free atmosphere for 3 hours. Germination occurred in the usual manner in abundance. Other tests were made in the same way except that the spore-charged water was covered with a film of paraffin oil. Spores treated in this manner behaved exactly as those in ordinary water. While these tests are somewhat crude, they indicate that free oxygen in the water is unnecessary for germination, and lead to the conclusion that the conidium contains within itself sufficient oxygen for germination.

Discussion and Conclusions

The conidia of *Phytophthora infestans* can germinate either indirectly, that is, by liberating zoöspores, or directly by the production of a germ tube, a fact first emphasized by De Bary (1863). Various theories have been proposed for explaining this dual nature of the Phytophthora conidium, none of which have been generally accepted or supported by any considerable amount of convincing experimental data. It is not my purpose, however, to discuss this question but simply to attempt to correlate the results of earlier investigators with my own as to the effect of external influences upon germination.

INDIRECT GERMINATION OF THE CONIDIA OF PHYTOPHTHORA

In an earlier paper it has been clearly shown that comparatively low temperatures aid indirect germination of the conidia of Cystopus and thereby favor infection. The optimum for germination was not definitely determined for Cystopus but the results tend to show that it is about 10° C., with the minimum very near zero, while the maximum, as De Bary has shown, is about 25° C. The fact that Phytophthora is closely related to Cystopus makes it especially interesting to compare the behavior of the conidia of the two species with reference to temperature. The experimental data with Phytophthora recorded in the preceding pages show clearly that temperatures below 20° C. are more favorable to indirect germination than high. The data in both Tables I and II show that germination can take place at any temperature from 2 to 9° C. and that the optimum is about 13° C. It appears, therefore, that the conidia of Cystopus and Phytophthora behave much alike with reference to temperature.

EVIDENCE FROM EARLIER FIELD OBSERVATIONS THAT LOW TEMPERATURES FAVOR EPIDEMICS OF LATE BLIGHT

Evidence that low temperatures favor the spread of Phytophthora is found in Jones' excellent observations in Vermont, extending over a period of 20 years. These have recently been assembled by Lutman (1911), so it is readily possible to see the moisture and temperature conditions at which epidemics developed. In 1891, when some blight was present, Jones writes as follows of the weather conditions: "The temperature was low and rainfall slight. The weather became very warm and on August 12 and 14 was followed by a fall in temperature and copious rains, and this was followed by another rise in temperature and more rains about the twenty-first. The conditions favoring the blight began thus about the twelfth." The following year, 1892, he writes: "From August 6 to 12 almost ideal conditions prevailed for the blight and reference to the records show that it in reality appeared August 10, and progressed with unprecedented rapidity, so that almost every potato plant was destroyed by it before August 20." The minimum temperature for

the period from August 6 to 12 ranged from about 15 to 18° C. Late blight was also very bad in 1893 when he again writes: "The later conditions favoring the blight began about August 22, and the disease is recorded as under full headway by the twenty-fifth, and as continuing into September with unusually destructive results." The minimum temperature for the period between August 22 and September 22 ranged from about 8 to 20° C., with 10 days above $15\frac{2}{3}°$ and 21 days below. Phytophthora was again severe in Vermont in 1902, when we find this statement regarding its advent: "The summer was moist and cool and the Phytophthora appeared at the earliest date on record here, July 13. * * * A similar period later in the season, August 18-27, finished up the later varieties, so that a canvass of the vicinity of Burlington on August 23 showed the plants in two-thirds of the fields entirely dead and rapidly dying in the remainder." The minimum for the period from July 14 to August 27 fluctuated from about 10 to 22° C. with 25 days below $15\frac{1}{2}°$ and 19 above. Similar obeservations by the same author occur in the Vermont reports 1891-1909 and might be correlated in like manner, but the four years cited are typical.

Halsted and Selby (1907) have made similar, but less extensive, correlations in New Jersey and Ohio, respectively. These show that an epidemic may develop when the minimum temperatures are prevailingly below 20° C., a condition at which, according to our laboratory tests, indirect germination is most abundant.

It is common knowledge according to Morse (1909), that an outbreak of *Phytophthora infestans* in Maine follows a period of wet weather during the months of July, August, and September. A very excellent description of the conditions that favor an epidemic of late blight is given by Ward (1901) as follows: "The now well-known spots * * * were observed during dull, cloudy, and wet weather, cooler than usual, when the temperature was saturated for days together in July and August. The actual amount of rain does not seem to have been excessive * * *. So rapidly did the disease run its course, that in a few days nearly all of the plants were a rotting, blackened mass in the fields."

GEOGRAPHICAL DISTRIBUTION IN RELATION TO SPORE GERMINATION AT LOW TEMPERATURES

It is not surprising that low temperatures are more favorable for the germination of the conidia of *Phytophthora infestans* than higher ones, when we consider the latitude and climatic conditions under which the fungus thrives best. In a recent paper by Jones, Giddings and Lutman (1912, p. 14) one finds this sentence: "This disease is common in the northeastern states, especially in northern New England and New York and also in adjacent Canada. Further south and west it is either unknown or more sporadic, unless it be in the northern Pacific coast regions." According to Jensen (1887) the potato fungus can not exist where the mean temperature exceeds 25° C. In other words, it seldom occurs south of latitude 40° in the United States and chiefly north of latitude 50° in Europe. Minor variations in rainfall and altitude of course also influence the distribution. The precipitation of these regions is from 20 to 40 inches and they are crossed by the mean annual isotherm of 50° F. (10° C.). A recent paper by Reed (1912) is interesting in this connection and shows the effect of altitude on the distribution of late blight. "It has been noted each year that the disease has not appeared until the advent of weather cool enough to bring lower temperatures during the night. At the altitude of Blacksburg, Virginia, (2,200 feet) the cool nights bring an abundant formation of dew which apparently gives proper conditions for the germination of the swarm spores of the fungus." He continues by saying that the disease appears earlier at high altitudes and is practically unknown below an altitude of 2,000 feet. These observations are interesting since they are made in a region south of the usual section infested by late blight, where a higher altitude, compensates for the difference in latitude.

COMPARISON OF PHYTOPHTHORA INFESTANS WITH OTHER PERONOSPORACEAE

Other parasites belonging to the same family as Phytophthora are similarly influenced by temperature. Pée-Laby (1899) has described the ravages of *Peronospora parasitica* on broccoli in southern France, where this plant is grown

during the winter months. He states that the Peronospora flourishes and is especially vicious during the coldest weather. The same fungus has been reported on cabbages in the southern states and southern California only during the winter by Orton (1910). I have noted (1912) that *Peronospora parasitica* is most prevalent on *Lepidum virginicum* in the spring and fall in the vicinity of Madison, Wisconsin, which further supports the contention that temperature influences the distribution of this fungus and shows, as well, its close likeness to the potato fungus.

Another clear case is offered in Istvanffi and Pálinkás' (1913, p. 25) studies of the grape mildew. In an extensive monograph they have recorded meterological conditions in conjunction with the infection periods of Plasmopara in Austria for the season of 1911. Their data show that the greatest number of infections took place in the spring and early summer. From May 5 to June 20 they followed the development of 9 different outbreaks of the mildew, only one less than they studied all of the remainder of the season. Their chart shows, also, that the normal temperatures between the above dates stayed below 20° C., with the minimum fluctuating mostly between 10 and 15° C. In view of this fact, it seems probable that a considerable proportion of the germination must have taken place below 20° C. Indeed, it is not improbable that many of the spores germinated in the range of the minimum (10 to 15° C.) when dew might well have existed, although the authors make no mention of this fact.

Besides moisture in the form of dew on the foliage of the host plant, it also occurs as rain. I have collected motile zoöspores of Phytophthora from potato foliage wet with rain on several occasions and also the zoöspores of *Plasmopara viticola* from the wild grape. Whetzel (1904) in his studies of the downy mildew of the onion, *Peronospora schleideniana*, reports that he took germinating spores from infected plants in the field during a rain and at no other time did he see the spores germinating. Gerneck (1912) has recently called attention to the fact that heavy general rain storms accompanied by high humidity and heavy dews facilitate the spread of the grape mildew. Halsted (1889) has on several occasions called attention to the correlation of rainfall and

the development of the Peronosporaceae, but makes no special mention of temperature assuming an important rôle. In my studies of the downy mildew of the wild grape it has repeatedly been noted that an outbreak of the disease followed a shower of rain or several days of cloudy weather. The conclusion seems perfectly justifiable considering my data and observations and those of other investigators on species belonging to the same family, that the low temperature conditions which prevail either during or following a rain are favorable for the germination of the spores of this fungus.

A considerable amount of data is at hand as to the optimum temperature and limits of conidial germination of *Plasmopara viticola*, which is worth comparing with the limits I have established for Cystopus and Phytophthora. According to Scribner (1886) the most favorable temperature for the germination of the conidia of Plasmopara lies between 25 and 35° C. Millardet (1886) found that germination took place in one and one-half hours at 9° C. Patrigeon (1887) reports that a temperature of 25–30° C. is the most favorable. Viala (1893) states that at 28–30° C. zoöspore germination takes place in from half an hour to an hour, but if they are subjected to temperatures below 20° C. the conidia do not germinate by zoöspores any longer, but push out a germ tube instead. It is very evident that Viala was influenced in his study of direct germination by the results of De Bary (1863) with Phytophthora, where direct germination is a common phenomenon, although recently Istvanffi (1913) has described and figured this type of germination as developing occasionally. Viala states further that it required two or three days for germination to take place at 10–17° C., and that below 5° C., no germination took place, although the spores did germinate when restored to 25° C. Recently Ravaz and Verge (1911) have published a short account of spore germination of the grape mildew in which they maintain that the minimum is 6.5° C. and the maximum very near 30° C; the optimum, using time as a basis of comparison, is near 27° C. The time gradually decreases as the temperature increases up to 27° C., while at higher temperatures it again increases. It is unfortunate that Ravaz and Verge did not describe their method in detail and duplicate their

experiments, so that their figures might carry conviction. That this is necessary is apparent from a very recent article by Gregory (1912). He summarizes his experience as follows: "Ordinarily germination is brought about by placing the conidia in a suspension of water in a van Tiegham cell. * * * At times, however, the conidia can not be germinated under these or any other conditions, so far as I have been able to determine. At best the germination is rather uncertain." I have experienced little difficulty in germinating the mature viable spores of Plasmopara, although, as stated earlier, no extensive study has been made with this fungus. In an excellent paper dealing with the grape mildew, Istvanffi and Pálinkás (1913) report that no germination took place at 2° C. and that at 8° C. zoöspores were formed only to a slight extent after 8 or 10 hours. At 14 or 15° C. the formation of zoöspores was abundant in 2 or 3 hours. Germination was most rapid, however, at 20 to 22° C., at which temperature only from one to two hours was required. At 28 to 30° C. only slight germination developed after 4 to 10 hours. No zoöspores were formed at 35° C. Here we have still another set of limits for the germination of the spores of Plasmopara. It is hard to interpret these data in the light of the observations of Lüstner (1905), Sorauer (1908), Gerneck (1912), and others to the effect that many days of wet weather and the conditions in spring and fall are most conducive to the development and spread of this disease. According to existing data on *Plasmopara viticola*, the optimum appears to lie somewhere between 20 and 30° C., a strikingly high temperature when compared with the optimum fixed for Cystopus and Phytophthora. The question naturally arises as to the presence of moisture on grape foliage, when the temperature ranged between 20 and 30° C., for a sufficient time to allow germination to take place. That a film of water is necessary has been repeatedly demonstrated by many other investigators. It seems to me that the question of spore germinaton of the conidia of the downy mildew of the grape is one worthy of further careful study in the light of my results with species belonging to closely related genera, and because of its direct relation to infection, spread of the disease, and its control.

THE EFFECT OF LOW TEMPERATURE ON THE GERMINATION OF THE SPORES OF OTHER FUNGI

Low temperatures not only have a beneficial influence on germination and infection with these various Peronosporaceae but also on a variety of other fungi. It has been known for a long time and has recently been re-confirmed by Hecke (1909), that the optimum for the germination and infection of some of the cereal smuts is about 10° C. In the case of our common wheat rust, Jaczewski has found that both the uredo and aecidiospores germinate best at temperatures slightly below normal (18° C.). Johnson (1912) has recently confirmed Jaczewski's (1910) results for *Puccinia graminis* and extended them to several of the other cereal rusts. It may well be that when we have more accurate knowledge concerning the relation of external influences to germination and infection of other parasitic fungi, the number known to show a preference for low temperatures will be materially increased, especially where there is as close a correlation between the presence of the fungus and climatic conditions as exists in the case of the potato fungus.

INTERMITTENT TEMPERATURE AND GERMINATION

Eriksson (1896) found that intermittent temperature, changing from low to high, facilitated the germination of the aecidiospores of *Puccinia graminis*. Similar tests with the spores of Phytophthora have given me no such results as reported by Eriksson but, on the other hand, it has been found that constant low temperatures are more favorable. Likewise a transition from high to low temperature had no stimulating effect and in fact prevented indirect germination absolutely if the time at the high temperature was over one hour. In this respect the conidia of Phytophthora behave strikingly differently from the spores of various species of Ascobolus studied by Dodge (1912), who proved that subjecting the spores to a dry heat of from 50 to 70° C. for from 5 to 10 minutes and then returning to ordinary temperatures materially aids germination. But, of course, the two types of spores are widely different from a morphological and physiological standpoint. The latter have thick walls and live saprophytically, while the former are thin walled and

live parasitically. The spores of Ascobolus have the nature of resting spores while those of Phytophthora are extremely ephemeral, remaining alive in dry air less than 24 hours.

Germination of the Zoöspores after Liberation from the Sporangium

A great diversity of statements exists in the literature as to the period of motility of the zoöspores. De Bary (1863) states that this period varies from 20 to 30 minutes. Farlow (1875) found it to be from 15 to 30 minutes. MacAlpine (1910) reported that they were motile only 8 to 10 minutes and Ward (1887) says that their motility varies from 1 to 20 minutes and that sometimes they give only one little flirt and then come to rest. In the case of the Phtytophthora infecting the Areca palm, the period of motility of the zoöspores in the field is from 30 minutes to $2\frac{1}{3}$ hours, according to Colman (1910).

The results presented in Table IV and Figure 4 show that the period of motility varies inversely with the temperature within certain limits, ranging approximately from 20 hours at 5–6° C. to 19 minutes at 24–25° C. This variation with temperature explains the diversity of the statements of previous workers and also shows that they worked with temperatures too high for the best indirect germination. That the period of motility should be shorter at 24° than at 6° C. is only to be expected when we consider what happens to the zoöspore in its process of losing motility. It gradually loses its lenticular form and deposits a cell wall at its periphery. This is a common and familiar phenomenon described first by Prevost (1807) and corroborated later by De Bary, Ward, and others. The deposition of a cell wall requires metabolic processes, or growth, and the rate of growth is much faster at 24 than at 6° C., because respiration and absorption of water increase with the temperature. This is further evident from the varying rate of growth of the germ tubes which develop from a quiescent zoöspore (Table V). A similar influence of temperature on the motility of the zoöspores of *Olpidium viciae* has been observed by Kusano (1912) who found the motile period shorter at 27° than at 15° C.

An interesting fact in this connection is the difference in behavior of the zoöspore and the conidium. The most favorable temperature for the germination of the liberated zoöspore is above 20° C. and probably about 25° C., while the optimum for indirect conidial germination is only 13°. The optimum for direct germination and germination of the liberated zoöspore is about the same. In both cases mycelium is developed. The optimum for its growth, according to Jensen (1887), is 23° C. His observations were made on the mycelium growing in pieces of potato tubers. This is slightly higher than that given by Orton (1911) and Jones (1912), who found that the fungus grew best between 16 and 19° C. on sterile potato blocks, but the difference in the methods used by these investigators may well explain the discrepancy. In another portion of this paper dealing with infection it will be shown that the optimum for the growth of the mycelium in leaf tissue is probably very near 25° C.

The difference in response of the liberated zoöspore and the conidium is probably due to the fact that indirect germination of the conidium requires but little energy. No new cytoplasm is formed. Cleavage, it seems, is brought about by the absorption of water and the translocation of the cytoplasm, due to a process of vacuolation. These processes can go on at low temperatures. The germination of the free zoöspore, on the other hand, requires the deposit of a cell wall and the development of a long germ tube, and these phenomena are accelerated by higher temperatures. This is in accord, also, with the physiological principle that temperature directly influences respiration, absorption of water, and growth within certain limits. Whatever may be the explanation of the difference, it is clear, as is graphically shown in Figures 4 and 5, that while the period of motility varies indirectly, the growth of the germ tubes varies directly with the temperature within the range given.

DIRECT GERMINATION OR DEVELOPMENT OF A GERM TUBE

The conidia of Phytophthora are different from those of the other members of the Peronosporaceae in that they can germinate either indirectly, that is by zoöspores, or directly, by a germ tube. When the latter type of germination takes place, a germ tube grows out from one end of the conidium.

This type of germination is not influenced by temperature in the same manner as the indirect type, and fewer of the spores in water germinate in this manner. For indirect germination the minimum has just been shown to be 2–3° C. For direct germination the minimum is much higher, lying between 10 and 13°; at least at this temperature a very low percentage germinated directly. At 23–24° C., the optimum, 30.2 per cent germinated. This is somewhat less than the percentage given by Jones (1912) who found that more than 50 per cent germinate directly at 25° and that this method of germination is exceptional at 10–20° C. It was plain that 30° C. was very nearly the maximum for germination. This maximum agrees with that fixed by Hecke (1898) for direct germination in a dilute leaf decoction, but my minimum and optimum are not in accord with those given by him. He found the minimum 7° and the optimum 20° C. in potato leaf decoction, about 5° lower in each case than the figures I obtained, using water. It is not strange that there should be this difference considering the medium used in each case. The density of the medium doubtless influences osmotic activities, which in turn probably react on the spores. It is interesting to note that direct germination has its minimum near the optimum for indirect germination and its optimum at the maximum for that type.

Hallier (1895) believed that young spores germinate directly and the older ones indirectly. De Bary (1863) concluded that direct germination was due to abnormal conditions of the conidium. It is interesting to note that Hecke reached the opposite conclusion from Hallier, namely, that the younger spores produce swarm spores and the older ones germ tubes. Farlow (1875) is inclined to believe that light influences the method of germination, at least to a certain extent, light favoring direct germination. According to McAlpine (1910) only the first crop of sporangia produces zoöspores, all others germinating directly. By Ward (1887) the development of a germ tube by the conidium was thought to be due to the nutrition of the germinating spore, the amount of oxygen present, the number of spores in a given drop, and the comparative maturity of the spore. In an earlier part of this paper I have shown that the addition of oxygen has little influence.

It has been clearly shown by Hallier that direct germination is not an abnormality, as suggested by De Bary. Hecke has since substantiated Hallier. It is hard to believe with them that the method of germination depends upon the age of the conidium, or with McAlpine that all except the first crop germinate directly. Before it can be established that state of maturity bears any relation to type of germination, a criterion of degree of maturity must be established. This is only possible by making careful histological studies of the various stages in the process of development of the conidium. Livingston's (1900) work on Stigeoclonium is interesting in this connection. He found that osmotic pressure was the controlling factor in determining the form of the plant. When the osmotic pressure was low, zoöspores were formed, but when it was high, increased vegetative activity resulted, inhibiting the production of zoöspores. It may well be that osmotic pressure is the determining factor in the case of the germination of the spores of Phytophthora.

Very little evidence is at hand as to the role played by direct germination in causing infection either to potato foliage or tubers. It has been shown that direct germination, at least, seldom occurs on the potato foliage in the morning dew. Hecke (1898) has suggested that this type of germination functions partly in causing tuber infection in the soil, but presents no direct experimental evidence. He argues that, since direct germination is the only type of germination that can take place in a potato leaf decoction with more than 5 per cent solid matter (a solution comparable with a mixture of soil and water), and since old spores always germinate directly, considerable tuber-infection must result from direct germination. Further study of this problem is in progress and will be discussed in a later paper.

TOXIC EFFECT OF VARIOUS CHEMICALS AND FUNGICIDES ON THE GERMINATION OF THE SPORES OF PHYTOPHTHORA AND PLASMOPARA

In the preceding chapter I have discussed the conditions favorable for the germination of the spores of *Phytophthora infestans*. It has been shown that temperatures below 20° C. are more favorable than those above and that the optimum is about 13°. The question arose as to the effect of various

chemicals and fungicides on indirect germination at such low temperatures. Although the behavior of the spores of Phytophthora in a large number of salts and acids is known, no tests have previously been made under optimum conditions for germination and no study has been made of the effect of Bordeaux mixture and calcium polysulphide on the germination of the spores of Phytophthora. Moreover, earlier workers put the spores directly into the mixture to be tested, a method not comparable with what takes place in the open when foliage is sprayed with a fungicide.

Method

The method first used by Burrill (1907) and later elaborated by Reddick and Wallace (1910) has been followed in this study. It consists of spraying the compound to be tested on a clean slide and allowing it to dry after which a water suspension of the spores is placed on the slide.

The slides that were used were allowed to remain in a cleaning mixture[4] for 24 hours. Following this treatment they were thoroughly rinsed and dried between filter paper. All the water used in making the dilutions was distilled over alkaline potassium permanganate to free it from ammonia. Whenever possible Kahlbaum's chemicals of the highest purity were used. In the case of the copper compounds the dilutions were made in terms of copper and not of the salt as a whole. In all the other chemicals the dilutions are in terms of percentage as usually figured, namely, one gram of the compound dissolved in distilled water and made up to 100 cc. In the case of the polysulphides, the dry weight was determined and dilutions made on the basis of percentage of solid matter. In Sherwin-Williams commercial lime sulphur, for example, it was found that each cubic centimeter contained 0.434 gms. solid matter. The dilutions were sprayed on slides immediately after preparation by means of a DeVilbiss atomizer and allowed to dry.[5]

The spore material used in these studies was selected with particular reference to securing high viability. Two species were studied, *Phytophthora infestans* and *Plasmopara viticola*, the latter being included primarily for purposes of com-

[4] Potassium bichromate, 800 gms.; commercial sulphuric acid, 4,600 cc.; water 3,000 cc.

[5] The author is indebted to Dr. O. Butler for preparing the solutions which have been studied in this chapter.

parison. Distilled water containing spores was stippled in small drops on the slides bearing the various salts to be tested. The cultures were immediately subjected to 13° C., the optimum temperature for indirect germination of the Phytophthora. Controls were always run with each experiment and if the germination in pure water was not normal, the result was discarded. The controls also served as an index as to the proper time to continue the experiment. The time of final examination of the various cultures varied from 2 to 24 hours, but in the great majority of tests the duration was less than 5 hours. No attempt was made to estimate the percentage of germination taking place. Only its presence or absence was noted.

EXPERIMENTAL STUDIES

It seemed desirable to repeat some of the earlier studies on the toxic effect of copper salts, making use of the method just described. Because of the different method employed, it was impossible to use the earlier studies of Prevost (1807), Millardet (1887), and Dufour (1890) as guides. They put the spores directly into the solution to be tested, while in the studies that follow, the spores were placed on the dry salt after it had been sprayed on the slides in solution and allowed to dry for 24 hours or longer.

TOXICITY OF COPPER SALTS

Some of the common copper salts were tested as to their toxic effect on germination in order to learn whether any marked difference exists in these. Trials were made with each, in dilutions ranging from 0.06 to 0.00015 per cent of copper. In the following table only dilutions within the limit of toxicity are given, although a large number of tests were made in both stronger and weaker solutions.

Five copper salts were tested, cupric nitrate ($Cu(NO_3)_2 3H_2O$), cupric sulphate ($CuSO_4 .5H_2O$), cupric acetate (neutral) ($Cu(C_2H_3O_2)_2.H_2O$), cuprammonium sulphate ($CuSO_4 4NH_2.H_2O$) and cupric chloride ($CuCl_2 .H_2O$). All the salts except cupric nitrate were used on the spores of the two fungi, Phytophthora and Plasmopara. From one to five tests were made with each of the different strengths of the various salts, and each test included triplicate cultures unless otherwise stated.

It should be noted that all of these copper salts are about equally toxic when they contain equal amounts of copper, except in the case of cuprammonium sulphate. Cupric nitrate prevented germination at 0.0156 per cent, and cupric chloride at 0.0233 per cent. Cupric chloride is less toxic than any of the other salts. It is also evident from the data set forth in Table IX that the spores of Phytophthora are

TABLE IX.—TOXIC EFFECT OF THE COPPER SALTS ON THE SPORES OF PHYTOPHTHORA AND PLASMOPARA

Copper salts	Copper	Salt	With *P. infestans*			With *P. viticola*		
			Expts.	Cultures		Expts.	Cultures	
				Germ.	Not germ.		Germ.	Not germ.
	Per cent	Per cent						
Cupric nitrate	0.0312	0.119				1		2
Cupric nitrate	0.0156	0.059				1		2
Cupric nitrate	0.0078	0.030				2	4	0
Cupric nitrate	0.0038	0.014				2	4	0
Cupric sulphate	0.0159	0.0628	3	0	6	3	0	6
Cupric sulphate	0.0079	0.0314	4	1	8	4	0	9
Cupric sulphate	0.0039	0.0157	3	2	4	4	1	11
Cupric sulphate	0.0019	0.0078	4	4	6	4	2	10
Cupric sulphate	0.0012	0.0039	4	15	0	4	11	0
Cupric sulphate	0.0006	0.0019	5	19	0	5	14	0
Cupric acetate	0.0199	0.0628	1	0	3	2	0	9
Cupric acetate	0.0099	0.0314	2	4	3	3	2	6
Cupric acetate	0.0049	0.0157	2	7	0	2	3	6
Cupric acetate	0.0024	0.0078	2	7	0	1	0	3
Cupric chloride	0.0233	0.0625	2	0	4	1	0	2
Cupric chloride	0.01165	0.0312	2	3	1	1	0	2
Cupric chloride	0.0058	0.0151	2	4	0	1	0	2
Cupric chloride	0.0029	0.0075	1	2	0	1	2	0
Cuprammonium sulphate	0.079	0.0314	2	0	6	1	0	2
Cuprammonium sulphate	0.00395	0.0153	2	0	6	1	0	2
Cuprammonium sulphate	0.00197	0.0076	2	0	18			
Cuprammonium sulphate	0.00098	0.0038	2	6	12			
Cuprammonium sulphate	0.00049	0.0019	2	18	0			

more resistant to the toxic effect of the copper salts than are the spores of Plasmopara, a fact that has previously been emphasized by Wüthrich (1892).

TOXICITY OF CERTAIN COPPER MIXTURES

Having studied the copper salts as such, attention was turned to the various copper mixtures employed for the control of plant diseases. The mixtures studied and their composition, in the order cited in Table X are as follows: (1) Dauphiny mixture, 1 part copper sulphate crystals + 1.84 parts crystallized sodium carbonate; (2) Soda Bordeaux mixture, 1 part copper sulphate crystals + approximately 0.33

parts sodium hydroxide; (3) Woburn Bordeaux mixture, 5 parts copper sulphate crystals+1 part calcium oxide; (4) Bordeaux mixture, alkaline to phenolphthalein; (5) Bordeaux mixture, 1 part copper sulphate crystals+1 part calcium oxide; (6) Bordeaux mixture, 1 part copper sulphate crystals+2 parts calcium oxide; (7) Bordeaux mixture, 1 part copper sulphate crystals+1 part calcium oxide—calcium carbonate; (8) Bordeaux mixture, 2 parts copper sulphate crystals+3 parts calcium oxide; (9) Commercial Paris green, copper acetoarsenite; 3 aceto-arsenite of copper. According to Merck's Index it should contain about 27.7 per cent copper.

TABLE X.—TOXICITY OF VARIOUS COPPER MIXTURES USED IN PRACTICE FOR THE CONTROL OF PLANT DISEASES

Fungicide	Per cent copper	Per cent copper sulphate	Results					
			With *P. infestans*			With *P. viticola*		
			No. expts.	Cults. germ.	Cults. not germ.	No. expts.	Cults. germ.	Cults. not germ.
1. Dauphiny mixture	0.0079	0.0314	1	0	4			
	0.00395	0.0157	1	1	3			
	0.00197	0.0078	1	4	0			
2. Soda Bordeaux mixture	0.0159	0.059	2	0	8	1	0	2
	0.0079	0.0314	2	5	0	1	2	0
	0.00395	0.0157	1	3	0			
3. Woburn Bordeaux mixture	0.0159	0.059	4	0	11	3	0	11
	0.0079	0.0314	2	1	6	3	0	6
	0.00395	0.0157	2	3	4	2	0	9
	0.00197	0.0078				1	0	6
4. Bordeaux mixture	0.00395	0.0157	2	0	7	2	0	9
	0.00197	0.0078	2	6	0	2	0	9
5. Bordeaux mixture	0.00395	0.0157	1	0	3	1	0	3
	0.00197	0.0078	2	6	0	2	6	3
6. Bordeaux mixture	0.00395	0.0157	1	0	3	2	0	9
	0.00197	0.0078	1	3	0	2	6	3
7. Bordeaux mixture	0.00197	0.0078				1	0	6
8. Bordeaux mixture	0.0079	0.0314	1	0	3	2	0	6
	0.00395	0.0157	1	1	2	2	0	9
	0.00197	0.0078	1	3	0	1	6	0
9. Paris green	0.5*		2	2	2	3	5	2
	0.25*		2	2	2	3	5	2
	0.125*		2	4	0	3	7	0

* Percentage Paris green, not copper.

Soda Bordeaux, a mixture consisting of one part copper sulphate plus one part sodium hydroxide, killed the spores at 0.0159 per cent, but did not kill them at 0.0079 per cent.

Dauphiny mixture, which consists of one part copper sulphate and 1.84 parts of crystallized sodium carbonate, killed the spores of Phytophthora at 0.0079 per cent, but one culture out of four showed germination at 0.00395 per cent. This last mixture is more toxic than soda Bordeaux. The current idea is that Bordeaux mixtures vary in efficiency with the amount of lime present. In order to shed light on this subject from the standpoint of toxic effect on spore germination, the series of mixtures numbered 2, 3, 5, 6, and 8, in Table X varying in amount of lime, were prepared and tested. These Bordeaux mixtures were of approximately the following composition. The exact proportions of copper are given in Table X.

Mixture	Parts $CuSO_4 .5H_2O$	Parts CaO
3	1	0.2
8	1	0.6
5	1	1.0
6	1	2.0

A comparison of the data recorded in Table X shows that increasing the lime does not increase the toxicity to spore germination, and that the various mixtures are quite equal in their action. Plasmopara spores are here again more easily killed by copper mixtures than are the spores of Phytophthora.

Commercial Paris green did not prevent slight germination in 0.5 per cent solutions of its total dry weight. No analysis was made to determine the amount of copper present. It is doubtful, judging from the results obtained with commercial Paris green, whether it is sufficiently toxic to prevent germination, and it therefore has little fungicidal value. This is especially interesting in view of the fact that it has been thought by various writers to have quite marked fungicidal value.

Fungicidal Value of Certain Calcium and Sodium Salts

When calcium hydroxide and sulphur are brought together under the influence of heat and moisture, there results a mixture of variable composition, containing among

other compounds one or more polysulphides of calcium. Under ordinary conditions of exposure, these decompose, giving rise to various compounds, among which are free sulphur, sulphite, and thiosulphate. The thiosulphate appears to break down still further into sulphur and sulphite, both of these being finally oxidized more or less completely to sulphate.

TABLE XI.—TOXICITY OF SOME OF THE COMPOUNDS OF SODIUM POLYSULPHIDE

Compound	Dry weight	With *P. infestans*			With *P. viticola*		
		No. expts.	Cults. germ.	Cults. not germ.	No. expts.	Cults. germ.	Cults. not germ.
	Per cent						
Crystallized sulphur	5	4	12	0	4	12	0
Powdered sulphur	5	4	12	0	4	12	0
Sodium sulphide	1	1	10	0	1	10	0
Sodium sulphide	.5	1	4	0	1	4	0
Sodium thiosulphate	1	1	3	0	1	6	0
Sodium thiosulphate	.5	1	3	0	1	6	0
Sodium sulphite	1	1	6	0	1	6	0
Sodium sulphite	.5	1	6	0	1	6	0
Sodium hydroxide	1	1	0	2	1	0	2
Sodium hydroxide	.5	1	2	0	2	2	3
Sodium hydroxide	.25	1	2	0	2	2	3
Sodium hydroxide	.125	1	2	0	2	5	0
Sodium sulphate	1	1	3	0	2	9	0
Sodium sulphate	.5	1	3	0	2	9	0
Sodium hydrogen sulphide	1	1	1	1	1	1	1
Sodium hydrogen sulphide	.5		2	0	1	2	0
Hydrogen sulphide	[1]	1	0	6			
Hydrogen sulphide	[2]	1	0	6			
Hydrogen sulphide	[3]	1	6	0			

[1] Saturated solution.
[2] Saturated solution diluted with equal volume water.
[3] Diluted with three parts water.

The first substance tested was sulphur, both crystallized and powdered, because it is advocated as having toxic properties. Four experiments of three cultures each were made. Germination took place in every case as easily as in pure water. This is what one would naturally expect, in view of the fact that sulphur is not soluble in water. Six compounds of sodium were tested in various strengths, ranging from 0.125 to 2 per cent. As shown in Table XI, a one-per cent solution was necessary to prevent germination. Of these, sodium hydroxide was the most toxic compound. Normal germination took place in all the sodium compounds at one per cent, except as stated above, in the sodium hydroxide. Sodium hydrogen sulphide was slightly toxic at at one per cent. but at one-half per cent germination was

normal. Hydrogen sulphide (Mercks), saturated solution, prevented germination, and so did a solution obtained by mixing equal volumes of water and hydrogen sulphide. A solution of one part in three of water was not toxic. From the data at hand it again seems that Phytophthora is the more resistant of the two fungi under observation.

It is clearly evident from the data in Table XI that under the conditions of this experiment, sulphur in water is not toxic to germination. Also that none of the constituents of sodium polysulphide are toxic at one per cent, except sodium hydroxide and hydrogen sulphide. The former of these is toxic at one per cent and the latter at one-half per cent. No attempt has been made to determine the exact limits of their toxicity, the object being to learn which compound was toxic and at what strength.

The Fungicidal Action of Several Polysulphides

Attention was now turned to a study of several polysulphides, such as calcium, sodium, and potassium polysulphides and the Sherwin-Williams commercial polysulphide (lime sulphur).

Table XII.—Toxicity of Four Polysulphides

Fungicide and proportion of ingredients	Dry weight	Dilution	Results					
			With P. infestans			With P. viticola		
			No. expts.	Cults. germ.	Cults. not germ.	No. expts.	Cults. germ.	Cults. not germ.
	Per cent							
Calcium polysulphide								
11.5S:5CaO	2.	1:21.7	2	0	4	1	0	2
11.5S:5CaO	1.	1:43.4	2	2	2	4	9	1
11.5S:5CaO	.5	1:86.8	2	2	2	3	5	2
11.5S:5CaO	.25	1:173.6	2	4	0	2	4	0
Sodium polysulphide								
12.5S:8NaOH	1.	1:43.4	2	0	4	3	6	2
12.5S:8NaOH	.5	1:86.8	2	1	5	4	9	2
12.5S:8NaOH	.25	1:173.6	2	6	0	3	8	0
Potassium polysulphide								
Not determined	1.	1:43.4	1	2	0	2	2	3
Not determined	.5	1:86.8	2	4	0	3	7	0
Not determined	.25	1:174.8	2	4	0	3	7	0
Sherwin-Williams lime sulphur								
Not determined	4.	1:10.8	2	0	4	2	0	5
Not determined	2.	1:21.7	3	0	7	4	7	4
Not determined	1.	1:43.4	3	1	6	3	6	2
Not determined	.5	1:86.8	3	8	2	3	6	2
Not determined	.25	1:173.6	1	3	0	2	6	0

The compounds named in Table XII were evaporated to dryness, and the dry weight determined. The strengths are stated in the table in terms of percentages of dry weight and ratio of polysulphide to water. The proportions of lime and sulphur are also given for the calcium and sodium polysulphides. A 2-per cent solution of potassium polysulphide killed the spores, while a 1-per cent solution killed only part of them. A solution of the same strength of sodium polysulphides prevented the spores of Phytophthora from germinating, but not those of Plasmopara. The strength of this compound required to prevent germination is undoubtedly close to 1 per cent. Potassium polysulphide was non-toxic at 1 per cent. Sodium and calcium polysulphides seem from the data at hand to be like in their effect on spore germination, in both cases 1-per cent solution being required. With the Sherwin-Williams commercial polysulphide, a 2-per cent dry weight solution was non-toxic to the spores of Plasmopara, though quite toxic to those of Phytophthora. A 1-per cent solution permitted germination of some of the spores of both Phytophthora and of Plasmopara, but the number was less in the case of the latter fungus. This behavior of Plasmopara is of special interest in view of the fact that it has been heretofore more easily killed than Phytophthora. In conclusion it can be stated that the polysulphides here studied are toxic, but not markedly so. Sherwin-Williams calcium polysulphide at 2 per cent dry weight, or 1 part in 21.7 parts water, is less toxic to Plasmopara than to Phytophthora.

DISCUSSION AND CONCLUSIONS

Copper salts and Bordeaux mixtures.—It has been found, as has been shown by Millardet (1886 and 1887) and others, that the toxic constituent of Bordeaux mixture is the copper sulphate. A 0.00003-per cent solution of this copper salt in water was found by Millardet to be sufficient to prevent zoöspore germination of the conidia of *Plasmopara viticola*. More recently Wüthrich (1892), using the same fungus, found that a solution four times as strong as that said to be toxic by Millardet permitted germination, while it required a 0.00124-per cent solution to prevent germination. Both of these investigators placed the spores directly in the solution

of copper salt, a method not comparable with the conditions taking place when a fungicide is applied as a spray mixture. In practice it is sought to deposit a thin film of the fungicide over the leaves to be protected. The object is, of course, to prevent new infection and not to check infections already under way. It is well established that when a fungus has obtained a foothold in the tissues before spraying, it continues to fruit even though the surface of the leaves is covered with a layer of Bordeaux mixture. The fungicide on the foliage becomes effective only when water and spores come in contact with it. The point I wish to make clear is that the conditions under which fungicides act in the open are much different from those secured by plunging the spores directly into a solution, as was done by Millardet, Wüthrich, and others. This fact has also been emphasized by Wallace (1911), who elaborated the method followed in this paper.

It requires a 0.0315-per cent solution of copper sulphate to prevent the conidia of *Plasmopara viticola* from forming zoöspores when the salt is sprayed on glass slides as previously described, a method comparable with that generally in practice. By comparing these figures with Wüthrich's, it is evident that according to the method I have used a solution over 25 times as strong as his is required to prevent germination. The contrast is similar though less extreme when we compare the figures given for *Phytophthora infestans*. Wüthrich found that a 0.0124-per cent solution of copper sulphate in water was sufficient to prevent its germination, whereas in my studies a 0.0628-per cent solution was required, i. e., nearly five times the strength. It is not surprising that a stronger solution should be required by my method since the fungus spore comes in contact with only a fraction of the whole spray mixture applied thus to the slide. The action of the lime sulphur in the liquid and dry state is nicely shown by Wallace (1911). It required a much stronger solution when the mixture was sprayed on slides and allowed to dry. This is easily understood when we remember that the spores are subjected only to the action of the film of spray mixture upon which the droplet of spore suspension rests, a comparatively small area. When this fact is considered, the discrepancy between my results given above and those of earlier workers is not so marked. Indeed

it may well be that they are wholly alike. Be this as it may, the significant point in my studies, not clearly brought out heretofore, is the fact that when spray mixtures are applied, as in practice, a stronger solution is required than shown by previous studies on the toxic action of copper salts.

The question of the influence of temperature on the toxic action of fungicides and on the reaction of the spores is an interesting one. Prevost (1807), in his study of the effect of dissolved copper on the spores of *Tilletia tritici*, found that germination was materially retarded in a 1:1,000,000 solution, at a temperature of from $6\frac{1}{4}$ to $7\frac{1}{2}$° C., while it was not retarded in a 1:200,000 solution at higher temperatures. This condition in the smuts is just the reverse of that existing in the case of indirect germination of Phytophthora.

The most efficient 2-per cent Bordeaux mixtures contain nearly 200 times as much copper sulphate as is needed to prevent indirect germination of the spores of *Phytophthora infestans*. This suggests that a reduction in the amount of copper sulphate can be made in Bordeaux mixture used for the control of Phytophthora if these laboratory tests are a true representation of conditions existing in the open. It has already been shown by Istvanffi (1903) and others that a one- and even a one-half-per cent Bordeaux mixture control Plasmopara on the cultivated grape. A one-fourth per cent mixture has been used for the control of apple diseases by Clinton (1912) with promising results. Hawkins (1912) has shown that a Bordeaux mixture (3–2–50), with 2 pounds of resin fish-oil soap as a sticker, is very efficient in controlling the black rot of grape. Should it be found that a reduction in the amount of copper sulphate, say from 1 to 0.5 per cent, can be made in the spraying of potatoes, it would mean a considerable saving financially, even though this salt is not very expensive.

It is quite generally agreed that the fungicidal value of any copper spray mixture is due to the action of the contained copper, as such. The data tabulated in Table X bring interesting evidence to bear up this hypothesis. It will be seen that toxicity is governed by the amount of copper present in the compounds. It makes no difference whether it is combined as nitrate, sulphate, or acetate; practically the same percentage of copper (about 0.0156 per cent) is required to

prevent germination in each case. When copper is combined as a chloride a slightly stronger solution is necessary, 0.0233. Cuprammonium sulphate cannot be directly compared with the other salts because it ionizes in a different way from the other copper salts studied. A solution only about one-eighth as strong as that required when using the copper salts named above was necessary to prevent germination. This fact supports the contention made by certain writers that it is more efficient than Bordeaux mixture. Its use as a spray mixture is not practical because of its toxic action on the foliage.

The toxic limits of the Bordeaux mixtures studied are about the same from the standpoint of copper present, as already brought out for the copper compounds. Here again is evidence tending to support the theory that the copper ions, as such, are the fungicidal constituents. This, however, raises the question as to the action of the copper on the fungus spores. It is generally agreed by students of this question that it is the soluble copper that is active, but there is wide disagreement as to the way the insoluble basic copper salts are brought into solution. It is held by Millardet and Gayon (1887), Crandall (1909), Pickering (1910), and Butler (1914) that the insoluble basic copper salt is brought into solution by some purely chemical means, e. g., by the action of carbon dioxide or free ammonia, present in meteoric water and the air. On the other hand, it is held by some that the exudate liberated from the host tissues through wounds or other abrasions may dissolve enough of the insoluble copper to prevent germination. My experiments, in which the mixture was allowed to dry on slides and later droplets of water charged with spores were placed on the mixture, show that the fungicidal action takes place even though the solvent action of the leaf is excluded. Frank and Krüger (1894), Aderhold (1899), Clark (1902), Schander (1904), Barker and Gimingham (1911) are quite agreed that the fungus spore has the power to dissolve the insoluble salt in sufficient quantity to destroy its germinating capacity.

It has also been shown by Aderhold and by Barker and Gimingham that the filtrate of Bordeaux mixture after standing a time is non-toxic, while the gelatinous precipitate is toxic. This is ascribed to the action of the spore on the

insoluble copper salt, and they believe that the proximity of the spores to the copper particles is a matter of primary importance. It does not seem improbable to assume that the action of the spore in its immediate vicinity is one that should be considered where a germ tube develops, i. e., that it may have solvent powers due to its chemical actions on the medium in which it lies. On the other hand, it is not probable, although possible, that the conidia of Phytophthora when producing zoöspores, excrete substances having solvent action. As already pointed out, little, if any, growth takes place in this process, it being largely absorption of water and rearrangement of the cytoplasm within the conidium, followed by cleavage. These processes are not comparable with the growth of a germ tube such as occurs, for example, in the rusts. It is thus evident that the action of the copper on the spores presents some interesting problems not solved by these experiments and is a matter that deserves further careful study.

Polysulphides.—Lime sulphur has come into prominence during the last two decades and has in a measure replaced Bordeaux mixture as an orchard spray.

Only one proprietary mixture of this type has been studied—the Sherwin-Williams lime sulphur. This may, without any particular unfairness, be taken as typical of many other commercial mixtures. It has been found that it is not decidedly toxic to the germination of the spores of Phytophthora and Plasmopara. The latter fungus is apparently the more resistant.

Since I have used Wallace's (1910) method, direct comparison of our results, within certain limits, is possible. Such comparison makes it evident that the spores of Sphaeropsis and Venturia, as tested by Wallace, are more resistant than those of Phytophthora and Plasmopara. This is in accordance with what we should expect, considering the difference in the type of spores. Sclerotinia is more like these mildews, both in type of spore and in its susceptibility to poisons as shown by Wallace's trials. The relative resistance of Venturia as compared with Plasmopara to copper sulphate has also been pointed out by Crandall (1909).

"Solutions perfectly effective against grape mildew do not have even a retarding action on the growth of spores of the scab fungus."

The behavior of Phytophthora spores in my laboratory tests is interesting in view of recent field trials of lime sulphur as a potato fungicide. Stewart (1912), in New York, found that it injured the potato plant, although in the absence of the disease he could not judge of its fungicidal value. Pethybridge (1912), in Ireland, pronounced it worthless for the control of *Phytophthora infestans*. This can easily be understood in the light of my results, which show that a 1:21 solution is necessary to prevent the germination of the spores, a solution stronger than that commonly used in practice. Sherwin-Williams' commercial calcium polysulphide is about equally toxic with the other polysulphides tested.

Potassium polysulphide, another commercial mixture used for spraying, has been studied from the standpoint of toxicity to spore germination by Forman (1911). He found it toxic to Botrytis spores when it carried 0.25 per cent of either potassium or sulphur. My own tests have shown it to be only slightly more toxic, if any, than commercial lime sulphur (calcium polysulphide). A comparison of Forman's results with those in this paper is not readily possible because he placed the spores directly in the solution. He also studied some of the decomposition products of the mixture which are of interest in the light of my tests on the same or similar compounds. He found that sodium hydroxide was the most toxic of the compounds tested. My results confirm his findings, except that a slightly stronger solution was required. Forman showed that a 0.33-per cent solution was sufficient to prevent the germination of the spores of Botrytis, while a solution one-half as strong was not toxic. Potassium and calcium hydroxide of the same strength permitted germination, although a 0.66-per cent solution checked it. Sodium sulphide, sodium thiosulphate, sodium sulphite, and sodium hydrogen sulphide were all found to be non-toxic at 1 per cent. Two facts have been learned from the study of the compounds in sodium and calcium polysulphides: first, that only two of the more common compounds, namely, sodium hydroxide and hydrogen sulphide, are toxic, and,

second, they are not toxic at the strengths at which they can occur in the polysulphides.

A STUDY OF INFECTION OF POTATO FOLIAGE WITH PHYTOPHTHORA

Earlier in this bulletin it has been shown that temperature markedly influences spore germination. Because of the close relation that exists between germination and infection, from the pathological standpoint, it was thought advisable to determine whether there is a similar relation of termperature to infection. Other closely related problems were also given consideration, such as susceptibility of the upper and lower surfaces of leaves and relation of method of germination to infection.

METHOD

The method used is in general that described in an article published in Phytopathology, (Melhus, 1912). Potato plants were grown in pots and were infected when from four to twelve inches tall. The spores of Phytophthora were placed in water and sprayed on the potato plants with a De Vilbiss atomizer. When the fresh conidia were used, the plants were chilled for from 6 to 24 hours in order to allow ample time for the conidia to germinate. When the plants were exposed to infection by using zoöspores instead of conidia, they were held constantly at the greenhouse temperature. Infection usually became visible in from two to eight days after the plants were subjected to infection, depending upon the environmental conditions and the spore material used.

EXPERIMENTAL STUDIES

Following the above method, plants have been infected and treated in a variety of ways in order to give us a clearer understanding of how infection takes place and what external factors favor or retard it. The first experiments were planned to determine the influence of temperature on infection.

INFLUENCE OF TEMPERATURE ON INFECTION

Plants have been exposed to infection and held for a short time at different temperatures ranging from 10 to 32° C., as shown in Table XIII. It will be apparent by referring to

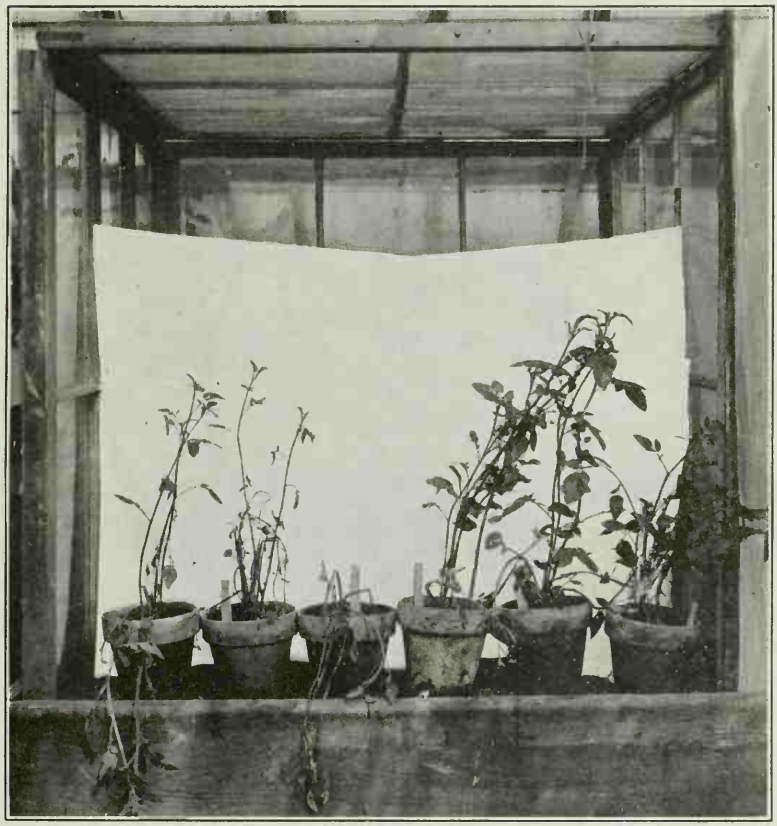

FIG. 5.—RELATION OF TEMPERATURE TO INFECTION BY PHYTOPHTHORA

Six potato plants were exposed to infection by spraying them with a suspension of conidia of *Phytophthora infestans* on August 21, 1911. The three plants at the left were chilled, 13° C., for eight hours following the application of spores, whereas the three plants at the right were held continuously at the higher temperature, 25-27° C. The chilled plants showed 98 per cent of the leaves infected, while those not chilled, at the right showed less than 10-per cent infection.

this table that in general at the low temperatures, 10–13° C., from 95 to 100 per cent of the leaves became infected in from 4 to 6 days. At 17° only 85 per cent showed infection and as

the temperature increased above this the infection percentage decreased until at 25–30° C. there were few or no infections. Figures 5 and 6 show photographs of some of these plants. In Figure 6 the plant held at 28° C. shows no infection, the one at 13° was totally killed. It is plain, therefore, that temperature influences the behavior of the spores of the leaves in the same way that it does when they are

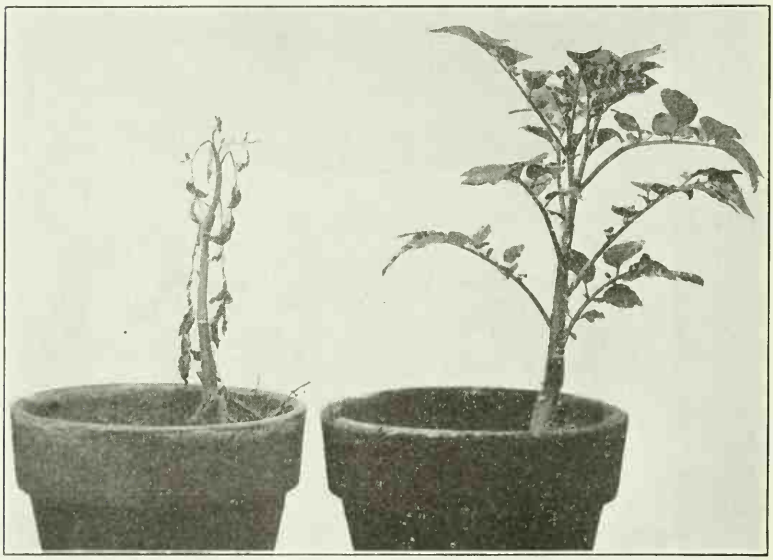

FIG. 6.—RELATION OF TEMPERATURE TO INFECTION BY PHYTOPHTHORA

These two plants were treated as described for Figure 5, except that the plant at the right was held at 28° C. It did not become infected, while the control at the left which was chilled at 13° C. was totally killed.

placed on glass slides. The presence or absence of infection is determined primarily by the behavior of the spores. At high temperatures these fail to germinate and therefore the possibility of infection is precluded.

These results are in general accord with those previously published (Melhus, 1911) for Cystopus.

TABLE XIII.—EFFECT OF TEMPERATURE ON THE AMOUNT OF INFECTION OBTAINED BY EXPOSING POTATO PLANTS TO PHYTOPHTHORA CONIDIA

AT LOW TEMPERATURES					AT HIGH TEMPERATURES				
Plants	Refrigeration[1]		Incubation[2]	Leaf infection	Plants	In moist chamber		Incubation[2]	Leaf infection
	Hrs.	Degrees	Days	Per cent		Hrs.	Degrees	Days	Per cent
4	21.5	11	4	95	4	21.5	19–20	4	50
1	9	12	4	95	2	9	19–25	4	5
1	9	12	4	95	2	9	26–32	4	0
2	10.5	12	4	95	2	12.5	26–29	6	0
3	18	11	5	95	3	18	17–18	5	85
2	22	13	6	95	4	22	18–19	6	57
2	20	14	5	95	1	20	26–32	5	0
2	30	10	5	98	7	30	15–30	5	50
2	18	13	4	100	2	18	19–25	5	33
1	18	13	4	80	1	18	19–25	5	10
1	18	13	4	74	1	18	19–25	5	4
1	18	13	4	34	1	18	19–25	5	17
5	8	13	5	98	3	8	25–27	5	10

[1] *Refrigeration* period in this table, indicates the period the plants were held at the temperatures given.
[2] *Incubation* period indicates the time from the date the plants were exposed to infection to the development of visible evidence of the same.

DOES CHILLING INCREASE THE SUSCEPTIBILITY OF THE POTATO PLANT

It is also of interest to learn whether the potato plant becomes more susceptible when chilled a few hours. In other words, does a comparatively low temperature influence the reaction of the host to the fungus? In order to secure evidence on this point, it was necessary to devise some method of inoculating potato plants at both high (23–27° C.) and low (12–15° C.) temperatures. As shown in the discussion of Table XIII, infection does not take place readily unless the plant is held at 12–15° C. This was due primarily to the less vigorous germination of the spores. In order to avoid this difficulty, the spores were germinated in water in a beaker and later the zoöspores were applied to the plants. It has been shown in an earlier part of this paper that when the zoöspores are liberated from the conidium they grow more rapidly at about 24 than at 13° C. Experimental data bearing upon this point are recorded in Table XIV.

Two lots of plants (var. Irish Cobbler) were sprayed with zoöspores and subjected to like conditions in all respects except as to temperature. In one lot the plants were chilled at 12–14° C. for from 8 to 24 hours.

An equal number constituting the second lot were not chilled, but held constantly in a greenhouse where the tem-

perature varied from 23 to 27° C. Table XIV shows that approximately the same amount of infection developed on both the chilled and unchilled plants. It is worthy of note that it required nearly a day longer for the plants held at

TABLE XIV.—COMPARATIVE EFFECT OF LOW TEMPERATURE ON THE SUSCEPTIBILITY OF THE POTATO PLANT

Infected	Height of plants	Exposed to infection at 12–15° C.					Exposed to Infection at 23–27° C.		
		Plants	Chilled		Infection		Plants	Infection	
			Time	Temperature	Visible	Percentage		Visible	Percentage
	In.		Hrs.	Degrees					
Jan. 24	7	3	8	12–13	Jan. 28	95	3	Jan. 28	80
Jan. 25	8	3	8	14–15	Jan. 28	80	3	Jan. 28	80
Jan. 29	6	3	19	12–13	Jan. 31	90	3	Jan. 31	90
Feb. 5	6	3	24	13–14	Feb. 8	90	3	Feb. 7	95
Feb. 6	6	3	18	12–13	Feb. 9	85	3	Feb. 8	90
Feb. 12	12	4	24	12–15	Feb. 16	95	4	Feb. 15	95
Feb. 14	10	4	24	10–13	Feb. 18	85	4	Feb. 17	95
Feb. 24	12	5	24	12–14	Feb. 28	95	5	Feb. 27	85
Feb. 25	8	2	24	12–13	Mar. 2	95	2	Mar. 1	85

the lower temperature to show visible signs of infection than for those held at the higher temperature. The fact that the chilled plants were slower in showing infection was probably due (1) to the longer period of motility of the zoöspores on the plants chilled, and (2) to the fact that their subsequent rate of growth was retarded by the lower temperature. In an earlier part of this paper it has been shown that low temperature retards both loss of motility and germination of the liberated zoöspores. This fact considered, the time required for infection to become visible on the chilled and unchilled plants was not materially different. Likewise the amount of infection in each case was very nearly the same. It is plain, too, that chilling for from 8 to 24 hours does not make the host more susceptible.

THE RATE OF DEVELOPMENT OF PHYTOPHTHORA INFECTION

Various statements exist in the literature in regard to the time required for infections of Phytophthora to become visible. The usual time given is 6 days, although many investigators allow even 10 days. It has been suggested in the above paragraph that temperature is a factor. In the series of experiments recorded in Table XV, the plants were

sprayed with a water suspension of zoöspores obtained in the same way as described in the discussion of Table XIV and held at different temperatures, namely, 12-16° and 25-27° C.

Infection readily took place on both lots of plants. The amount was equal, but the rate of development or spread of the fungus in the potato foliage was more rapid in the plants held at the higher temperature (23–27° C.). This shows

TABLE XV.—COMPARATIVE EFFECT OF HIGH AND LOW TEMPERATURE ON THE RATE OF DEVELOPMENT OF PHYTOPHTHORA INFECTION IN POTATO FOLIAGE

Infected	Height of plants	Plants at 12-16° C.			Plants at 23-27° C.		
		No. of plants	Infection		No. of plants	Infection	
			Visible	Percentage		Visible	Percentage
	In.						
Jan. 16	7	3	Jan. 19	90	3	Jan. 18	90
Jan. 15	6	3	Jan. 18	94	3	Jan. 17	94
Jan. 11	6	2	Jan 15	95	2	Jan. 14	95
Jan. 7	6	2	Jan. 11	93	2	Jan. 10	93
Jan. 25	8	3	Jan. 29	90	3	Jan. 27	80
Feb. 1	8	3	Feb. 4	98	3	Feb. 3	98
Jan. 29	8	3	Feb. 2	90	3	Jan. 31	90

that the optimum for the growth of the mycelium in the foliage is much higher than that required for indirect germination of the conidia, already shown to be about 13° C. Just what the optimum may be for the growth of the mycelium was not determined, but it is clear from the results at hand that it is above 20° and it may be between 24 and 25° C. The behaviour of the zoöspores as described earlier would also indicate that it was probably this higher limit.

INFECTION BY DIRECT CONIDIAL GERMINATION

The preceding infection studies have been concerned chiefly with indirect germination. In order to learn whether or not foliage infection can take place by direct germination, viable conidia were placed in 10-per cent dextrose solutions and sprayed on plants held at 20° C. It was found that only direct germination occurred in this solution under the conditions of the experiment. Controls were exposed to zoöspores at the same time. Infection appeared in four days on both sets, but the amount of infection was much less on

the plants sprayed with the conidia in dextrose solutions than on the controls subjected to zoöspores.

Still another experiment, of interest in this connection, showed that infection can take place at about 25° C. Since this is the maximum temperature for indirect germination and the optimum for direct, it is most probable that infection resulted from conidia germinating directly. In another experiment four potato plants were exposed to conidial infection and two of the plants placed in a moist atmosphere at 30° C., while the other two were held at 12–13° C. Infection developed on only two leaves of one of the plants held at 30° C., and the other remained wholly free from infection. The controls at the low temperature were heavily infected as usual. There can be no doubt that these infections at 30° C. came from direct germination. That infection was so sparing is in accord with my germination tests previosuly reported, which show that direct germination is not abundant at this high temperature.

THE DIFFERENCE IN SUSCEPTIBILITY BETWEEN THE UPPER AND LOWER SURFACES OF THE LEAF

When it had been established that low temperature was most favorable for infection, the question of relative susceptibility of the upper and lower surfaces of the potato leaves was studied. Müller-Thurgau (1911) has shown very conclusively that Plasmopara seldom infects the cultivated grape through the upper surface of the leaves. The question naturally arises whether this is characteristic of the related fungi, including Phytophthora. Many experiments were made to determine this, some of which are tabulated in Table XVI in order to show the general trend of the results obtained.

The general methods in these studies recorded in Table XVI were the same as in those already described. The plants were in all cases kept under conditions of temperature and moisture favorable to infection. In some cases fresh conidia were used, in others, motile zoöspores. In order to prevent any possibility of confusion the spores were applied to only one spot on each leaflet and this was marked with a circle of India ink. Care was exercised when the infection was made in a drop of water on the upper surface to prevent its

TABLE XVI.—SUSCEPTIBILITY OF UPPER AND LOWER SURFACES OF POTATO LEAVES TO PHYTOPHTHORA INFECTION

Date	Upper surface				Lower surface			
	Leaves	Result			Leaves	Result		
		Date	Infections	Percentage of infection		Date	Infections	Percentage of infection
Sept. 28	14	Oct. 4	3	21	18	Oct. 4	16	89
Dec. 7	12	Dec. 12	2	17	3	Dec. 12	3	100
Dec. 7	26	Dec. 12	13	50	11	Dec. 12	11	100
Dec. 3	3	Dec. 8	2	67	2	Dec. 8	2	100
Dec. 15	6	Dec. 21	6	100	6	Dec. 20	6	100
Feb. 24	8	Feb. 28	7	88	8	Feb. 27	8	100
Feb. 25	10	Mar. 1	8	80	10	Mar. 1	10	100
Total	79		41		58		56	
Average				52				97

running over the margin to reach the lower surface. Of 79 exposures made on the upper surface, 41 gave infection, as compared with 58 on the lower surface with 56 infections. It is obvious from these results that the upper surface of the potato leaf is susceptible to Phytophthora infection, though less so than the lower surface.

The experiments tabulated in Table XVII gave further evidence that infection may take place when the spores are applied on the upper surface of the potato leaf. In this series of tests, a water suspension of zoöspores was sprayed on either the upper or the lower surface of the leaf, except in

TABLE XVII.—DIFFERENCE IN SUSCEPTIBILITY OF THE UPPER AND LOWER SURFACES OF POTATO LEAVES WHEN SPRAYED WITH A WATER SUSPENSION OF ZOÖSPORES OF PHYTOPHTHORA

Date	Plants	Height of	Upper surface		Lower surface		Upper and lower surface	
			Plants	Infection	Plants	Infection	Plants	Infection
		In.		Per cent		Per cent		Per cent
Jan. 16	6	6	2	20	2	90	2	100[1]
Jan. 18	6	6	2	25	2	80	2	100[2]
Jan. 21	6	7	2	14	2	65	2	100[2]
Jan. 23	12	8	4	2	4	19	4	100[3]
Feb. 5	9	8	3	50	3	95	3	100[4]
Feb. 6	9	8	3	10	3	90	3	100[4]

[1] An equal amount of zoöspore suspension was sprayed on the upper or lower surface, respectively, of all the plants exposed to infection on this date.

[2] One cc. was applied to each plant except the checks.

[3] Less was applied to the upper surface than to the lower.

[4] Zoöspore suspension was much diluted, and one cubic centimeter of same applied to either the upper or the lower surface.

the controls, where the suspension was sprayed on both surfaces. In this way all the leaves on a plant could be exposed to infection in much the same way as when they are in the open, although the method is less exacting than described in connection with Table XVI. There was a greater possibility that some of the spores might reach the under surface, but this imperfection in the method was overcome, it is believed, by infecting a large number of leaves. It will be seen by referring to Table XVII that a total of 48 plants was used; 16 were sprayed on the upper leaf surface, 16 on the lower, and 16 controls sprayed on both the upper and the lower surfaces. The infection that developed on the controls in each case was considered perfect and rated as 100 per cent, and the amount of infection on the others rated proportionately. The amount of infection on the controls was always greater than that in any of the other plants infected, doubtless due to the application of a greater number of zoöspores. The results again show that infection may take place through the upper surface of the leaf, although less readily. The comparative effect on the plant when sprayed on the upper leaf surface as compared with the lower is shown in Figure 7. It was also evident that the bud of the plant and the upper portion of the stem were more susceptible to infection than the upper leaf surface. (See Figure 7.)

It is probable that the difference in number of stomata on the two leaf surfaces influences materially the entrance of the fungus. Stomata occur on both surfaces, but are four or five times as numerous on the lower side as on the upper. The amount of spore suspension, whether applied on the upper or the lower surface, had no effect other than to increase or decrease the relative amount of infection.

DISCUSSION AND CONCLUSIONS

If low temperature facilitates germination, it should also aid infection, since the latter is dependent upon the former. The effects of different temperatures ranging from 10 to 30° C. are shown in Table XIII. It is quite clear that low temperatures are more favorable than high. (See Figure 5.) These results with Phytophthora confirm in general my results obtained in similar tests with *Cystopus candidus* on the

common radish. There is, however, this difference, that in the case of Phytophthora a greater amount of infection takes place at temperatures between 20 and 30° C. This is doubtless due to the ability of the conidia of Phytophthora to germinate directly at the higher temperature. In the case of

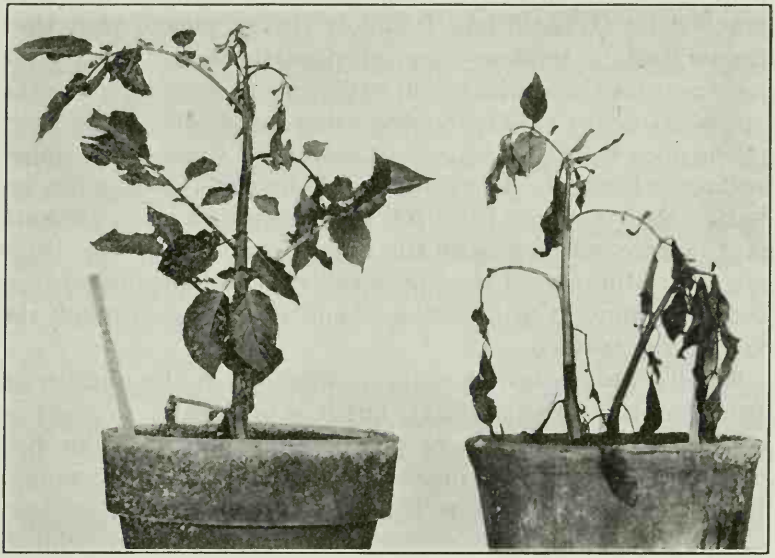

FIG. 7.—RELATIVE AMOUNTS OF INFECTION THROUGH UPPER AND LOWER LEAF SURFACES

The potato plants (var. Irish Cobbler) were exposed to infection February 6, 1913. Both plants were sprayed with a suspension of Phytophthora zoöspores and held for 18 hours in a saturated atmosphere at greenhouse temperature (18–22°C.). In the case of the plant at the right, the spores were applied to the lower surfaces and showed infection estimated at 90 per cent. The plant at the left, infected on the upper surface, showed much less, estimated at 10 per cent. The buds and young stem tissue in the latter case made up a considerable portion of the infection shown.

Cystopus the conidia do not germinate directly and consequently, when indirect germination is prevented, no infection takes place.

In my work on Cystopus, the effect on the host of chilling was not studied, but in the infection experiments with Phytophthora this question also has been considered. It seems clear that chilling influences infection only in so far as it facilitates germination.

Whether infection can or can not take place through the upper surface of the potato leaf is of considerable interest in view of the results of recent investigation with *Plasmopara viticola*. Ruhland and Faber (1909) concluded that Plas-

mopara infection seldom if ever takes place through the upper surface of the leaf. The following year this statement was confirmed by Müller-Thurgau (1911), who showed by a carefully planned series of experiments that infection seldom takes place through the upper surface of the cultivated grape leaf. More recently the same general conclusion has been reached by Istvanffi and Pálinkás (1912), except that they found a slight tendency for infection to occur through the upper surface when infection experiments were made in the open. Gregory (1912), on the other hand, also made tests in the open but failed to get any infections through the upper surface. Recently Istvanffi and Pálinkás (1913) have reported that there are from 200 to 400 times as many stomata on the lower surface as on the upper. Since, however, there are some stomata on the upper surface, it seems logical that a certain amount of infection should take place through the upper leaf surface.

On the potato leaf there is a difference in the number of stomata on the two surfaces, but it is not nearly so great as on the grape leaf. There are probably only four or five times as many stomata on the lower surface as on the upper. Experiments tabulated in Tables XVI and XVII show that 52 per cent of the leaves exposed on the upper side developed Phytophthora infection, while 97 per cent of those exposed on the lower surface gave infection. The relative effect on the plant of infection resulting from applying zoöspores on the upper and lower surfaces of the leaves is also shown in Figure 7. Where the zoöspores were applied on the upper surface, more of the foliage is alive. From these data it is plain that infection can take place through the upper as well as through the lower surface. The difference in amount of infection may be due to the difference in the number of stomata on the respective surfaces. It is suggested by these tests that the entrance of the fungus into the leaf may depend, as it probably does in the grape mildew, upon the presence of stomata.

The question naturally arises in this connection as to whether the difference in susceptibility is sufficient to warrant spraying the under surface of the potato leaf in order best to control the late blight. There can be no doubt that such a treatment would be more efficient if practical, but the

present method of spraying potatoes by making the application on the upper surface has proved quite efficient and the results of these studies do not warrant any change from this practice.

SUMMARY

Spore Germination of Phytophthora

The spores of *Phytophthora infestans* may germinate either indirectly by the production of zoöspores or directly by germ tubes. The type of germination is determined chiefly by external influences, such as temperature, moisture, and the medium in which the spores are placed.

Temperatures below 20° C. have been found more favorable for indirect or zoöspore germination in water than higher temperatures. The minimum lies between 2 and 3° C., the optimum between 12 and 13° C., and maximum between 24 and 25° C.

For direct or tube germination the limits are all higher. Direct germination was very scanty below 15° C. Above 20° C., it became more abundant, increasing with the temperature. The minimum for this is probably between 10 and 13° C., the optimum about 24° and the maximum very near 30°.

Indirect germination occurs generally in a 10-per cent dextrose solution, sparingly in a 16-per cent solution, and not at all in a 20-per cent solution. Indirect germination is replaced by some direct germination in the last mentioned strength.

The time required for the spores of *Phytophthora infestans* to germinate depends upon two factors: (1) the viability of the spores, and (2) the external influences. The shortest period for indirect germination was 45 minutes, although it usually required from one to three hours. The time decreases as the temperature increases up to 13° C. (See Figure 1, Curve B.) Above this point the ratio is reversed. Direct germination is a slower process.

The number of spores germinating was also dependent upon the temperature. Eighty per cent germinated at temperatures between 10 and 13° C. At either higher or lower temperatures, however, the percentage decreased, showing that the optimum lies between 10 and 13° C.

Intermittent temperatures, changing from high to low, or vice versa, do not particulary favor germination.

The period of motility of the zoöspores was also influenced by temperature. Its duration varied inversely with the temperature, ranging from 22 hours at 5–6° C. to 19 minutes at 24–25° C. (See Figure 3.) The further development of the zoöspore after coming to rest, i. e., growth of germ tubes, is more rapid at 23–24° C. than at lower temperatures. (See Figure 4.) It is probable that the optimum for the growth of the germ tubes and for direct germination in water are the same (about 24° C.).

The spores of *Phytophthora infestans* are killed in from 6 to 24 hours when exposed to such dry atmospheric conditions as exist in an ordinary room.

A frost that kills the tissues of the host plant is also sufficient to kill the conidia of Phytophthora.

Leaf juices resulting from the softening of infected tissues have an inhibiting effect on germination.

Light, either direct or diffuse, does not hinder germination so long as the temperature is not above the optimum.

Indirect germination takes place in the morning dew and rain on potato foliage under field conditions. Direct germination was not observed to occur in the open on the foliage.

Increasing the amount of nascent oxygen in the medium containing the spores does not stimulate germination, but on the contrary, inhibits it. It may be that sufficient oxygen exists in the spore to allow indirect germination to take place.

TOXICITY OF CERTAIN SALTS AND FUNGICIDES

Studies of toxicity have been carried on with two parasites, *Phytophthora infestans* and *Plasmopara viticola*, using chiefly the glass-slide method.

When the spores were subjected to optimum temperature conditions for indirect germination, 0.0159 per cent of copper was necessary to prevent germination.

It is quite generally agreed that the fungicidal action of copper sulphate is due to the copper contained. This is quite clearly brought out by the experiments here reported showing that 0.0159 per cent copper, as such, is required to prevent germination, whether it is in the form of copper nitrate, copper acetate, or cupric chloride. Cupram-

monium sulphate is about eight times as toxic as the other copper salts tested. This is possibly due to the different ionization of this salt.

Slight changes in the amount of calcium oxide in Bordeaux mixture do not materially change its toxicity. A Bordeaux mixture low in lime (Woburn formula) was no more toxic than one high in lime.

Various polysulphides were studied in the same way as the Bordeaux mixtures in order to gain some idea of their toxic effect on the germination of the spores of Phytophthora and Plasmopara. It has been found that calcium polysulphide (1:21.7) is necessary to prevent the germination of the spores of Phytophthora, and a stronger solution is required for the spores of Plasmopara. Chemically pure calcium polysulphide was slightly more toxic than the commercial article used as a fungicide. Sodium and potassium polysulphides are about equally toxic, preventing germination at one per cent.

The spores of Plasmopara were slightly more resistant to the polysulphides than those of Phytophthora.

Sodium hydroxide and hydrogen sulphide were the most toxic compounds of sodium polysulphide tested. None of the other compounds studied were toxic in one-per cent solutions.

Infection Studies of Potato Foliage

Infection of the potato plant with *Phytophthora infestans* takes place at conditions favorable for germination. Plants chilled for periods of from 12 to 24 hours at 10–13° C. showed a greater amount of infection than the controls held at higher temperatures, i. e., the amount of infection decreased as the temperature increased above 13° C. (Figure 5.) This was due to the effect of temperature on the fungus (spore germination) rather than on the host, since chilling has no tendency to increase the susceptibility of the plant. (Figure 8.)

Infection becomes visible in two or three days at temperatures between 23 and 27° C. It requires a longer period at lower temperatures.

The most favorable temperature for the growth of the mycelium in the tissue (probably about 24° C.) is about the same as the optimum for direct germination in water and

considerably higher than the optimum temperature for indirect germination (13° C.).

Foliage infection may take place when only direct germination occurs.

Infection may take place through either the upper or lower surface of the leaf. The plant is, however, less liable

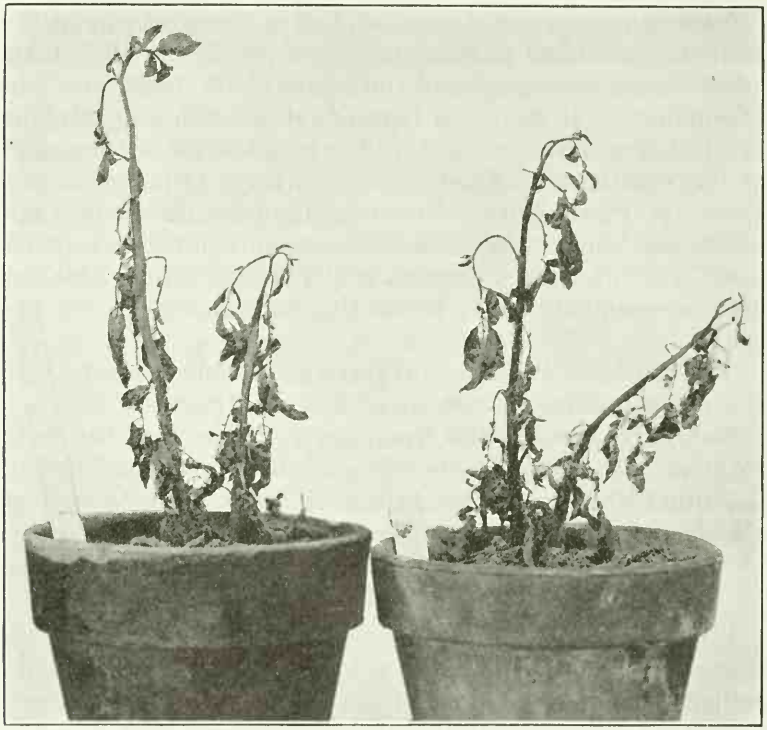

FIG. 8.—COMPARATIVE EFFECT OF CONIDIAL AND ZOÖSPORE INFECTION

The plant at the left was sprayed with a suspesion of zoöpsores in water and held in a saturated atmosphere in the greenhouse for 18 hours at a temperature of 25–27° C. The other plant was sprayed with a suspension of conidia and chilled for 24 hours at 12–14° C. The chilling for 24 hours did not increase its susceptibility. Practically the same amount of infection developed on the two plants exposed to zoöspores and conidia.

to infection through the upper surface than the lower. (Figure 7.)

The difference in susceptibility of the upper and lower surface of the leaf is attributed to the difference in the relative number of stomata.

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The Control of Cabbage Yellows Through Disease Resistance

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The Control of Cabbage Yellows Through Disease Resistance

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The Cabbage Industry in Wisconsin

Cabbage-growing as a specialized industry has assumed considerable proportions in Wisconsin. In Racine county it began on a commercial scale some thirty years ago with a small acreage by one man, according to Moyle (1913). It has since continued to develop in southeastern Wisconsin, notably in Racine and Kenosha counties, until in certain sections it has become the dominant cash crop. An even earlier beginning in commercial cabbage culture was made in the vicinity of Green Bay[1]. Here also the local success stimulated growers in nearby towns to take up the crop. This has proved highly profitable, especially westward of Green Bay in Outagamie county where Shiocton and neighboring places are large shipping points. Most Wisconsin growers produce winter cabbage chiefly for storage and for the southern markets. With the development of kraut factories a few centers have given increased attention to growing the summer or kraut types for local manufacture, e. g., in Grant, La Crosse, Racine, Rock, Sheboygan, and Waupaca counties. Cabbage growing in a somewhat experimental way is now being undertaken in various other sections of the state and it seems that with the reclamation of marsh lands attention to cabbage culture is certain to increase.

There are no full or reliable statistics available as to the yearly production of cabbage in Wisconsin. The Annual Reports of the Wisconsin State Board of Agriculture give yields by counties for the six years 1907–1912. These are, however, based upon the returns sent in by the county clerks

[1] George B. Smith of Green Bay has kindly reported on conditions in Brown and Outagamie counties. He writes that his father began shipping cabbage from Brown county in 1867, and that the industry has had a steady growth ever since; also that in the Shiocton region, Outagamie county, cabbage culture on a commercial scale began over twenty years ago and the acreage there is increasing each year.

and are quite irregular and evidently inaccurate. No figures are given for cabbage before 1907 or since 1912.

The yields, in tons, of the five leading counties in 1911, the last year when complete lists are given in these reports, are as follows: Outagamie, 20,630; Milwaukee, 5,041; Racine, 3,461; Eau Claire, 3,397; St. Croix, 3,188; Brown, 2,789. Kenosha doubtless should be included in this list, probably about equalling Racine, but for some reason no report was sent from that county.

The acreage and yield from Milwaukee county has been generally uniform during this six-year period, but a comparison of Outagamie and Racine counties shows a significant change in both actual and relative production.

	1907 Tons	1908 Tons	1909 Tons	1910 Tons	1911 Tons	1912[2] Tons
Outagamie county	220	2,324	6,120	12,037	20,630	37,000
Racine county	12,155	5,144	15,555	2,829	3,461	3,300

From field observations in Racine county we are confident that the above figures considerably understate the production there[3] and it is quite possible that they do for Outagamie county also. It is probably safe to assume, however, that they indicate for each county approximately the relative production year by year.

These figures show two peculiarities of the cabbage crop, (1) its intensive development in certain localities, and (2) its fluctuation, with the possibility of rapid decline after a few years. The reasons for local specialization in cabbage production are to be found in part in the fact that the cultural and marketing methods are somewhat peculiar, and in part in the further fact that cabbage is best suited to certain soil types (Fig. 1). Although it will grow on any good soil, it thrives best on reclaimed swamp land or similar deep rich land with abundant humus, and, in addition, it requires high manuring. Since, under such favorable conditions, it frequently brings an unusually high return in proportion to the labor investment, the temptation is strong, upon ground

[2] For 1912, only acreages were given in the board's report and from these we calculated the yields, assuming that they would be approximately the same per acre as in 1911 when both acreage and yield were given.

[3] We also sought figures from the railroad freight departments showing annual car shipments. These were not available for 1912 or earlier but for 1913 they indicate a shipment probably totaling 1,500 cars from stations in Racine county, a figure so much in excess of the report of the Board of Agriculture for the preceding years as to indicate that the figures of the board are probably much below the actual production.

FIG. 1.—A PROFITABLE CABBAGE FIELD

Cabbage is a highly profitable crop when grown on rich, deep black, soil. This field was recently drained and plowed for the first time.

suited for cabbage, to grow it on the same soil in successive years. Not infrequently in the earlier days, Racine cabbage growers continued to grow cabbage on a field for from five

to ten years and at Green Bay even longer cropping is practiced without rotation[4]. In such cases, the fertility is usually kept up by frequent dressings with stable manure. Nevertheless the outcome in the Racine sections has been that sooner or later these fields have tended to become "cabbage sick" so that instead of the uniformly profitable crops, partial or complete failures occurred (Fig. 2); hence the extreme fluctuations already noted and the tendency to abandon cabbage in Racine county. The first complaints of such failures came in 1895-1896 from the Racine growers where Dr. Russell (1898), upon investigation, found the cause to be the bacterial disease, black rot. This malady has continued to plague the cabbage fields of that vicinity and now is found over the state generally. Meanwhile, other serious parasitic diseases have been introduced and are rapidly spreading. In short, growers are finding that the cabbage crop is no exception to the rule that intensive culture and continuous cropping bring an accumulation of diseases in their train, and that ability to cope with these diseases becomes the deciding factor in continued success with the crop.

THE VARIOUS CABBAGE DISEASES

There are a number of serious diseases which may attack the cabbage in Wisconsin. All are due to parasites which when once introduced persist in favorable soil. Although the present publication will deal with only one of these, the so-called yellows disease, it has been necessary to give consideration to all of them in connection with these investigations, and it will be helpful to describe briefly some of the others in order to forestall possible confusion. These include, as of major importance, black rot, soft rot, club root, and black leg, any one of which under certain conditions may ruin a crop. Besides these, there are some minor diseases not requiring mention here.

Black rot. This is due to a bacterial parasite, *Pseudemonas campestris* (Pam.) Smith, (*Bacterium campestre*),

[4] We are again indebted to George B. Smith of Green Bay for the following statement. "One of my neighbors grew cabbage continually for seventeen years and then changed to another crop, not because he had poor cabbage, for the cabbage was fine. This land was, however, located in a valley where the wash from neighboring hills brought some new soil to it each year. I have gradually grown into the practice of rotation of all crops myself so as to prevent sickness. I think four or five years is as long as I have ever planted cabbage continuously on level land and the last year it showed some yellows."

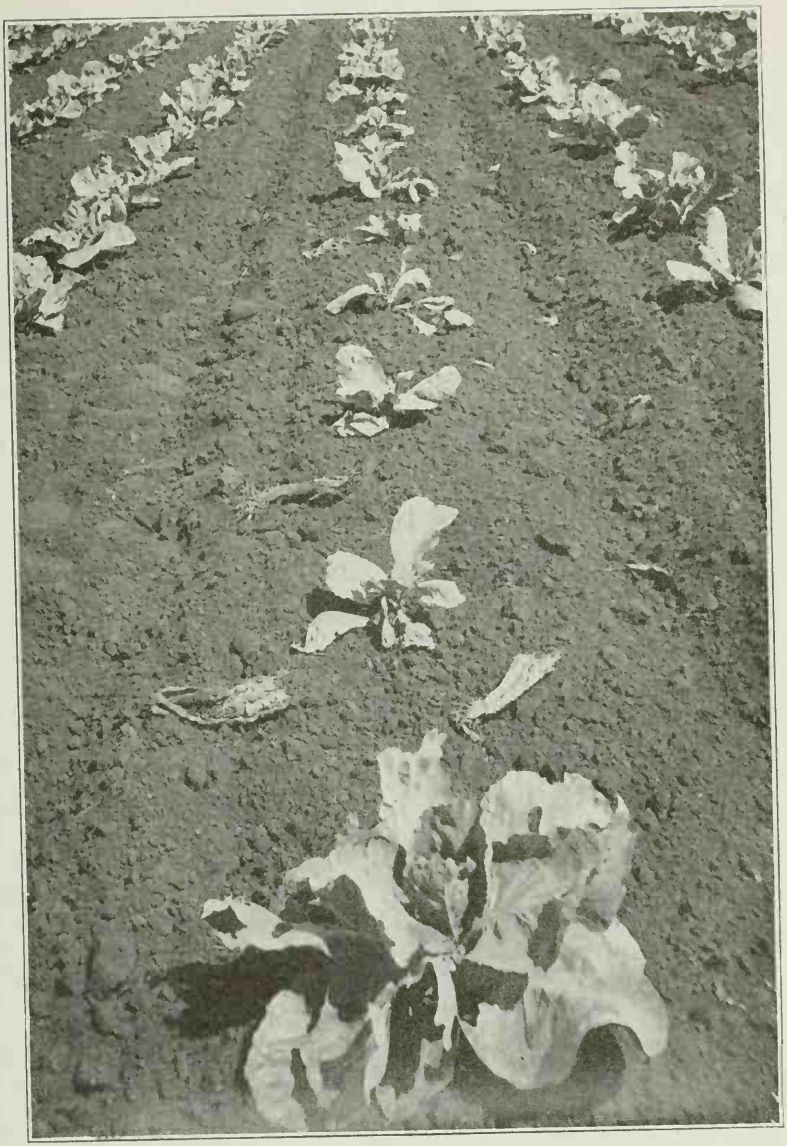

FIG. 2.—THE BEGINNING OF YELLOWS

This was the second successive crop of cabbage on new land. Note the old cabbage stumps on the ground. The plant in the foreground is healthy but the next eight in this row all showed "yellows". Note dwarfing and shedding of lower leaves. Probably a single plant in this place, possibly one of these stumps, was diseased the previous year. Spots like this, scattered throughout the field, mean the end of profitable cabbage culture on this land.

which finds its way into the plant at the leaf margins or through the vascular system of the plant and causes a black rot which starts with the veins. This seems to be carried on the seed according to Harding, Stewart, and Prucha (1904) and also on seedlings and is widespread in the state. Russell, as already noted, found it common and highly destructive in southeastern Wisconsin in 1895-1896, when it was the chief factor in the cabbage losses then prevalent in the Racine district. It has also caused similar severe losses elsewhere, but the degree of its destructiveness varies widely with climatic and other conditions. It is more likely to be confused with yellows than is any other malady. The yellows and black rot may be distinguished by field characters through the differences in both season and mode of attack. These will be pointed out following the discussion of yellows. The primary measure looking to the control of black rot as shown by Harding and his associates is seed disinfection, a practice quite inapplicable to yellows, as will be shown later.

Soft rot. Black rot kills the tissues but does not rot them rapidly. Their destruction is usually hastened and completed by another bacterial disease, soft rot, caused by a distinct organism, *Bacillus carotovorus* Jones. This may also follow other maladies or work independently, getting started through wounds, insect punctures, etc. It works rapidly, reducing stem and leaves to a soft vile-smelling mass. This occurs more or less in practically all cabbage fields and is often termed "stump rot" by the growers.

Club root. Club root is caused by the parasitic slime mold, *Plasmodiophora brassicae* Wor. It is an introduced pest, but is already widely scattered through Wisconsin, especially in the vicinity of Green Bay. It becomes evident by the stunting of the plants and their tendency to wilt, but is recognized with certainty by the enormously swollen and deformed roots. The use of lime is the specific remedy, a treatment which our later discussion will show has no value for yellows.

Black Leg. Black leg is caused by a fungous parasite, *Phoma lingam* (Tode) Desm. (*Phoma oleracea* Sacc.)[5]. It develops on the leaves, stems, and roots. It spots the leaves,

[5] M. P. Henderson, while working recently in this department, made a special study of black leg and the above statements represent his conclusions relative to the organism and its nomenclature.

but this is not a serious matter. The chief harm results when the stems and tap root are attacked near the ground or below, whereupon they blacken and die, hence the name "black leg." With the rotting of the stem, the leaves wilt and the plant slowly perishes. This disease occurs quite generally over the state, but has proved most destructive in La Crosse county. Seed disinfection and sanitation have proved important measures in its control.

Cabbage yellows. This is the disease with which the present publication is primarily concerned. It is widely scattered in Wisconsin, at least as far north as Green Bay and west to La Crosse. It is caused by a soil fungus, *Fusarium conglutinans* Wollenw. Cabbage yellows has been known to American phytopathologists for nearly two decades, but has not been recorded from any other country. It was first observed by E. F. Smith as occurring seriously in the eastern United States in 1895 although he did not publish his records until some years later (1899, a, b). Later Orton and Harter (1909, 1912) continued work upon it in the southeastern states and Manns (1911) published upon it from Ohio. Work was begun in Wisconsin in 1910. Comparative study of the organism and its description under the name *Fusarium conglutinans* was made by Wollenweber (1913)[6].

Fusarium attacks the roots under favorable conditions, either in the seed bed or within a short time after transplanting. The attacked plants are stunted and the foliage assumes a pale, lifeless yellow. Sometimes the plant is uniformly attacked, more often the symptoms appear earlier and continue worse on one side, and this one-sided check results in a lateral warping or curving of stem and leaves (Fig. 3). The trouble apparently begins, however, with the invasion of the fibrous roots, and from these it passes to the stem tissues showing first in the vascular bundles. These appear simply water soaked to begin with, but soon darken and finally become brownish-black in the later stages, while the cortical tissues overlying them gradually die and collapse. These invasions of root and stem result in diminution of water and food supplies from the soil and hence

[6] Since the genus Fusarium is a most complex one and Wollenweber's description is meager, it may be worth recording that in a letter, July 2, 1913, he states "I based the *Fusarium conglutinans* on your (Wisconsin) material because it gave me the first normal conidia and was identical with a strain from Dr. E. F. Smith and one from Mr. Harter."

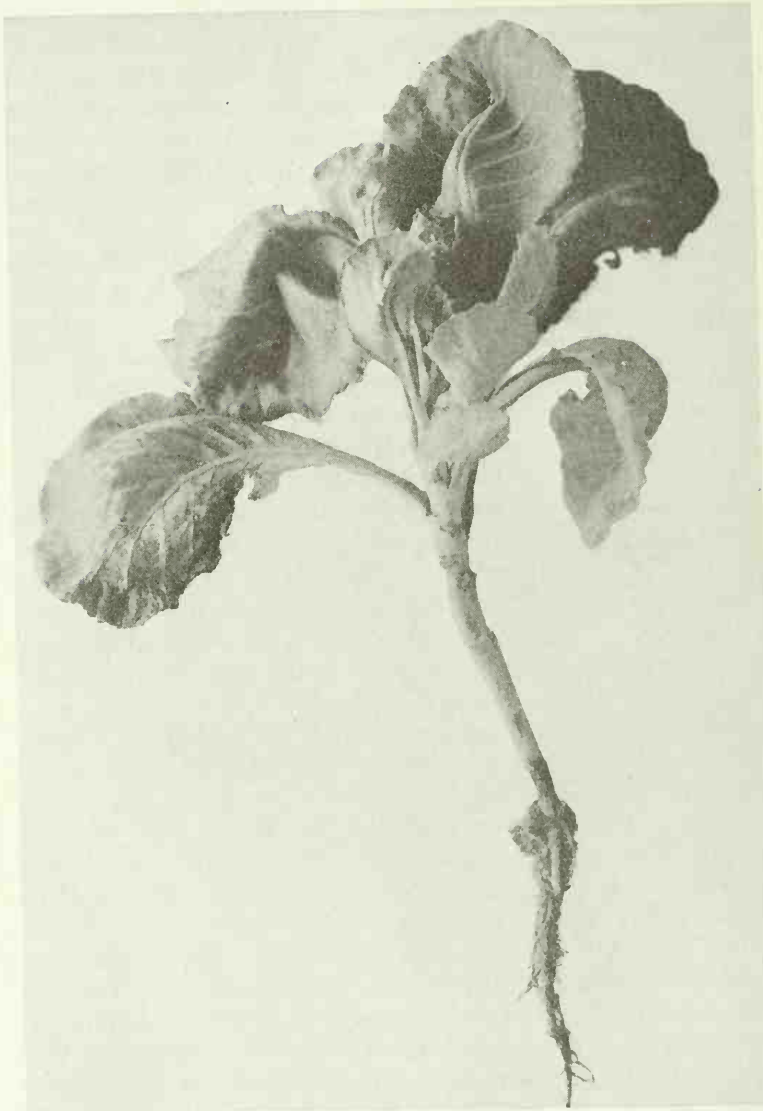

FIG. 3.—CABBAGE SEEDLING ATTACKED BY FUSARIUM

The early stage is characterized by general dwarfing and especially a one-sided check in the growth of leaves. In addition there is a general yellowing not shown, of course, in the photograph. The normal plant of this age should be twice as large.

the foliage symptoms noted above. Meanwhile, the fungus passes into the vascular system of the upper parts, stem and leaves. The invaded plants begin early to shed their lower leaves while making a weak attempt to continue growth above. Death may result in the worst cases within a week or so after transplanting. The majority of the diseased plants continue a sickly existence for a month or more, then succumb; a few less severely attacked, live through the summer, but rarely forms heads (Fig. 4).

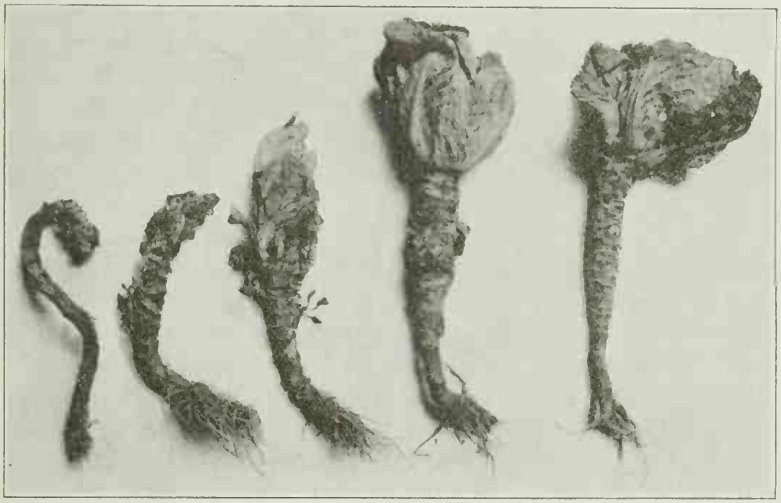

FIG. 4.—CABBAGE YELLOWS, LATER STAGES

Where the attack is not too severe or the plant is somewhat resistant, the plants may continue a sickly existence through the season. Such plants are yellowish and the lower leaves keep dying and falling. The attack is often worse on one side, warping or curling the stems.

YELLOWS DISTINGUISHED FROM BLACK ROT

As already noted, black rot is the only disease liable to confusion with yellows. The two may occur in the same field. More often, however, according to our observations and the testimony of experienced growers, they appear at their worst in separate seasons or situations. While this is in part a result of the accident of introduction, it is largely because the conditions favorable to one are repressive to the other.

The diseases differ in these respects as follows:

Season of attack. Both may appear in the seed bed, but the yellows more conspicuously. Following transplanting the yellows appears promptly, some evidence of the disease being seen within a week and it is at its height within three weeks, providing climatic conditions favor. Thereafter, there are few new infections. The black rot does not appear seriously until the plants begin to head up; thereafter it spreads rapidly, and continues its development into the autumn.

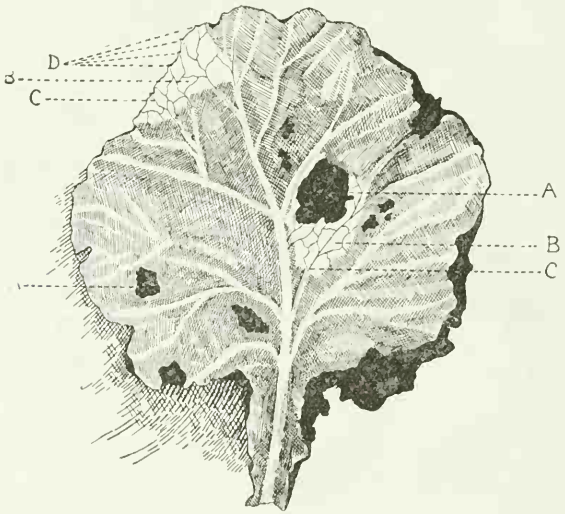

FIG. 5.—BLACK ROT STARTING ON THE LEAF

Diseased areas (B) unshaded except blackened mesh of veinlets. (A) Hole eaten by insects. Disease introduced at this point, and spreading backward to main rib. (C) Blackened veinlets affected by disease. (D) Water pores through which disease germs gain a foothold, producing marginal infection. (After Russell.)

Climatic conditions. The most serious attack of yellows is conditioned upon a period of dry, hot weather, following transplanting.[7] The development and rapid spread of black rot, on the other hand, require an abundant water supply with cooler conditions, especially at night.[8]

[7] The detailed studies which justify the above conclusions, including the consideration of *Fusarium conglutinans* in relation to host and to environment, will be the subject of a separate publication by J. C. Gilman. (Cabbage yellows and the relation of temperature to its occurrence) in the Annals of the Missouri Botanical Garden, Vol. 3. (In press.)

[8] Russell (1898) states with reference to loss from black rot in Wisconsin: "the season of '96 was extremely severe while the losses in '97 were relatively small. The reason for this is that climatic condiditions exert a profound effect . . . . . atmospheric influences that favor the formation of water beads have a direct influence . . . . . the disease spreads more rapidly in vigorous, rapidly developing plants than in those whose growth is retarded by insufficient supply of water." This is exactly the opposite of our observations with the Fusarium disease.

Symptoms and progress of the disease. The yellows first invades the roots and the base of the stem. It is often worse on one side, giving lop-sided plants, but, in any case, the entire plant is soon involved and off-color without local leaf-spotting. The black rot, in general, invades the plant through the leaves, causing a local leaf-spotting either along the margins or through insect wounds in the interior (Fig. 5). These spots show blackened veinlets, and from these the progress of the parasite, as indicated by the blackened veins, is backward through the mid-veins of the leaves into the stem. With the yellows, the discoloration of the vessels proceeds from the base of the stem upwards. In the advanced stages of yellows, the vascular system of the stem and of the mid-ribs of the leaves is much darkened, but it is rarely as black even in the last stages as the black rot is from the outset and in no case does it affect the finer veinlets of the leaves.

The organisms. In the case of yellows, the fungus parasite *Fusarium conglutinans* is prevalent in all discolored vascular elements and is easily secured in culture. Bacteria may be associated with it, especially the soft rot organism, *Bacillus carotovorus*; we have not, however, found the black rot organism is these tissues. In the case of black rot, the bacterial parasite, *Bacterium campestre*, is present and easily isolated, and we have not found the Fusarium associated with this.

CABBAGE YELLOWS IN WISCONSIN

Occurrence. As already stated, cabbage yellows has been observed widely scattered in the state, including all of the important cabbage-growing regions, e. g., Racine, Kenosha, Union Grove, Milwaukee, Oshkosh, Green Bay, Shiocton, and La Crosse. Its most serious developments are, however, confined to the southeastern section, notably Racine and Kenosha counties. Whether this distribution is to be attributed primarily to the more widespread introduction of the parasite here or to more favorable conditions for its development will remain in doubt until further observation. We are inclined to think that both factors are involved,

with the climatic factor favoring the more rapid spread in the southern region.[9]

Destructiveness. Where this Fusarium occurs and conditions favor, it is one of the most destructive parasites we have ever observed. In highly fertile soils otherwise specially suited to cabbage culture, the loss on even moderately infected fields frequently amounts to from 50 to 75 per cent of the crop and may be greater. This, be it understood, is on fields such as are shown in Figures 10 and 16 from which the grower supposed he would get a profitable yield. Most experienced growers have learned better than to plant cabbage on a thoroughly cabbage-sick soil, but if one is so foolhardy as to attempt it and the season favors the fungus, his loss will be practically total.

Introduction and spread. The general evidence indicates that cabbage yellows appeared destructively in the Racine-Kenosha district about 1900, although doubtless present in lesser degree earlier. Within a few years after its first outbreak cabbage-growing had to be abandoned on the older lands and the industry has been pushed westward across these counties. The disease has, however, followed persistently so that it is only a matter of brief time when it will invade the remotest cabbage fields of that section of the state.

No one knows where it originated. All the evidence indicates that it was introduced, not endemic. Inasmuch as Smith (1899) recorded that it was present and highly destructive in New York some years earlier and New York-grown cabbage seed was at that time being planted commonly in Wisconsin, it seems probable that it was introduced with such seed. Some growers hold positively to the idea that it appeared with a certain lot of seed about fifteen years ago, and cite circumstantial evidence.

While we may accept this evidence as to its introduction, it does not seem probable that it is now being brought in on the seed with any frequency. As a matter of fact, in five years of experimental work we have never seen any development of yellows in the seed bed attributable to seed infection. In a region where the disease is widely prevalent the chance of infection from other sources is far greater than from seed.

[9] See Gilman (1914) on the relation of temperature to the infection of cabbage by *Fusarium conglutinans*.

The cabbage seed used in Wisconsin is practically all grown in one or the other of three regions: (1) Europe, chiefly Holland, Denmark, and Germany; (2) New York, chiefly Long Island; (3) the Puget Sound region in the state of Washington. It is important, therefore, as bearing on this question of seed introduction, to know whether the Fusarium disease occurs in these regions.

As already stated, it is frequent in New York. On the other hand, all the evidence indicates that it is not known in Europe. There is no reference to it in European literature. In addition, we have been so fortunate during the progress of this work as to have had a personal visit to Wisconsin of the leading plant pathologist of each of the above nations, Dr. F. Kølpin Ravn of Copenhagen, Dr. Johanna Westerdijk of Amsterdam, and Dr. Otto Appel of Berlin. They all agree that the disease is not known to them in Europe. The senior author has made a personal inspection of portions of the Puget Sound cabbage seed-growing region and found no signs of the Fusarium disease there.

These lines of evidence are, moreover, the more convincing because they accord with our observations on the relation of climatic conditions to the development of yellows. New York conditions are like those of southern Wisconsin, favorable to the Fusarium, but the continued cool summers of the Puget Sound region and of northern Europe preclude the possibility of the serious development of the fungus even if it were introduced.

Field surveys, with the cumulative experience of cabbage-growers, afford abundant circumstantial evidence that, once introduced, the organism is quickly carried from field to field in a variety of ways. These include the following. (1) By diseased plants from infected seed beds, a common way. (2) By water. Much of the cabbage land is lowlying and flat so is subject to flooding by water which has drained from infected fields.[10] (3) By wind blowing the dust from field to field (Fig. 6). (4) By vehicles and animals. Various cases have been noted where the disease appeared

[10] On the Chandler farm at Corliss, Wisconsin, this mode of dissemination was clearly illustrated in 1912. Here the disease was much more severe along a drainage ditch than in any other part of the field. The ditch drained a field three-fourths of a mile distant which was badly infected the previous year.

The same year, W. E. Thompson, Kenosha, found a similar condition occurring on his farm. Here he found yellows on soil that had never been planted to cabbage before, but the disease was found only along a shallow ditch which served to drain land that was in cabbage in 1908 and was diseased at that time.

first along roadways, especially where these crossed fields. There is no reason, however, to hold insects responsible in any serious degree for the distribution of the Fusarium.

Persistence. Once introduced into the soil, it is most persistent. It was formerly common practice to grow cabbage three or four years in succession in the same field, applying stable manure each year to maintain the fertility. If this is done now in districts where the disease occurs, an occasional head may show yellows the first year, many diseased areas

FIG. 6.—YELLOWS ON NEW LAND

The causal organism was probably introduced with dust, blown or otherwise carried, from the field in the background at the right which was badly diseased old cabbage soil.

will appear in the field the second year (Fig. 2), and, if the grower is so injudicious as to plant cabbage on such a field a third year, the disease will be universal, and he may lose practically all of his crop. Moreover, once established in the soil in this way, evidence, to be cited later, shows that it may persist indefinitely (Fig. 7), a fact of the greatest importance in determining control measures.

CONTROL MEASURES PREVIOUSLY RECOMMENDED FOR YELLOWS

The seriousness of this disease and the need of more attention to control measures has been emphasized in turn by each pathologist who has considered it. Smith (1899 b) pointed out with great clearness, about fifteen years ago, the danger of such infestation of agricultural soils with a persistent parasite, and the difficulty of combating it when once established. Some helpful suggestions have been made, based upon observations and general knowledge of the nature of

FIG. 7.—THE CABBAGE FUSARIUM PERSISTS LONG IN THE SOIL

This shows a field at Berryville, on which cabbage was so "sick" in 1900 that it was seeded down and lay in grass until 1914. It was then again planted with cabbage with the above results, the loss being practically all due to Fusarium. Evidently rotation offers little hope as a remedy for yellows.

the disease, especially by Manns (1911). Harter (1912), the last to publish on this subject, summarizes possible control measures as follows: (1) Disinfect seed; (2) make seed bed on disease-free soil, or sterilize the soil of the seed bed; (3) avoid contaminated manure; (4) reject diseased plants when transplanting; (5) avoid the use of contaminated surface water when setting transplants; (6) pull and destroy diseased plants; (7) do not allow stock to pass from diseased

to healthy fields; (8) practice crop rotation covering a period of from 4 to 8 years. Manns (1911), in addition to suggestions which covered most of these points, directed attention to the possibility of remedying the trouble by more care in the source of seed, recommended the use of home-grown seed, and expressed "much hope . . . . . of the possibility of securing resistant strains." None of these writers was able, however, to follow up his suggestions with critical experimental trials. In this respect we have had more favorable opportunity.

TRIALS OF CERTAIN CONTROL MEASURES IN WISCONSIN

Beginning in 1910, we have for five seasons been working on control measures. The problem that arose as soon as the nature of the disease was understood was as to whether cabbage culture must be permanently abandoned on this Fusarium-infected, cabbage-sick soil, or whether this crop can be reinstated on a reliable basis. The incentive is unusually strong for attempting such restoration, since in these regions the soil and market conditions are peculiarly favorable for cabbage and farmers prefer this to any other cash crop. All possible control measures were, therefore, given consideration and the results are recorded in the following pages.

SEED AND SEED BED

Seed disinfection. The evidence already discussed justifies the conclusion that the Fusarium spores may be carried with dust on the surface of the seed. Seed disinfection seems, therefore, a logical initial control measure. Unfortunately, in the northeastern United States the Fusarium is already so widely distributed and so rapidly spread locally by other agencies that seed disinfection cannot be relied upon to help much in its control.[11]

[11] While warning against reliance upon seed disinfection as a chief safeguard from Fusarium, especially in the older cabbage centers, we wish at the same time to emphasize our belief in its importance as a general preventive measure against cabbage diseases, especially black rot and black leg. We, therefore, recommend the disinfection of cabbage seed as follows: soak the seed for 20 minutes in a solution of 1 part of standard formaldehyde (40 per cent solution) in 250 parts of water (1 oz. in 2 gals.), then wash in water, and dry. This practice was first recommended by Harding, Stewart, and Prucha (1904) for black rot control and is in general accord with the advice of later writers, Manns (1911) and Harter (1912). Its merits were recently proved by the results of trials by M. P. Henderson in the investigations of black leg already referred to.

Selection of seed bed. The importance of the selection of new soil each year for the seed bed deserves to be emphasized most strongly. Examples have frequently come under our observation of even experienced cabbage-growers continuing their seed bed on the same soil year after year. The inevitable result is that one or more of the cabbage parasites is soon established. This applies especially to black leg, club root, and yellows. The arguments that follow for rotation in the field apply even more emphatically to the seed bed. The safe rule is to make the seed bed each year on soil which has never grown cabbage before. Here again, however, we stop short of the final solution of the problem since sound plants from a healthy seed bed contract the disease promptly when transplanted to an infected field. The control of the disease after transplanting is the final essential to success and it is to this that most attention has been given in these studies.

CROP ROTATION

Since the Fusarium is a soil parasite, the possibilities of crop rotation naturally received early consideration. At the outset a careful canvas was made among experienced cabbage-growers working under a variety of soil conditions with reference to their experience upon this point. The results showed with practical unanimity that when once a soil becomes thoroughly infested, i. e., cabbage sick, no reasonable rotation will eliminate the fungus. These conclusions have been further confirmed in connection with our experiments.

We secured abundant evidence from farmers that the disease will appear seriously upon such sick soil which has been used for crops other than cabbage for from three to eight years. For example, Benjamin Bones, an expert truck-grower who had the longest experience in cabbage-growing of any man in the Racine district, stated that after his soil was once infested rotation did not as a rule rid it of the fungus. In one case, he seeded cabbage-sick soil to clover and timothy, let it lie thus for five years, broke it up, replanted to cabbage, and did not get a sound head from the entire five acres; indeed, he not only sold none, but was obliged to get cabbage from his neighbors for the use of his own family. At this time, however, Mr. Bones did not de-

termine whether the disease was black rot or yellows. From his description, and from subsequent observations on his farm, we believe it was yellows.

In 1910, in order further to test out this matter, we planted experimentally on this same farm a piece of cabbage-sick land on which no cabbage had been grown for at least six years. During this time it had been in grass for three years. The outcome was that more than one-half of the stand died of yellows and there was considerable disease in the balance of the crop.

In only one case in Mr. Bones' experience had he succeeded through long rotation. This was where cabbage-sick soil was put into strawberries for two years, then seeded to grass for five years. At the end of the seven years he broke it up, decided to again try cabbage and "to his surprise" got a good crop with no serious amount of yellows. The fact that there was a little of the disease in this case, however, shows that only one crop of cabbage could be secured even then, following which a return must be made to other crops for another long period.

A. L. Curtis, another Racine grower, said that when forced by cabbage-sickness to discontinue cabbage culture on a piece of land, about twelve years previously, he raised good truck crops of other kinds, onions, beets, and potatoes, but cabbages tested twice during the twelve years had shown the disease in the soil without evident abatement.

In still another case where cabbage failed from disease, the field was seeded down for fourteen years, then plowed, in 1914, and planted to cabbage. The result was a large percentage of loss from yellows (Fig. 7).

While none of these cases carries the conviction of experimental evidence, as there is always the possibility of reinfection of such soil in the interim, nevertheless the cumulative evidence has convinced us that the fungus persists indefinitely in the soil. Smith (1899 a) reports that the disease reappeared when cabbages were planted on soil from a sick field which had been kept in dry storage for over three years.

While, therefore, we were forced to abandon the idea of crop rotation of any reasonable length as insuring complete recovery of the cabbage-sick soils, we were convinced that when beginning with healthy soil in a region liable to yellows, attention to proper rotation will prolong the usefulness of

the soil for cabbage culture. As already noted, it is not uncommon in cabbage-growing sections to find cases where cabbage is grown continuously on a field for from five to ten years and even longer. While this may sometimes be done with success, disaster is almost inevitable as soon as the Fusarium or, indeed, any other soil parasite is introduced. The more rational method for long-time practice is to grow cabbage not oftener than once in three years in rotation with other crops.

FERTILIZATION

While little evidence favorable to crop rotation as a specific remedy for cabbage-sick soil was received from the growers frequent testimony favored one or another mode of fertilization. This seems natural, since it is known that the cabbage is one of the rankest feeders among cultivated crops, profiting from enormous applications of stable manures and responding well on some soils to commercial fertilizers, especially to potash salts. The use of lime, gypsum, ashes, and common salt was urged as helpful by one or anotheranother grower and one man testified to the benefits of a complete commercial fertilizer. Harter (1909) has shown that cabbage is sensitive to malnutrition following improper fertilization. It seemed reasonable to suppose that even with the liberal use of stable manures, an unbalanced condition of soil fertility might obtain following prolonged cabbage culture which might in turn predispose toward disease. There was also the specific suggestion from the work of Hart and Peterson (1911) that there might be a deficiency in sulfur. Cabbage tissue is especially rich in this element and they show that an average crop of cabbage will remove about 100 pounds of sulfur trioxide from the soil. Such considerations led us to plan a comprehensive series of fertilizer trials designed to cover fully the possibilities suggested.

FERTILIZER TRIALS, 1910[12]

A careful survey of the older cabbage-growing section where the yellow disease prevails was made in the spring of 1910 and areas of cabbage-sick land were selected on four farms for the fertilizer trials.

[12] Before this work was undertaken, A. J. Rogers, of the horticultural department, had become interested in the question and he cooperated in these trials the first year.

Field I. On the farm of Witcheber Brothers, Kenosha. Soil—black alluvial loam, tile drained, received 15 tons stable manure per acre previous autumn. Cabbages on land the year before showed some disease. Applied fertilizer on one-fourth acre plots at the following rates, with controls on either side untreated. Applications of fertilizer made on surface of cultivated field May 27, field subsequently disked, and cabbage planted June 25.

Plot 1. Potassium chloride	300 pounds per acre
Plot 2. Acidulated bone meal	450 pounds per acre
Plot 3. Potassium sulfate	150 pounds per acre
Plot 4. " "	300 pounds per acre
Plot 5. Calcium sulfate	500 pounds per acre

Field II. On farm of Matt. Broesch, Kenosha. Soil—strong clay loam, tile drained in high state of fertility. Recent history of field as follows: For preceding 15 years cabbage on field about every second year, alternating with either corn or potatoes; during first few years cabbages healthy and yields large; since about 1900 disease troublesome; some eight years before disease (yellows?) so severe that every head was lost on ten acres; last cabbage grown on here two years before (1908), badly diseased, loss 75 per cent; last year (1909) potatoes. Light applications of stable manure nearly every year; about 15 loads per acre in 1908; none in 1909; about 15 loads per acre again in winter 1909-1910 and plowed under in early spring.

For our experiments fertilizers at the following rates were applied on the surface and disked in on May 27; cabbages planted June 25.

Plots 2, 6, 8, control, no fertilizer added, balance treated as follows:

Plot 1. Potassium chloride	250 pounds per acre
Plot 3. Potassium sulfate	250 pounds per acre
Plot 4. Acidulated bone meal	250 pounds per acre
Plot 5. Potassium sulfate 250 pounds and bone meal 250 pounds per acre	
Plot 7. Air-slaked lime	120 bushels per acre
Plot 9. Calcium sulfate (gypsum)	600 pounds per acre

Field III. On farm of A. L. Curtis, Berryville. Light sandy soil, good surface drainage; no stable manure for 5 years previously; 125 pounds per acre of commercial fertilizer[13] with sugar beet crop, 1909.

[13] Mr. Curtis states that he used Homestead Complete Fertilizer M. & I. Special carrying fertilizing elements in the proportions N. 3; P. 8; K. 6.

In this experiment, the soil was fitted and fertilizer applied at the following rates, May 27; cabbage planted June 18.

Plot 1. Potassium chloride	250 pounds per acre
Plot 2. Potassium sulfate	250 pounds per acre
Plot 3. Potassium sulfate 250 pounds and bone meal 250 pounds per acre	
Plot 4. Control (no fertilizer)	
Plot 5. Acidulated bone meal	250 pounds per acre
Plot 6. Calcium sulfate (gypsum)	600 pounds per acre
Plot 7. Air-slaked lime	120 bushels per acre

Field IV. On farm of Benjamin Bones. Strong clay loam, surface drainage, fair state of fertility, cabbage last grown on it eight years before.

Experimental application of fertilizer at following rates made about June 1; cabbage planted June 20.

Plot 1. Potassium sulfate	400 pounds per acre
Plot 2. Potassium chloride	400 pounds per acre
Plot 3. Control, no fertilizer or manure	
Plot 4. Stable manure, liberal application	

Outcome of fertilizer experiments of 1910. The season was favorable for the disease and the yellows began to show in all of these fields, without evident relation to fertilization, within two weeks after transplanting. On July 30, when careful notes and counts were made, the conditions were as follows: I. Witcheber field: 10 per cent of plants dead and 10 per cent of balance diseased; no evidence of difference attributable to fertilizer. II. Broesch field: over 90 per cent of plants dead and no evidence of difference in favor of any fertilizer. III. Curtis field, the same. IV. Bones field not visited at this date.

In October, 1910, when the final notes were taken upon these plots, there was absolutely no evidence of gain from any type of fertilizer in any field. In the Witcheber field, about one-third of the crop was dead or diseased, the disease appearing worst in irregular areas or spots which stood in no relation whatever to the fertilization. In the Broesch field of one-half acre, only about twelve plants headed up and these were scattered irregularly over the field. Most of these showed symptoms of yellows. In the Curtis field, only one plant headed in the entire field. In the Bones field, the loss was over 50 per cent from yellows, scattered over the field regardless of fertilization lines. No encouragement

could be found in these results for the hope that fertilization would control the disease.

FERTILIZER EXPERIMENTS OF 1911

Plan. Although the outcome of the fertilizer experiments of 1910 was purely negative and seemed to show that no method of fertilization under trial had any effect upon the disease, it seemed wise to make similar trials in 1911 upon one field, using heavier applications. Since the Broesch land had proved most seriously diseased and was by uniformity and other conditions admirably suited for experimental work, it was decided to repeat the same applications on the identical plots. This was done May 5, 1910, except that the gypsum on plot 9 was added at the rate of 1200 pounds per acre, twice as much as in 1910. The fertilizers were harrowed in and the ground lay until planting time. On June 20, before planting, the field was disked and on one-half of each fertilized plot, Nos. 1, 3, 4, and 6, the original application was repeated. The result was that in the case of each commercial fertilizer under trial there was the single application made in 1910, repeated in 1911 on one-half of the same plot and doubled on the other half. With the slower-acting gypsum and lime it was considered that the single heavy application each year would do all of which these chemicals are capable. The soil was uniform and owing to the general occurrence of the disease in the crop of the preceding year, it was known to be uniformly infected unless the chemicals were exerting an influence.

Results of fertilizer trials, 1911. The cabbages were planted June 20. A period of dry, hot weather such as most favors the disease, followed, thus giving ideal conditions for a severe, but fair, test of the efficacy of the chemicals.

On July 8, examination showed yellows abundant over the entire field with no evidence whatever of any difference either in the diseased condition of the plants or in their general vigor except that in the portion of the potassium chloride plot which had received a double application the plants were very slightly larger. It was doubtful, however, if there was really less disease even here. There seemed, however, to be a little less disease on the limed plant. (Fig. 8 shows the appearance of the field on July 8.) During

the next week the disease worked rapidly on all plots alike and by July 16, 90 per cent of the plants were dead, or practically so, and most of the remainder were diseased. The slight advantage which the potassium chloride plots showed at first was lost, showing that although it gave a little stimulus to growth it had no appreciable influence upon the disease resistance of the plants. The same was true of the lime plot.

FIG. 8.—EXPERIMENTAL FIELD, 1911, BROESCH FARM

This soil was thoroughly "cabbage sick" through Fusarium infestation. Practically every plant in it was attacked by yellows in spite of applications on different sections of lime, gypsum, various commercial fertilizers, sulfur, and soil disinfectants as described in the text (see summary Table 3). That this soil is fertile and in fine tilth was shown by the fine stand of potatoes at the left and of corn and oats at the right. A photograph of the latter crops is shown in Figure 9. Figure 1 shows a neighboring healthy field of the same day.

At the end of the season, in October, the outcome was fully convincing. Only 8 plants were alive on the entire series of plots where over 3,000 were started, and only one of these formed a head. This was upon the potassium sulfate plot, but its location was evidently purely accidental, its survival being attributable to its individual disease-resistant qualities rather than to the fertilizer.

POSSIBLE INFLUENCE OF SULFUR

As already explained, especial consideration was given to the idea that sulfur-depletion of the soil might be a factor in

predisposing the crop to yellows. It would seem that the sulfur compounds used in the preceding trials, potassium and calcium sulfates, should have had a beneficial effect if sulfur were lacking. No such benefits followed their use, however. The possible influence of sulfur was further tested by applications, on two fields, of flowers of sulfur, 500 pounds per acre. This was made in connection with the trials of soil disinfectants, to be discussed later; but this again was absolutely without effect.

SOIL ANALYSES

Further evidence bearing upon the relation of fertilization to the disease was furnished by chemical analyses of the soil from two of these experimental fields, made by the department of agricultural chemistry.[14]

For this purpose, in 1910, samples of the soil were collected from the middle of the cabbage-sick fields on the farms of A. L. Curtis, Berryville, and M. Broesch, Kenosha. These were the fields selected for the 1910 fertilizer trials already described and of which the history has been given. We also secured similar samples of virgin soil from the permanent fence rows near by, which the owners in each case said had never been cultivated or cropped. In securing these, the first two inches were rejected and the underlying soil taken to a depth approximating six inches. As before explained the cultivated soil in both fields had received liberal applications of stable manure in previous years and on the Curtis field commercial fertilizer had been used each of the last three years. The percentages of sulfur trioxide found were as follows:

Curtis field, virgin soil 0.108 per cent, cultivated soil 0.115 per cent.

Broesch field, virgin soil 0.119 per cent, cultivated soil 0.140 per cent.

This chemical analysis justifies the conclusion that the sulfur content of these soils "was maintained and even slightly increased" by the liberal application of farm manures and fertilizers. Sulfur depletion could not, therefore, be the cause of the cabbage sickness.

[14] We are indebted to E. B. Hart of the department of agricultural chemistry for advice in the collection of these samples as well as for the supervision of the soil analysis recorded above. These results are quoted from the publication already referred to, Wis. Exp. Sta. Res. Bul. 14, p 13.

CONCLUSIONS SUMMARIZED

These results seemed fully conclusive. The trials were made upon four representative types of cabbage-sick soil; they extended over two seasons, involving in some cases repeated applications, and included the following chemicals: potassium chloride, potassium sulfate, phosphates from both rock and acidulated bone, calcium sulfate (gypsum), calcium hydrate (lime), and sulfur. The yellows disease was abundant on all the fields both seasons, and its occurrence

FIG. 9.—CABBAGE SICK SOIL IS NOT LACKING IN FERTILITY

Excellent corn and oats grew on the land bordering the experimental field of 1911. The picture was taken July 8. Note that the cabbage plants are all dying from yellows. (Compare Figure 8.)

was not influenced in any way by any fertilizer. Moreover, chemical analyses of sick and neighboring healthy soils showed no evidence of the depletion of the former in sulfur. The conclusion seems clear that the trouble was simply due to the occurrence of the parasitic Fusarium in the sick soils and that the application of fertilizers did not influence the inter-relation between the host plant and the fungus parasite.

SOIL DISINFECTION

In view of these facts the question next pertinent relates to soil disinfection. One who has had experience with soil inhabiting parasites knows how difficult it is to dislodge them by any mode of soil treatment which is practicable under field conditions. Nevertheless, from the outset of these investigations efforts were made to do this. The nature of this work and the conclusions reached will be briefly summarized.

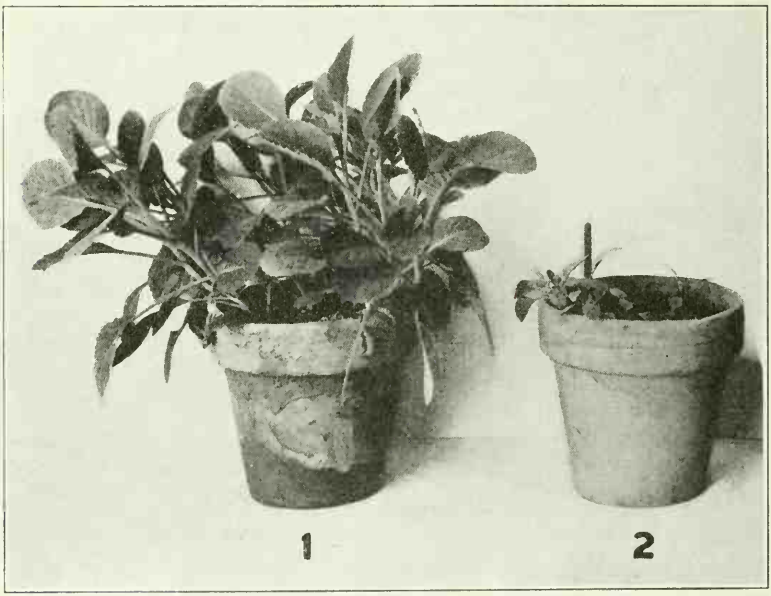

FIG. 10.—STEAM STERILIZATION PREVENTS YELLOWS

The soil in pot 2 was as taken from an infected cabbage field. Cabbage seed was planted in it, under greenhouse conditions, and all the seedlings promptly developed Fusarium yellows and most of them died. Pot 1 contained like soil but was autoclaved 1½ hours at 11 pounds pressure.

Steam sterilization. F. D. Bailey (1912) while working in our laboratories, made trial of steam sterilization of cabbage-sick soil and found that it was efficacious in preventing yellows. This method has been employed frequently in our greenhouse trials, (see Fig. 10). Here would be a sure remedy if practicable. It is not feasible, however, to practice steam sterilization of soils for cabbage culture in Wisconsin even for the seed beds. It might seem at first that

the methods of steaming seed beds recently shown by Johnson (1914) to result so advantageously for tobacco culture in Wisconsin would be applicable to cabbage. There are two difficulties in the way of this, either of which would be fatal in practice. (1) Wisconsin cabbage-growers plant their seed beds in rows wide enough apart to permit clean culture of the seedlings in order to get the strong plants desired for machine setting. This requires seed fields altogether too large for steam-sterilization. (2) The chief problems in fighting the yellows are not concerned with seed bed conditions. It is true that a contaminated seed bed is very serious where it occurs, but we have found the best growers so alert to this fact that upon realizing the danger they have generally been able to avoid it by making the cabbage seed bed on uncontaminated soil, a practice at once simple and effective.

If, therefore, soil treatment is to contribute to the control of cabbage yellows it must be applicable to one or the other of two things.

(1) To disinfecting the soil in the immediate vicinity of an infected plant. If this could be done inexpensively one might at least lessen the rate of spread of soil infection in the early stages of the disease by pulling the few "yellowed" plants as soon as seen and applying the soil disinfectant.

(2) In checking the invasion by the Fusarium of the healthy seedling following its transplantation into infected soil. The evidence, discussed elsewhere, leads us to conclude that this invasion occurs soon after transplanting and is dependent upon certain soil conditions favorable to the fungus. It is conceivable that the presence at this stage of some fungicidal element in the soil, especially if close to the cabbage roots, might so inhibit the fungus as to prevent infection.

With these possibilities in mind, trial was made of certain fungicidal compounds, first in the greenhouse with the cooperation of F. D. Bailey, and later in the field. These involved the use of formaldehyde solution, potassium sulfide solution, flowers of sulfur, and a proprietary soil fungicide supplied by the Sherwin-Williams Company. Without going over the experimental details, the results may be summarized as follows:

Formaldehyde. Formaldehyde was applied to cabbage-sick soil in greenhouse flats 4 inches deep, until the soil was thoroughly wetted. One per cent and 2 per cent solutions were tried. The flats were covered for twenty-four hours, then left uncovered to air out for three days, and planted to cabbage seed. The effect of the formalin was to retard slightly the germination of the cabbage and to reduce somewhat the percentage of disease in the early stages of its development. Nevertheless, considerable disease developed later, showing that even this drastic treatment was not fully effective.

Although these results gave little encouragement, trial was also made in the field as follows: Upon setting healthy cabbage plants in a soil known to contain the Fusarium, one-half-pint of formaldehyde solution was poured into the hole with each plant. In one row a 1 per cent solution was used, in another a $\frac{1}{2}$ per cent solution. Control rows were planted with water.

The result was that the treated plants either failed to start, evidently because of root injury by the chemical, or were less vigorous than the untreated plants. Within three weeks the surviving plants in all rows, both treated and untreated were attacked by yellows. At the end of the season, out of 150 plants set with formalin solution, only 6 were alive and none formed heads, whereas the corresponding two control rows averaged 12 alive with 1 headed in one row and 2 in the other. (See details in the table at the close of this chapter). It is evident, therefore, that formaldehyde solution is ineffective in checking the Fusarium, whether applied to the entire soil in amount sufficient to inhibit germination of the seed, or about the roots when transplanting, in amount sufficient to be decidedly injurious to the cabbage plants.

Potassium sulfide. Much the same methods were used in testing the effect of potassium sulfide. In the field trials with one row, each plant received one-half pint of 2 per cent potassium sulfide about the roots in the planting hole, and another row received a like amount of 1 per cent solution. The injury to the plants was even greater than from the corresponding formalin treatments so that most of the plants failed to establish themselves and renew growth. Those which

did, however, were all killed by yellows, showing that there is no hope from this treatment.

Sulfur. As already stated there was some hope that sulfur might have value as a fertilizing element with the cabbage crop. It is also understood that sulfur has value as a soil fungicide, having been recommended by various investigators in America and Europe as a preventive of potato scab[15] and other soil inhabiting diseases. A heavy application, at the rate of 500 pounds to the acre, was, therefore, made on cabbage-sick soil in each of two experimental fields in 1911. This was applied the day before planting. The soil, which was in fine tilth, was first dragged. The sulfur was then broadcasted, and gone over with a plank which covered it well into the surface layer of soil. Within three weeks yellows was showing up generally on both fields with no appreciable difference where sulfur was used. By the end of the first month every plant, control and sulfured alike, on one field was either dead or badly diseased with yellows. On the other field, the disease was abundant throughout, with no difference as to treatment. At this time the sulfur was not only visible when the surface soil was stirred but one could smell it when in the field. At the end of the season very few plants were alive on either field, and there was no appreciable difference attributable to sulfur treatment. In one field, not one plant out of the 400 planted on the sulfur plot lived through the season. In the other, as shown in the later tabular summary, of the 300 planted, only 10 plants lived and none headed, whereas in the control alongside, 14 lived. Clearly, therefore, sulfur had no retarding effect upon the development of the Fusarium.

Soil fungicide. The Sherwin-Williams Company sent a soil fungicide for trial in connection with these experiments. They did not state the composition, but it was a white, finely granular substance having the appearance and odor of naphthalene. They advised its use at 200 or 300 pounds per acre. Knowing that we were dealing with a resistant soil fungus and wishing to be sure, that if this fungicide had any efficacy we might learn it, we applied about twice the

[15] Halsted (1897) recommended sulphur as the best soil treatment tested for potato scab. Bernhard (1910) states that 350 pounds of flowers of sulfur per acre was successful against potato scab.

amount recommended—500 pounds per acre. It was used on a plot next the one treated with flowers of sulfur, applied broadcast at the same time and in the same way, on the two cabbage-sick fields. The results were again entirely negative. The application, even at this strength, did not seem to harm the plants in any way. When the yellows appeared, however, it was equally severe on treated and control plots. In the plot treated to the soil fungicide, on one field where 400 plants were set not a plant survived the summer, all being killed by the yellows. On the other the disease was not quite so bad, but the results, as shown in the following tabular summary, were no more favorable for the soil fungicide. The untreated controls on either side averaged practically the same results as did the treated plot. It was evident, therefore, that this "soil fungicide" like the other chemicals tested, was without any influence whatever upon the cabbage Fusarium.

In order to place the essential results of the work with these fungicides in brief form for comparison, the following tabular summary is given of the yields on the less diseased of the two fields. As already stated the destruction on the other field was practically complete on all plots.

TABLE I.—RESULTS FROM FUNGICIDAL SOIL TREATMENTS

Plot	Treatment	No. of cabbage plants set out	Condition at end of season			
			Living		Headed	
			No.	Per cent	No.	Per cent
Applied broadcast						
A	Control	300	14	5	0	0
B	Sulfur	300	10	3	0	0
C	Control	300	14	5	2	0.7
D	Soil fungicide	300	21	7	2	0.7
E	Control	1500	103	7	12	0.8
Applied in planting hole						
F	Control	150	22	15	1	0.7
G	Formaldehyde	150	6	4	0	0
H	Control	150	13	9	2	1.3
I	Potassium sulfide	150	4	3	0	0
J	Control	150	4	3	1	0.7

Conclusion. The conclusion seemed clear, therefore, that these experiments furnish no basis for hope that any

fungicidal soil treatment will help even in a minor way in the control of this disease.

THE POSSIBILITIES OF CONTROL THROUGH DISEASE RESISTANCE

As soon as the disease was well developed in 1910, the first year it was under observation, it was noted that individual plants, even in the most severely infested fields, remained comparatively unharmed by the disease. The possible importance of this was at once apparent since it has been shown by Orton (1900–1909) and Bolley (1901, 1903) that certain Fusarium diseases of other plants can be controlled by the use of disease resistant strains or varieties.[16]

With the hope of similar results with cabbage[17], plans were developed to work upon this idea along two different lines involving (1) the comparison of commercial varieties as to relative resistance, and (2) the development of disease-resistant strains by selection. Although the work on these has gone on simultaneously, it will conduce to clearness to discuss the results of the first two years separately.

THE COMPARISON OF COMMERCIAL VARIETIES AS TO RELATIVE RESISTANCE

In the southeastern Wisconsin district where the yellows occurs only two types of cabbage are grown at all extensively.

[16] It is a matter of common experience that closely related varieties of cultivated plants often differ widely in their relative susceptibility or resistance to some fungus attack. Thus, the Fameuse apple is especially susceptible to apple scab (*Venturia inaequalis*), the Russets are resistant; the Transcendent crab and Yellow Transparent are highly susceptible to fire blight (*B. amylovorus*), the McIntosh relatively resistant. The Wealthy apple may be ruined by the rust (*Gymnosporangium juniperi-virginianae*), while the Oldenburg standing beside it is unharmed. It is only comparatively recently, however, that the importance of disease resistance as a factor in the control of plant diseases has received full recognition. Considerable progress has been made in Europe and America in securing potato varieties resistant to late blight and wheat varieties resistant to rust. The most encouraging results to date have, however, been secured with the Fusarium diseases where disease resistance seems to be the hopeful method of attack.

[17] With reference to the possibilities of disease resistance in cabbage, the following reports have come to our attention.

Edwards (1907 and 1908) reported that trials in Ontario had shown the variety Houser to be especially resistant to black rot.

Dr. F. Kolpin Ravn stated in correspondence (1912) that he has perfected a strain of turnip resistant to club root. Dr. Ravn also sent us a sample of this seed, but we have not had a favorable opportunity to test its resistance to this disease.

Manns (1911) in his publication from the Ohio Experiment Station expressed hope in the possibility of securing disease-resistant strains; and E. G. Arbzerger, writing us from the same station in 1911, stated that he had observed the variety All Season to show especial resistance to yellows in Ohio. He kindly sent us samples of the commercial seed from Ohio which were used in our 1911 trials

Close and White (1909) reported from their trials at the Maryland Experiment Station that there was a difference in the susceptibility of cabbage varieties to black rot, and in 1913 Professor White sent us a sample of seed selected for resistance to this malady which was included in our trials of 1914 and proved highly resistant to yellows.

These, as already explained, are the winter or storage cabbage and the kraut cabbage. Of each of these types more than one variety or race is used. Over 90 per cent of the acreage is given to winter cabbage and less than 10 per cent to the earlier or kraut types. While both types suffer badly from yellows, the winter cabbages generally suffer more severely, largely because they are planted later and so are likely to pass through a period of trying weather immediately thereafter. Inquiry among experienced growers showed that in practice no reliance was placed upon any variety or strain then in use as peculiarly resistant to disease. The belief was expressed by some, however, that the increasing loss of winter cabbage from disease in recent years over former years was because the seed formerly secured from Europe was of a hardier type.

Accordingly, the cooperation was secured of Dr. F. Kølpin Ravn, Plant Pathologist of Denmark, in arranging for the selection and importation of the most promising Danish-grown strains. Along with these, trial was made in 1911 of the two standard commercial strains of Hollander or Danish Ball Head in general use in this district and also of the summer or kraut varieties of most promise.

TRIALS OF 1911

Ferry's strain of Hollander	
Hansche's strain of Hollander, grown in Puget Sound	
Imported Danish No. I	(Amager long stem)
Imported Danish No. II	(Amager long stem)
Imported Danish No. III	(Amager short stem)
Imported Danish No. IV	(Amager short stem)
Imported Danish No. V	(Amager short stem)
Houser	
All Season	

Trials of these varieties and strains were made in two fields, both having soil thoroughly cabbage sick with Fusarium. The climatic conditions favored a serious attack of the disease, making the trial a severe one. The results follow.

Field A.—Hansche farm. Within three weeks after transplanting, the disease was prevalent throughout the experimental field so that it was estimated that from 75 to 90 per cent of the plants were either diseased with yellows or already dead. At this time the Puget Sound strain was showing up better than any of the imported strains yet it was

estimated that 75 per cent of these plants were diseased and many were already dead. The Houser was judged slightly more vigorous. The evidence in favor of the Houser increased from this time on and at the end of the season it gave decidedly the best returns, with the Puget Sound the best of the winter cabbage strains.

TABLE II.—TABULAR SUMMARY OF VARIETY TRIALS, HANSCHE PLOT: 1911

Row	Variety or strain	Number of plants set out	Condition at end of season			
			Living		Headed	
			Number	Per cent	Number	Per cent
1	Puget Sound Hollander	150	20	13	1	0.7
2	Imported Danish I	150	0	0	0	0
3	Imported Danish III	150	5	3	0	0
4	Imported Danish III	150	3	2	0	0
5	Imported Danish II	150	2	1	0	0
6	Imported Danish II	150	14	9	0	0
7	Imported Danish IV	150	1	0.7	0	0
8	Imported Danish V	150	0	0	0	0
9	Houser	150	58	39	8	5
10	All Season	150	8	5	3	2
11	Puget Sound Hollander	150	22	15	1	0.7

Field B.—Broesch farm. Fewer varieties were included here and the trial was, therefore, on a larger scale. The disease was even more severe than on the Hansche field. The loss recorded in the following tabular summary was entirely due to the Fusarium.

TABLE III.—TABULAR SUMMARY OF VARIETY TRIALS, BROESCH FIELD: 1911

Row	Variety or strain	Number of plants set out	Condition at end of season			
			Living		Headed	
			Number	Per cent	Number	Per cent
1, 5, 13, 17	Imported Danish I	1000	2	0.2	0	0
2, 6, 14, 18	Imported Danish II	1000	0	0	0	0
3, 7, 15, 19	Imported Danish III	1000	11	1.1	1	0.1
4, 8–12, 16, 20–22	Ferry's Hollander	2500	5	0.2	1	0.04

These figures show clearly the extreme destructiveness of the parasite under favorable conditions, since except for this disease the above fields would have shown practically a perfect stand with 95 to 99 per cent heading.

As it was, none of these varieties showed any special degree of resistance, two of the imported, Danish I and II, being inferior to the standard American-grown (Ferry) strain and the other, Danish III, being only slightly superior to it. The two heads which formed were saved in the hope of growing seed from them, but they were not strong enough to survive the winter.

Trials of these Danish strains alongside of the American commercial seed were made also in two commercial cabbage fields in the same neighborhood. In both cases there was enough of the yellows to give some data as to relative susceptibility and yet fair commercial yields were secured. None of these Danish strains showed any peculiar excellence as to either disease resistance or yield.

TRIALS OF 1911

Similar trials were made in 1912 on the same cabbage-sick soil in which were again included the same strains of imported Danish seed, along with a standard commercial "Hollander" strain grown in Puget Sound and the Houser. Trial was also made of the best kraut variety being grown in this neighborhood, the Brunswick.[18]

The yellows was not as destructive as in 1911 but the comparative outcome was similar. The varieties are grouped in Table IV in the order of resistance.

TABLE IV.—OUTCOME OF COMMERCIAL VARIETY TRIALS: 1912

No.	Variety name	Source of seed	Condition at end of season	
			Living Per cent	Headed Per cent
VI	Large winter	Imported, Denmark	14	0
II	Amager, long stem	Imported, Denmark	36	13
XI	Brunswick	Imported, Germany	47	21
XII	Houser	Commercial	64	18
XV	Hollander	Hansche's Puget Sound	24	24
III	Amager, short stem	Imported, Denmark	69	27

TRIALS OF 1913

The same varieties used in 1912 were tried again in 1913 along with several additional ones, including some of the

[18] For selecting this variety and supplying seed for these trials as well as those of subsequent years we are indebted to F. W. Gunther, kraut grower and manufacturer of Racine. This seed was imported by him from Germany.

standard early and kraut types. They were planted on the same cabbage-sick soil. While the disease was not as bad as in 1910 and 1911, it was rather worse than in 1912. The full list of varieties and the outcome was as follows:

TABLE V.—OUTCOME OF COMMERCIAL VARIETY TRIALS: 1913

No.	Variety name	Source of seed	Condition at end of season	
			Living Per cent	Headed Per cent
XVI	Hollander	Ferry's Commercial	30	0
VI	Large Winter	Imported, Denmark	7	2
XXI	Copenhagen Market	Commercial	23	3
II	Amager, long stem	Imported, Denmark	12	4
XVII	All season	Commercial	38	5
XVIII	Succession	Commercial	25	11
XV	Hollander	Hansche's Puget Sound	32	12
XXII	Early Summer	Commercial	59	15
XII	Houser	Commercial	46	16
XX	Early Jersey Wakefield	Commercial	29	18
XXIII	Charleston Wakefield		48	18
XI	Brunswick	Imported, Germany	45	19
III	Amager, short stem	Imported, Denmark	41	22
XIX	Volga	Commercial	83	48

Conclusions. The results of these three years' trials served to convince us that there are well-marked differences as to disease-resistant qualities between the commercial varities or strains of cabbage. It was evident from the first year, 1911, that the Houser was quite highly resistant and the trials of Volga during 1912 and 1913 have shown that to be even more so[19] (Fig. 11). If it were merely a question of producing a yield of cabbage regardless of season or quality, either of these would constitute a promising variety from which to breed or select. Unfortunately both of them, at least under Wisconsin conditions, are of the summer type, of no value for either winter storage or kraut manufacture. Our chief problem has been to get a resistant strain of winter or storage cabbage of the Hollander or Danish Ball Head type. Second to this has been the desire for a resistant strain of the kraut type.

The results have shown that none of the commercial varieties of the winter type have marked preeminence as to disease resistance, although it is evident that there are

[19] The variety Volga, which has shown such a promising degree of disease resistance, was first brought to our attention by Dr. J. T. Barrett, then at the University of Illinois. He reported (December, 1912) that an Illinois cabbage-grower was succeeding with this variety, growing his own seed, in a district where other varieties of cabbage failed, presumably because of the yellows.

differences worthy of note in the relative susceptibility of these. Thus, of the imported Danish seed, the strain designated III Amager, short stem, has proved consistently superior to the others. On the other hand, various field trials, of which we have not here included the details, made of this alongside the standard local types of Hollander, have shown even this one to be on the whole commercially inferior to them.

With the valuable kraut types, and the early market garden varieties the outcome has shown similarly that there

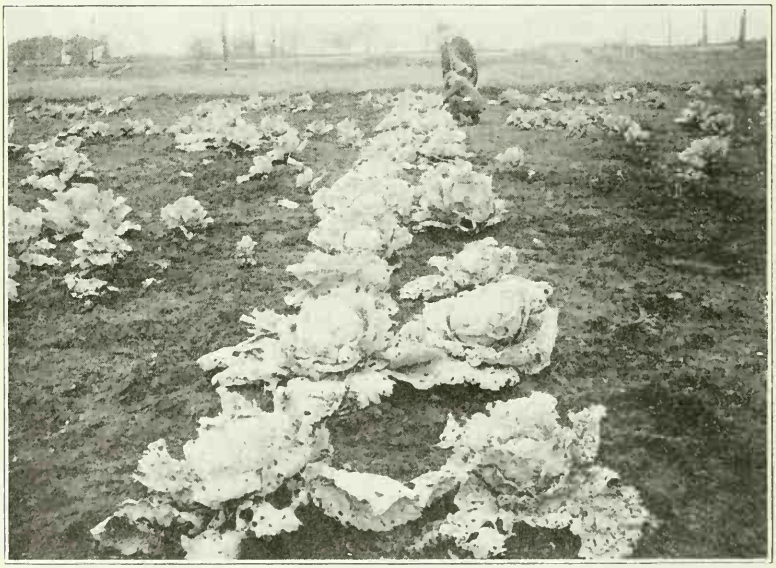

FIG. 11.—THE VOLGA IS THE MOST RESISTANT COMMERCIAL VARIETY TESTED

There are two rows of Volga in the middle of this trial field, 1913. The first two rows at the left of the Volga are Puget Sound Hollander; the first two at the right are Ferry's Hollander. In the trials of 1914 Volga made an even better showing; but unfortunately it is not suited for commercial culture. Figure 20 shows the results of a similar trial on this same field in 1911 when Houser proved the best of the varieties there tested, Volga not being in that trial series.

are differences, but none is sufficiently resistant to make its culture safe on cabbage-sick soil. Advantage has been taken of these differences, however, in making selections of resistant heads from the most promising of these varieties. From these we have secured seed for further trial of Volga and the German kraut variety, Brunswick. Trials of these

will be continued and through further selections it is hoped that Fusarium-resistant strains may be secured[20].

In addition selections from the two standard commercial types of winter cabbage were made in 1910 and trials and further selections made in the succeeding years. The results of these are discussed in the following pages.

THE DEVELOPMENT OF DISEASE-RESISTANT STRAINS BY SELECTION

THE PLAN OUTLINED

Very soon after the inception of the work in 1910 it became evident that this was a promising line of attack. As

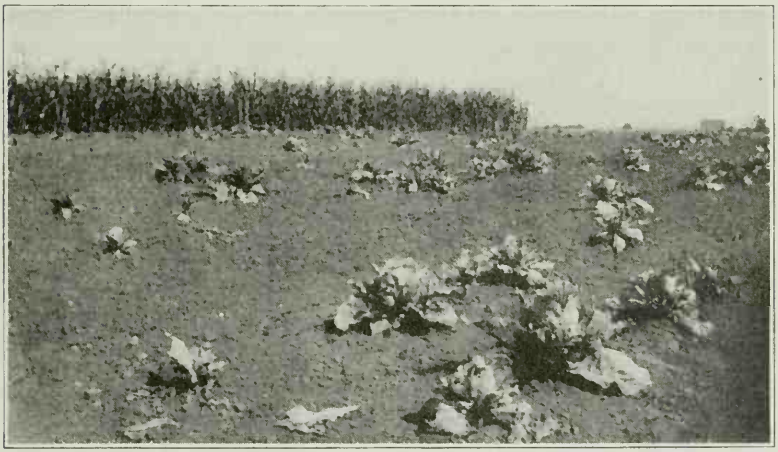

FIG. 12.—RESISTANT PLANTS ON UNIFORMLY DISEASED SOIL

It is characteristic of a Fusarium infected cabbage field that the individual plants vary widely in susceptibility, some appearing quite immune and maturing perfect heads. (Hansche field, Racine, Sept. 1910, from which selections were made. See Figure 13.)

already explained it is characteristic of the yellows that even in badly diseased fields of winter cabbage it rarely makes a clean sweep of any considerable area. Instead the plants are affected in varying degrees and individuals

[20] Through the cooperation of Professors A. D. Selby and S. N. Green of the Ohio Agricultural Experiment Station, we have also received seed of a strain of the favorite kraut variety, All Season, which has been selected at the Ohio Experiment Station for disease resistance. This will be tested in our 1915 series. Through the further cooperation of Professors J. W. Britton of the Ontario Agricultural College, Guelph, Canada, and Professor C. E. Myers, of State College, Pennsylvania, we have received samples of seed of the Houser variety, which has been selected in Canada by Edwards (1907, 1908) for resistance to black rot. This will also be included in our further field trials.

here and there appear normal and form sound, fully-developed heads. (Fig. 12.) Evidently there are two possible explanations for this; it may be due to difference in opportunity for infection, or, if infection is uniform, it may be due to different degrees of susceptibility or resistance of the individual plants. Even if the latter be the true explanation it remains a question of the greatest practical moment whether such individual differences are transmitted with constancy through the seed from generation to generation.

It was also obvious that in case they are, the practical solution of the problem was further conditioned upon finding such fixed and inheritable disease-resistant quality in combination with those other qualities which characterize the best commercial type of winter cabbage: keeping-quality, yield, texture, size, and shape of head. Steps were at once inaugurated to determine these points.

INITIAL HEAD SELECTIONS OF 1910

The disease was unusually severe in 1910. In the autumn selections were made in three different fields, all badly cabbage sick. In each case the aim was to select plants that were both sound and of the best commercial winter cabbage type. A. J. Rogers, of the department of horticulture, and F. D. Bailey, of the department of plant pathology, cooperated in this, and much reliance was placed on the judgment of W. J. Hansche, of Racine, an experienced cabbage grower and dealer. (Fig. 13.)

Proceeding in this way, about 100 heads were chosen and placed in storage, November, 1910, from the three fields, and of these 50 heads were finally selected for replanting in May, 1911, as follows:

From the field of Matt. Broesch, Kenosha, three heads; variety bought from D. M. Ferry Company as "Hollander or Danish Ball Head." This selection was from the worst-diseased field under observation. Upon the one-half acre of this only about twelve plants survived to form heads at the end of the season. Six of these were evidently diseased, and of the other six placed in storage, only three survived the winter for seed growing in 1911. These are the heads later designated as VIIIa, VIIIb, and IX3.

From the field of Witcheber Bros., Kenosha, 50 heads; variety the same as the last. This field was not nearly as badly diseased as Broesch's, and the plants had not, therefore, been subjected to as severe a process of natural selection. Of these, 30 heads passed through the winter in good condition, were replanted and produced seed in 1911. These are the heads later designated as VIIa-y and IX 5, 6, 24, 26, 32.

From the field of W. J. Hansche, Racine, 44 heads (Figs. 12 and 13). This was from seed of the Hollander type,

FIG. 13.—SELECTING DISEASE RESISTANT CABBAGES

The selections were made in 1910 in the fields where the yellows was as bad as could be found.[21] The above field would have shown a full stand except for Fusarium.

grown for Mr. Hansche in the Puget Sound region. Of these 16 were selected in the spring, 1911, replanted and produced seed. These are the heads later designated as IX 105, 116 and X 101–143.

It will thus be seen that we grew seed in the summer of 1911 from 49 seed-mother-heads, all selected for disease

[21] Mr. W. J. Hansche, an experienced cabbage grower and Professor A. J. Rogers of the horticultural department coöperated in selecting heads of the best commercial type as well as free from disease.

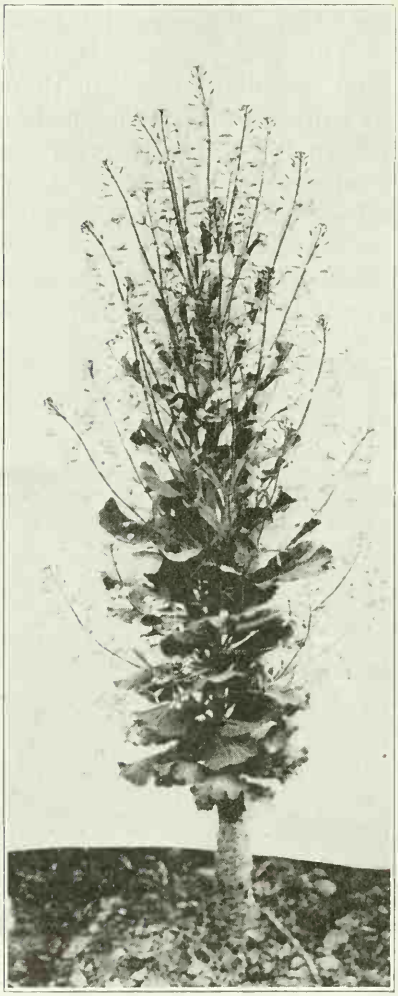

FIG. 14.—CABBAGE SEED PLANT

For seed growing in this climate the plant is pulled up by the roots in late autumn, stored in a cool cellar, or trench, and set out again the following spring. Such a plant will in general produce from an ounce to a quarter of a pound of seed.

resistance, secured from three fields, and representing two distinct commercial types.

An important question at the outset concerned the possibility or necessity of cross-pollination as between these heads. Cabbage seed-plants have a prolonged period of blossoming and are freely visited by bees and other insects which are instrumental in cross-pollination. Tracy (1906) advises that such cross-pollination seems essential, that a single isolated cabbage plant produces little or no seed and that for the best results seed-plants representing different types or strains should be set together. With these suggestions in mind the plantings were planned so as to be sure to get seed in one or all of three ways: (1) by self-pollination, (2) by cross-pollination between heads selected from the same field, and (3) by cross-pollination between heads selected from different fields. To this end the 49 cabbage heads were handled in four lots, each of which was planted by itself and with no other cabbage seed plants in its neighborhood.

The three lots collected from the three different fields were replanted, each by itself on the farm where grown, as follows:

(1) At Witcheber's, 25 heads, designated as Nos. VIIa-y.

(2) At Broesch's, 2 heads, Nos. VIIIa, VIIIb.

(3) At Hansche's, 14 heads, designated by numbers ranging between X 101 and X 143.

(4) In addition to the above, 8 heads representing selections from each of the three fields were planted, intermingled in close proximity, at Madison. These included the following, No. IX 3 (Broesch field,) IX 5, 6, 24, 26, 32 (Witcheber field), IX 105, 116 (Hansche field).

With lot 4 some of the branches were bagged, but no seed was secured from these. The uncovered branches produced seed abundantly, and it is assumed that this, in general, resulted from cross-pollination between the various heads representing the different fields.

Lots 1 to 3 were grown without bagging. Each of these fields was isolated with no other seed-cabbage in the vicinity. In each case, it is presumed much of the seed resulted from cross-pollination, but this was, of course, restricted in each lot to its own type, i.e., it was in each lot between plants selected from the same field. Since these had in each lot originated from the one commercial strain of seed and had, moreover, in all cases alike been selected for disease resistance and for uniformity to one type, such crossing commended itself to us as desirable rather than objectionable.[22]

The seed secured in 1913 from each of these 49 heads was collected and saved by itself and represents the beginning of one of the head strains. Each of these has since been maintained distinct in our later trials and bears the number by which it has just been designated, e.g., VIIIa, X 143, etc. The behavior of these in the trials of the succeeding years will now be considered.

TRIALS IN 1912 AND 1913 OF THESE FIRST-GENERATION SELECTED-HEAD STRAINS

Trials were made of these selected head strains in 1912 and 1913. The trials of 1912 included all of the head strains except certain of the VII series, a total of 38 strains. The

[22] Since the outcome from this latter method has proved highly satisfactory and better than that from lot 4, as will appear later, we have continued to follow this method in our subsequent work. Our associates, R. F. Howard and G. F. Potter, of the horticultural department, and L. J. Cole of the department of experimental breeding, have, however, undertaken to supplement this work by further breeding experiments, including close-pollination, and cross-pollination with these selected strains and with certain other commercial types.

1913 trials included a repetition of the trials of all tested in 1912 of which enough seed remained and in addition all of the strains VII omitted in 1912. These trials were made on the same cabbage-sick soil (Hansche field) where the disease was prevalent in 1910 (Figs. 12, 13) and which has been replanted to cabbage, experimentally, every year since. The soil has, therefore, become more sick from Fusarium infestation each year, if this be possible. Comparison of the losses in the control plots in these respective years shows that the

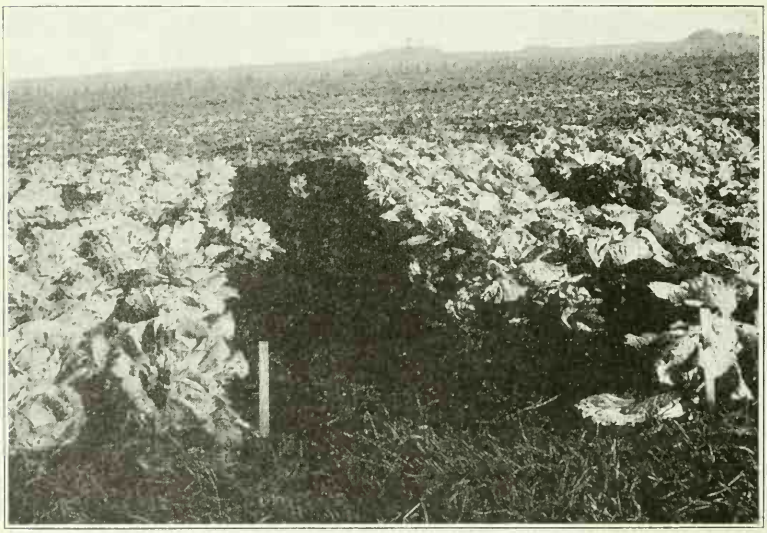

FIG. 15.—TRIALS OF SELECTED HEAD STRAINS, 1912

The three rows at the left of the center (IX 3) and at the extreme right (VIIIa) are both resistant Hollander strains. The three rows in right center (VI) imported Danish, non-resistant, have been nearly exterminated by the yellows. For further details see Table VII.

seasonal conditions were not, however, quite as favorable for the fungus in either 1912 or 1913, as in 1910 and 1911. There was, nevertheless, enough yellows both seasons to insure a thorough comparative trial. The trials were conducted as follows: The selected seed was planted along with controls in disease-free soil, where all developed with like vigor. A uniformly strong, healthy lot of seedlings from each strain was then selected and all transplanted on the same day, in the last week of June, into the infected trial field, in parallel rows, 45 plants of each strain being used in 1912 and 81 of each in 1913.

In 1912 a corresponding series of seedlings was at the same time transplanted to virgin soil, free from Fusarium, on another farm. On this latter field all alike produced a fine stand of healthy cabbage which headed up uniformly well. On the sick soil of the trial field the yellows appeared each season within two weeks of the time of transplanting and rapidly increased on the commercial varieties planted as controls. From the outset, and in both seasons alike, a marked difference was shown in the amount of disease in the selected head strains as compared with these commercial

FIG. 16.—TRIAL OF A SELECTED HEAD STRAIN, 1913

At the left is Leuker's Short Stem Hollander (non-resistant), at the right the resistant head strain VIIIa. This trial was on the Broesch farm, and was made to supplement the main field. (Compare Figure 15.)

controls. (Figs. 15 and 16). The results are brought together for convenient reference in the following table. They are expressed in percentages of the original stand except that, since there was always an occasional loss from insects

and in cultivation, where this occurred it was noted and proper deduction was made. There was no disease other than yellows responsible for any of the loss so that the results as tabulated represent correctly the loss due to the Fusarium attack, or yellows. In each season, 1912 and 1913, further selections of heads were made from the most promising head strains and saved for seed-growing. The less promising were ruled out from further trial as indicated.

Inasmuch as the commercial varieties of winter cabbage of the Hollander or Danish Ball Head type reported upon in the last chapter (Tables IV, V), were grown in this same field and constitute the controls in these trials, the results obtained with them are repeated at the beginning of the following table. (Table VII.)

SUMMARY OF RESULTS OF TRIALS OF 1912-1913

The significance of these results may perhaps be most clearly grasped by condensing and tabulating the averages for the two seasons as follows:

TABLE VI.—SUMMARY OF SIGNIFICANT RESULTS FROM TABLE VII

	Per cent lived	*Per cent headed
Commercial varieties		
Average 5 varieties, commercial seed	29	12
Puget Sound Hollander	28	18
Ferry Hollander	30	0
Selected head strains		
Ferry's Hollander from Witcheber field; mixed stand, average 5 strains	69	35
pure stand, average 25 strains	59	40
head strain, VIIy	86	60
head strain, VIIv	82	64
Ferry's Hollander from Broeseh field; mixed stand, average 1 strain	85	64
pure stand, average 2 strains	92	67
head strain VIIIa	94	64
head strain VIIIb	89	70
Puget Sound Hollander from Hansche field; mixed stand, average 2 strains	90	50
pure stand, average 14 strains	80	57
head strain X 135	98	78
head strain X 143	98	93

* The above figures for "per cent headed", although correct for 1913, fail to do full justice to the Ferry Hollander since the trials of 1911 and 1914 show that it is at least equal to other commercial varieties, if not superior, in disease resisting qualities.

TABLE VII.—TRIALS IN 1912 AND 1913 OF SEED FROM THE HEAD SELECTIONS OF 1910

Source and name	Number	Condition crop end season 1912		Condition crop end season 1913		Remarks
		Per cent living	Per cent headed	Per cent living	Per cent headed	
Commercial not selected (Inserted for comparison)						
Imp. Danish Large Winter	VI	14	0	7	2	Under trial 1911 also with poor record
Imp. Danish Amager long stem	II	36	13	12	4	Under trial 1911 also with poor record
Imp. Danish Amager short stem	III	69	27	41	22	Best of imported varieties.
Hansche's Puget Sd. Hollander	XV	24	24	32	12	Our selections X, 1910, were of this variety.
Ferry's Hollander	XVI	Not tested		30	0	Our selections VII and VIII, 1910, were of this variety.
Head selections of 1910, seed grown in pure plantations, 1911						
Ferry's Hollander selected from Witcheber field, 1910	VIIa	63	35	54	38	Discontinued
	VIIb	17	17	60	48	Discontinued
	VIIc	68	52	56	44	Discontinued
	VIId	56	40	40	30	Discontinued
	VIIe	71	52	50	36	Discontinued
	VIIf	81	63	42	22	Saved 10 heads, 1912 Discontinued, 1913
	VIIg	75	58	60	46	Discontinued
	VIIh	73	57	58	32	Discontinued
	VIIi	80	68	66	50	Saved 10 heads, 1912
	VIIj	68	41	43	33	Discontinued
	VIIk	67	53	46	28	Discontinued
	VIIl	44	21	35	25	Discontinued
	VIIm	24	24	75	59	Discontinued
	VIIn	65	43	60	44	Discontinued
	VIIo	not tested		70	46	Discontinued
	VIIp	not tested		50	30	Discontinued
	VIIq	not tested		53	18	Discontinued
	VIIr	not tested		43	12	Discontinued
	VIIs	not tested		64	32	Discontinued
	VIIt	not tested		62	28	Discontinued
	VIIu	not tested		60	34	Discontinued
	VIIv	not tested		82	64	25 heads selected, 1913
	VIIw	not tested		78	57	Discontinued
	VIIx	not tested		62	38	Discontinued
	VIIy	not tested		86	60	25 heads selected, 1913
Ferry's Hollander selected from Broesch field, 1910	VIIIa	96	80	92	48	Saved 35 heads, 1912
	VIIIb	87	67	91	73	Saved 29 heads, 1912
Puget Sound Hollander selected from Hansche field, 1910	X101	71	58	not tested		Discontinued
	X104	70	37	not tested		Discontinued
	X108	62	44	not tested		Discontinued
	X112	71	51	not tested		Discontinued
	X113	58	33	not tested		Discontinued
	X117	69	53	not tested		Discontinued
	X118	78	60	not tested		Discontinued
	X120	93	59	not tested		Discontinued
	X122	91	66	not tested		Discontinued
	X123	90	45	not tested		Discontinued
	X124	80	67	not tested		Discontinued
	X128	89	57	not tested		Discontinued
	X135	98	78	not tested		Saved 32 heads, 1912
	X143	98	93	not tested		Saved 38 heads, 1912
Head selections of 1910 grown in mixed plantation, 1911,						
Ferry's Hollander selected from Broesch field, 1910	IX 3	88	58	82	70	Discontinued
Ferry's Hollander selected from Witcheber field, 1910	IX 5	78	42	not tested		Discontinued
	IX 6	63	35	not tested		Discontinued
	IX24	60	28	70	46	Discontinued
	IX26	65	23	48	12	Discontinued
	IX32	75	41	78	53	Discontinued
Puget Sound Hollander selected from Hansche field, 1910	IX105	87	42	not tested		Discontinued
	IX116	91	51	92	57	Discontinued

DISCUSSION OF RESULTS OF THE TRIALS OF 1912 AND 1913

Table VII shows that even the poorest among the selected head strains averaged better than the best of the parent commercial strains from which selections were made. Table VI brings out still more strikingly the relative superiority of the selected head strains over the parent commercial strains. These averages prove conclusively the correctness of the principle of selection here practiced. On the other hand, the greatest stimulus to continued selection is found in the significant differences in the behavior of the various selected head strains, some of which show up far better than others.

It has already been explained that in most cases the seed-mother-heads of each kind or type have been planted separately but that certain of them each year have been grown in mixed plantation which would permit of cross-fertilization.

Comparison of the results from the strains where the seed plants from the three fields grew in mixed stand showed that the outcome as to disease resistance and yield was somewhat poorer than where each was grown in pure stand. Since the pure stand also yielded as much seed as the mixed stands it was decided to discontinue further trials with any of those mixed strains, which included all of the IX series.

It is also to be noted that the Ferry Hollander selections from the Broesch field (Series VIII) averaged considerably better than those from the Witcheber field (Series VII). This superior disease resistance of the VIII (Broesch) strains over the VII (Witcheber) strains, is, indeed, what was to be expected considering their history. The two fields were planted with the same commercial variety, Ferry Hollander, in 1910, but the disease was not nearly so severe on the Witcheber field, hence the opportunity for selection was less favorable. It should be recalled that on the Broesch field of one-half acre only three mother-heads survived to produce seed and heads VIIIa and VIIIb were the best of these.

It was also easy to select X135 and X143 as the best two head strains of the Puget Sound Hollanders, although it was unfortunate that no seed of this X series was available for further trials in 1913.

This prepared the way for focusing attention in the trials of 1914 upon the relative merits of these most promising head strains from each of the two types, viz., VIIIa and VIIIb, of the Ferry Hollander, and X135 and X143 of the Puget Sound Hollander. Seed of these was grown in 1913 from the heads selected in 1912.

SEED GROWING IN 1913 FROM THE SECOND-GENERATION HEADS SELECTED IN 1912

Selections were made in 1912 as already explained in Table VII of heads for the continuation of the trials. It will be noted that the chief attention in these second-generation head selections was given to the four head strains already shown to be most promising, viz., VIIIa and VIIIb, X 135, and X 143. These heads were in all cases selected as apparently healthy on badly diseased ground. A few heads were lost in winter storage. In 1913 seed was grown from the balance. The same general plan was followed as in seed-growing in 1911, i.e., part was grown in isolated plantations at Madison, and the balance in mixed plantation at Racine. The seed from each head was again kept separate. Following is a list of these together with the yields of seed in grams per head.

TABLE VIII.—SEED GROWN IN PURE PLANTATIONS, MADISON: 1913

Strain	Weight grams	Head strain number	Weight grams	Head strain number	Weight grams	Head strain number	Weight grams
VIIf 1	82	VIIIa16	58	VIIIb16	9	X 135-23	56
VIIf 4	38	VIIIa17	30	VIIIb17	2	X 135-30	38
VIIf 5	103	VIIIa20	24	VIIIb18	32	X 135-33	57
VIIf 7	100	VIIIa21	13	VIIIb19	28	X 143- 1	22
VIIf 9	67	VIIIa22	39	VIIIb20	2	X 143- 2	40
VIIf 10	6	VIIIa23	23	X135- 2	54	X 143- 3	75
VIIi 1	70	VIIIa25	38	X135- 3	65	X 143- 4	23
VIIi 2	127	VIIIb 1	27	X135- 4	13	X 143- 7	35
VIIIi 3	33	VIIIb 2	21	X135- 5	48	X 143- 8	26
VIIi 4	3	VIIIb 3	38	X135- 6	31	X 143- 9	43
VIIi 6	63	VIIIb 4	30	X135- 8	85	X 143-10	69
VIIIa 2	5	VIIIb 5	5	X135- 9	28	X 143-12	16
VIIIa 3	22	VIIIb 6	6	X135-11	58	X 143-13	24
VIIIa 5	15	VIIIb 7	27	X135-12	30	X 143-14	24
VIIIa 6	1	VIIIb 8	10	X135-13	41	X 143-15	20
VIIIa 7	32	VIIIb 9	39	X135-15	10	X 143-16	11
VIIIa 8	16	VIIIb10	4	X135-17	76	X 143-18	52
VIIIa 9	6	VIIIb11	8	X135-18	63	X 143-20	31
VIIIa12	15	VIIIb12	1	X135-19	122	X 143-21	72
VIIIa13	54	VIIIb13	50	X135-20	59	X 143-24	15
VIIIa14	19	VIIIb14	53	X135-21	64	X 143-25	65
VIIIa15	35	VIIIb15	3	X135-26	6	X 143-28	40

TABLE IX.—SEED GROWN IN MIXED PLANTATION, RACINE: 1913

Head strain number	Weight grams	Head strain number	Weight grams	Head strain number	Weight grams	Head strain number	Weight grams
VIIf 2	5	VIIIa 33	15	VIIIb 29	9	X 135–32	20
VIIf 3	49	VIIIa 34	33	X 135–24	29	X 143–29	47
VIIf 8	22	VIIIa 35	33	X 135–25	26	X 143–30	16
VIIi 9	42	VIIIb 22	28	X 135–26	28	X 143–31	9
VIIIa27	30	VIIIb 23	12	X 135–27	22	X 143–33	2
VIIIa28	8	VIIIb 24	24	X 135–28	25	X 143–34	18
VIIIa29	14	VIIIb 25	15	X 135–30	38	X 143–35	5
VIIIa32	6	VIIIb 27	3	X 135–31	11	X 143–38	68

TRIALS OF SELECTED-HEAD STRAINS 1914

The number of head strains available for trial in 1914 had become so large that it was feasible to make trial of only a select list of the more promising of each series. Therefore, in order to eliminate any especially weak plants only those were included which produced one ounce or more of seed in 1913. This gave 44 head strains as listed in the Table X. Along with these selected strains commercial controls were introduced.

The procedure was as heretofore, to make the seed bed in disease-free soil, select uniformly strong seedlings, and transplant to Fusarium-infested soil. In order to make these 1914 trials as convincing as possible, trials were made in three series as follows:

A. The larger numbers were planted on the W. J. Hansche farm on the same cabbage-sick soil which had grown experimental cabbage each year since 1910. Owing to the large number of plots only 81 heads could be planted in each plot in this field.

B. A smaller number of the most promising selections were planted on the farm of Matt. Broesch, Kenosha. Mr. Broesch selected the area as representing the worst cabbage-sick soil in a field where he had abandoned cabbage culture because of successive losses of his crop through yellows. This being a larger field with fewer strains, about 240 plants were set of each head strain under trial and twice as many of the commercial controls.

C. A sample of seed of a single head strain was given to each of several farmers in the neighborhood with instructions to test it alongside of the best commercial seed on cabbage-sick soil.

The climatic conditions of 1914 were favorable for a severe attack of yellows. Although there were copious rains im-

FIG. 17.—TRIAL OF SELECTED HEAD STRAINS, 1914, HANSCHE FIELD

At the left of the center are 3 rows of commercial Puget Sound Hollander (Nos. 67–69), non-resistant. At the end of the season 20 per cent headed. At the right of the center are 3 rows (Nos. 64–66) of selected head strain. X 135–33, resistant, but not one of the best; at the end of the season 78 per cent headed. Other selected head strains are shown in the background at the extreme right and left. For further data from this field see Table X.

mediately following the transplanting which gave the plants a satisfactory start, these were followed by a period of dry, hot weather through the middle of July which so stimulated the Fusarium development that before August 1 the disease was much in evidence on all the non-resistant commercial varieties in the trials fields. The outcome of these trials is shown in Table X.

In order to enable a better analysis of the results to be made fuller data are included in this table than in those of the

FIG. 18.—A FARMER'S TRIAL OF RESISTANT STRAINS VS. COMMERCIAL VARIETIES

Wm. Braid of Berryville made a trial of resistant strains in 1914. He planted for his general field two varieties of commercial seed, both of which were ruined by yellows. Through the middle of the field one row was set with plants of the selected head strain X 143–18 as shown in the figure. This row matured 174 heads; the row at the right, Imported Danish, matured 17 heads; the row at the left, Puget Sound Hollander, matured 8 heads.

TABLE X.—TRIALS IN 1914 OF SELECTED CABBAGE STRAINS; FIELD A, HANSCHE FARM (FIGURE 17)

Description of seed				Condition at end season			Marketable heads produced		
Source and kind	Rows number	Year grown	Number	Yellows	Living	Headed*	Per cent of total	Average weight	Yield per acre
(Rows 1–69, Puget Sound Hollander)				Per cent	Per cent	Per cent	Per cent	Lbs.	Tons
Commercial control	**1–3**		**XV**	**82**	**35**	**13**	**6**	**3.8**	**0.9**
Second generation selection	4–6	1913	X 143- 2	12	93	83	67	4.3	11.4
	7–9		X 143- 3	10	91	83	68	3.9	10.4
	10–12		X 143- 9	35	91	63	44	3.5	6.1
	13–15		X 143-10	15	91	81	67	4.	10.4
	16–18		X 143-18	5	100	97	89	3.8	13.
	19–21		X 143-21	19	86	80	68	3.8	10.5
Commercial control	**22–24**		**XV**	**86**	**34.6**	**19**	**15**	**3.9**	**2.3**
Second generation selection	25–27	1913	X 143-25	40	86	79	73	3.5	10.1
	28–30		X 143-28	17	90	84	84	3.8	12.4
	31–33		X 143-29	4	100	100	91	3.7	13.
	34–36		X 143-38	48	56	53	47	4.3	8.
First generation selection	37–39	1911	.....	19	91	86	77	4.2	12.7
	40–42		X 135	20	91	81	78	3.8	12.3
Second generation selection	43–45	1913	X 118- 4	28	83	75	75	4.	11.8
Commercial control	**46–48**		**XV**	**90**	**26**	**15**	**11**	**3.2**	**1.8**
Second generation selection	49–51	1913	X 124- 2	25	85	79	72	3.5	11.1
	52–54		X 135- 2	7	96	93	86	3.9	13.2
	55–57		X 135- 3	1	100	99	97	3.5	13.6
	58–60		X 135-20	21	90	79	80	4.	12.9
	61–63		X 135-23	24	83	72	78	4.	12.2
	64–66		X 135-33	27	84	73	78	3.6	11.2
Commercial control	**67–69**		**XV**	**84**	**37**	**26**	**20**	**2.6**	**2.**
(Rows 70–126 Ferry's Hollander)									
Second generation selection	70–72	1913	VIIIa 7	3	100	85	81	3.5	11.4
	73–75		VIIIa 13	0	100	94	83	3.5	11.6
	76–78		VIIIa 15	0	100	96	88	3.5	12.2
	79–81		VIIIa 16	1	100	97	86	3.8	13.1
	82–84		VIIIa 17	7	100	99	88	3.4	11.8
Commercial control	**85–87**		**XV**	**74**	**62**	**30**	**27**	**1.8**	**1.8**
Second generation selection	88–90	1913	VIIIa 22	0	100	99	94	4.6	17.1
	91–93		VIIIa 25	0	100	100	94	4.9	18.3
	94–96		VIIIa 27	0	100	97	94	3.8	14.3
	97–99		VIIIa 34	1	99	94	75	3.1	9.3
	100–102		VIIIa 35	0	100	94	64	3.1	8.
Commercial control	**103–105**		**XVI**	**75**	**51**	**33**	**23**	**2.**	**1.9**
Second generation selection	106–108	1913	VIIIb 3	0	100	99	92	3.4	12.2
	109–111		VIIIb 4	9	99	91	91	3.7	12.2
	112–114		VIIIb 9	7	100	95	91	3.6	11.9
	115–117		VIIIb 13	4	99	90	85	3.9	12.6
Commercial control	**124–126**		**XVI**	**71**	**55**	**34**	**34**	**2.1**	**2.**

*The data for this column were taken October 1 and are based on a count of all plants which had then formed a hard head, *regardless of size* of the head. The next column represents the precentage of heads of marketable size at time of cutting, in November. It will be noted that the figures in this first column in most cases are a little higher than those in the second. They are included partly because they represent rather more exactly than the next column the growth conditions but chiefly because they correspond to the data taken in the years 1911-1913 on this same field and furnish the best basis for a comparison of developments during these four years.

preceding years, including percentages of disease, average weights, and yields.

FARMER'S TRIALS

In addition to the trials in these two experimental fields planned and planted under our immediate supervision, a

number of farmers' trials were made in 1914. In each such case a 10-gram sample of one of the selected strains was given to a farmer who had experienced loss from cabbage yellows

TABLE XI.—TRIALS IN 1914 OF SELECTED CABBAGE STRAINS; FIELD B, BROESCH FARM (FIGURE 19)

Description of seed			Condition at end season			Average weight	Yield per acre
Source and kind	Row	Number	Yellows	Living	Headed		
(Rows 1-17, Puget Sound Hollander)			Per cent	Per cent	Per cent	Pounds	Tons
Second generation selection	1	X 135- 8	46	76	53	3.7	7.8
	2	X 135 17	38	88	63	3.2	8.1
	3	X 135-18	69	53	38	3.7	5.6
	4	X 135-19	31	88	71	3.5	10.1
	5	X 135-21	63	70	48	4.7	8.9
Commercial control	**6**	**XV**	**95**	**15**	**8**	**4.1**	**1.3**
	7	**XV**	**95**	**8**	**5**	**4.8**	**0.9**
Second generation selection	8	X 143- 2	63	69	48	4.5	8.6
	9	X 143- 3	51	81	62	3.7	9.
	10	X 143- 9	87	43	25	3.9	3.8
	11	X 143-10	78	61	39	3.4	5.2
	12	X 143-18	48	82	63	4.4	10.9
	13	X 143-21	55	70	56	3.8	8.3
	14	X 143-25	66	59	45	4.2	7.4
	15	X 143-28	63	67	52	3.9	8.1
	16	X 143-29	20	94	80	4.	12.7
	17	X 143-38	89	35	18	3.6	2.5
Leuker Hollander, short stem*	18	XIII 11	97	29	8	2.8	.9
Leuker Hollander, long stem*	19	XIV 18	96	23	8	2.2	.6
(Rows 20-40, Ferry's Hollander)							
Second generation selection	20	VIIf 5	47	83	62	4.5	11.1
	21	VIIf 7	64	75	46	3.3	5.9
	22	VIIf 2	55	71	54	4.4	9.3
	23	VIIf 6	57	72	52	4.7	9.8
	24	VIIIa 7	9	99	88	4.	10.6
	25	VIIIa 13	9	98	91	3.6	8.7
	26	VIIIa 15	8	99	93	4.	9.
	27	VIIIa 16	11	99.6	94	4.	11.1
	28	VIIIa 17	23	93	83	3.6	9.8
	29	VIIIa 22	2	99.6	99	5.4	18.2
	30	VIIIa 25	3	100	96	6.	19.2
	31	VIIIa 27	6	99	91	4.5	11.8
	32	VIIIa 34	8	98	89	3.8	10.1
	33	VIIIa 35	5	100	96	4.1	12.8
Commercial control Ferry	**34**	**XVI**	**89**	**37**	**16**	**3.6**	**2.3**
	35	**XVI**	**89**	**35**	**18**	**3.2**	**2.2**
Second generation selection	36	VIIIb 3	4	99.6	93	5.	18.2
	37	VIIIb 9	6	99	95	4.	15.1
	38	VIIIb 13	11	97.4	86	3.8	13.
	39	VIIIb 14	17	94	83	3.3	9.
	40	VIIIb 18	22	95	78	3.7	10.9

*Rows 18 and 19 were Hollander cabbage of two strains which have been grown for eight years by Theodore Leuker, of Racine. They are excellent commercial cabbages but these and other trials have shown that they are fully as susceptible to yellows as the ordinary commercial Hollander strains.

with the request that he put the plants for trial on sick old cabbage land alongside of the standard commercial variety of cabbage he considered best. Owing to the small amount of seed available each sample was necessarily taken from a different strain. In all cases samples from the same strain were also under trial in one or both of our experimental fields as reported above.

Naturally some of these were not so managed as to have experimental value, but in a number of fields where the disease was bad the outcome was quite as convincing as on our experimental fields. Some of these results are worth citing.

FIG. 19.—TRIALS OF SELECTED HEAD STRAINS, 1914, BROESCH FIELD

The cabbage at the right is resistant head strain VIIIb–3, yielding 219 heads per row, or an equivalent of 18.2 tons per acre. At the left are two rows of commercial (non-resistant) Ferry's Hollander, averaging 40 heads per row, or only 2.3 tons per acre. At the extreme left are resistant head strains, VIIIa Series, which yielded 19.2 tons per acre. For further data see table XI.

Graber field. N. Graber planted in 4 parallel rows on cabbage-sick soil, 138 plants each, 2 rows of commercial Puget Sound Hollander and 2 rows of our selected head strain X 135–34. . The condition as to yellows was recorded August 21 and counts made of the numbers living and headed at the end of the season. The results were as follows:

	Yellows		Living		Headed	
	Plants	Per cent	Plants	Per cent	Plants	Per cent
Commercial	110	40	210	76	189	68
Selected head strain X 135–34	80	3	268	97	262	95

Drummond field. W. S. Drummond planted his commercial cabbage field with the commercial Puget Sound Hollander. Through the middle of the field he set one row with 328 plants of the selected-head strain X 143–29. The soil was supposed to be not badly infested, but as the season progressed considerable yellows developed. The following figures show the relative amount of disease as well as the conditions at harvest time of the selected row as compared with the commercial rows on either side.

	Yellows		Living		Headed	
	Plants	Per cent	Plants	Per cent	Plants	Per cent
Commercial (average 2 rows)	142	43	322	98	202	62
Selected head strain X 143–29	10	3	326	99	311	95

Mr. Drummond was so favorably impressed with this outcome that he saved the heads of the selected strain for seed-growing.

A. and S. Hansche field. A. and S. Hansche planted a small field with commercial cabbage seed of the Puget Sound Hollander and planted at one side of it 5 rows of the selected head strain X 135–19. Examination on August 22 showed 30 per cent of yellows among the commercial plants and 3 per cent in the rows of the selected strain. At the end of the season 97 per cent of the selected strain developed heads as compared with 75 per cent in the commercial rows.

Piper field. A. J. Piper planted a small area for purposes of this trial on old cabbage ground which he knew to be decidedly cabbage sick. He planted 6 rows through the middle of the field with the selected head strain X 143–10, with 5 rows on one side and 3 rows on the other of commercial Puget Sound Hollander. The yellows developed quite badly in this field and many plants in the commercial rows died early with very little disease in the selected strain. No counts were made of the original stand and no exact percentage records of yellows were made during the summer. The yields in the autumn in the same length of row averaged,

commercial strain, 156 heads, selected head strain, 303 heads. Moreover, the difference in size of heads was fully as striking as the difference in number, the heads in the selected row being apparently twice as heavy. Mr. Piper was so well pleased with the outcome that he saved these heads of the resistant strain for seed growing.

Braid field. William Braid planted a large field with cabbage, supposing his soil to be not sufficiently infected to prevent his growing a profitable crop. He used two commercial varieties, Puget Sound Hollander and an imported Danish seed from Copenhagen. Through the middle of his field between these two varieties he planted one row of the selected head strain X 143-18 (Fig. 18). The yellows appeared in very severe form on this field so that when counts were made on August 21 it was found that 75 per cent of the commercial plants showed the disease and in the row approximately 600 feet long only 44 plants were still living. There was considerable disease, 29 per cent, in the selected-head row, but 219 plants were then living. At the end of the season the counts were as follows on the resistant row and the two commercial rows immediately alongside.

	Living	Headed
Commercial Puget Sound Hollander	25 plants	8 plants
Selected head strain X 143-18	219 "	174 "
Commercial Imported Danish	29 "	17 "

Although comparison with our main field plots will show that this was not one of the best strains, it looked so good to Mr. Braid that he saved the best heads from this row for seed-growing.

Krause field. William Krause made a trial of selected-head strain VIIIb 3 by planting one row of this and one row of commercial cabbage through the middle of a field where he knew the soil to be cabbage sick, the balance of the field being planted to sugar beets. The percentage of yellows on August 21 and the condition at the end of the season were as follows:

	Yellows	Living	Headed
Commercial variety	84%	55 plants	16 plants
Selected-head strain VIIIb 3	8%	293 "	233 "

J. Hansche field. J. Hansche planted a row of selected-head strain VIIIb 9 through the middle of his trial field the balance of which was planted to the commercial cabbage of the Danish Ball Head variety known as Grenadier. Yellows was very severe on this field also, so that on August 21 counts showed 88 per cent of these commercial plants diseased as compared with 9 per cent on the selected-head row. At the end of the season counts on the resistant row and the commercial rows on either side of it gave the following figures:

	Living	Headed
Commercial variety Grenadier	45 plants	8 plants
Selected head strain VIIIb 9	277 "	238 "
Commercial variety Grenadier	65 "	18 "

Broesch field. Henry Broesch was given the selected head strain VIIIa 13. For his commercial cabbage crop he used seed of two varieties, Danish Ball Head Grenadier, and Ferry's Hollander, planting several acres of each. Through the middle of this field where these two varieties met he planted two rows of the selected strain. Although he had supposed the field suitable for a successful cabbage crop, considerable yellows developed so that on August 21 counts showed an average of 59 per cent diseased along the commercial varieties with only 1 per cent in the selected head rows. At the end of the season Mr. Broesch reported the outcome as follows and our counts subsequently substantiated the figures:

Conditions (2 rows each)	Ferry's Hollander	Commercial Selected Strain VIIIa 13	Commercial Danish Ball Head
Number plants at beginning	524	509	519
Died from yellows	236	1	352
Died from other causes	14	4	7
Lived but did not head	30	2	30
Heads harvested	244	502	136
Percentage that lived	52	99	32
Percentage that headed	47	98.6	26

Since this is from the VIIIa series which included the most promising strains in our own trials, we encouraged Mr. Broesch to save the heads of this strain for seed-growing with the expectation that he would be able to supply some others as well as himself in this way.

DISCUSSION OF RESULTS OF 1914 TRIALS

The results of the 1914 trials serve fully to establish our confidence that the disease-resistant characteristics of the selected strains are fairly well fixed, constant, and inheritable. Examination of the above tables shows that in every case the selected head strains stood up much better than the best commercial strains. One of the encouraging things is the evidence that on the whole the second generation selections showed some improvement over the first generation. It will be noted, however, that among these there is a considerable variation, indicating probable opportunity for further improvement through continued head selection. The general facts justifying these conclusions may perhaps be brought out more clearly by summarizing the more important results from each of the two trial fields.

TABLE XII.—SUMMARY HANSCHE TRIAL FIELD: 1914*

Description of seed	Yellows	Living	Headed	Marketable heads	Average weight of heads	Yield per acre
General averages	Per cent	Per cent	Per cent	Per cent	Pounds	Tons
Commercial, averages of all kinds	**80.1**	**42.9**	**24.1**	**18.9**	**2.8**	**1.8**
Selected head strains, averages of all	12.5	93.5	87.2	79.5	3.8	11.7
Puget Sound Hollander						
Commercial averages of all 12 rows	**85.5**	**33.2**	**18.3**	**13.**	**3.4**	**1.8**
First selection head strain X 143	19	91	86	77	4.2	12.7
Second selection head strain X 143 averages all	21	88.4	80.3	69.8	3.9	10.5
X 143–29 the best of this series	4	100	100	91	3.7	13
First selection head strain X 135	20	91	81	78	3.8	12.3
Second selection head strain X 135 averages all	16	91	83	83.8	3.8	12.6
X 135–3, the best of this series	1	100	99	97	3.5	13.6
Ferry's Hollander						
Commercial averages of all 9 rows	**73**	**56**	**32**	**28**	**1.9**	**1.9**
Selected head strains VIIIa series, average all 30 rows	1	100	96	84.7	3.7	12.2
VIIIa 25, the best of this series	0	100	100	94	4.9	18.3
Selected head strains VIIIb series, averages of all 12 rows	5	99	95	89.7	3.7	12.2
VIIIb 3, the best of this series	0	100	99	92	3.4	12.2

*Averages from Table X.

TABLE XIII.—SUMMARY BROESCH TRIAL FIELD: 1914*

Description of seed	Yellows	Living	Heads	Average weight	Total yield per acre
Puget Sound Hollander	Per cent	Per cent	Per cent	Pounds	Tons
Commercial, averages of all kinds	**95**	**12**	**7**	**4.5**	**1.1**
Selected head strains X 143 series, average all	62	66	49	3.9	7.7
X 143-29, the best of this series	20	94	89	4	12.7
Selected head strains X 135 series, average all	49	75	55	3.8	8.1
X 135-19, the best of this series	31	88	71	3.5	10.1
Ferry's Hollander					
Commercial, averages of all kinds	**89**	**36**	**17**	**3.4**	**2.3**
Selected head strains VIIIa series, average all	8.4	99	92	4.3	12.1
VIIIa 25, the best of this series	3	100	96	6	19.2
Selected head strain VIIIb series, average all	12	97	87	4	13.2
VIIIb 3, the best of this series	4	99.6	93	5	18.2

*Averages from Table XI.

The results in these two trial fields show beyond doubt the superiority of the VIIIa strains. The evidence, as shown in the field by the uniformity, general thrift, and healthy appearance of these plants, was even more convincing than the figures as tabulated. A committee of local cabbage growers and buyers of long experience went over these fields in the autumn and all agreed as to the superiority of these strains in commercial quality as well as in respect to disease resistance. It was their unanimous verdict that these strains represent a highly satisfactory commercial type of Hollander cabbage.

Moreover, from a comparison of these results in the two fields it is evident that the head strain VIIIa 25 is somewhat superior to any other of the head strains. The only one to rival it was VIIIa 22, but a comparison as they grew side by side in the two fields showed the same slight superiority of VIIIa 25 evidenced by the figures in the tables. The following summary serves to bring out these points.

Averages from the Broesch and Hansche Fields, VIIIa strains.

	Yellows Per cent	Living Per cent	Heads Per cent	Average Weight pounds	Total yield per acre
Commercial, Ferry	81	46	24.5	2.65	2.1
VIIIa, all strains	5	99.5	94	4.0	12.2
VIIIa 22	1	99.8	99	5.0	17.7
VIIIa 25	1.5	100.	98	5.45	18.8

For further seed-growing and selection looking to the maintenance or possible improvement of the present standards, a series of 25 of the best heads of the VIIIa 25 strain

were selected from the Broesch field, where the disease was worst, and these will be planted by themselves for seed-growing in 1915.

The balance of the heads of all the VIIIa strains grown in 1914 were also saved for seed-growing in 1915. These include 2000 heads from which the seed will be available for commercial cabbage-growing in 1916 under the name Wisconsin Hollander No. 8. Since various experienced cabbage growers are coöperating in this seed-growing it is believed that there will be no difficulty in so organizing and directing their efforts that they will attend hereafter to the commercial aspects of producing the seed of the resistant strains, always growing their mother plants on cabbage-sick soil. This will leave us free to focus attention upon the maintenance and possible improvement of the parent strains by further trial and selection.

OTHER QUESTIONS OF PRACTICAL AND FUNDAMENTAL INTEREST

The nature of disease resistance. Naturally in connection with this work numerous questions have arisen which deserve further attention. Chief of these is the fundamental one as to the nature or cause of the disease resistance shown in these selected strains. While much thought has been given to this we can as yet offer no satisfactory reply other than to note that it seems to be associated with a high degree of general vegetative vigor. We do not wish to imply, however, that such vigor, by itself, necessarily carries with it resistance to Fusarium. While further attention will be given to these questions no early or easy solution is to be expected.

Disease resistance in relation to seed production. There is a wide range of variation in the amount of seed produced by different cabbage heads. Tracy (1909) calls attention to the belief frequently expressed by growers that the amount of seed produced stands in inverse ratio to the quality of the cabbage. While it is doubtless true that the wild-cabbage type would be more prolific than the cultivated, head-forming strains, still there seems no good reason for supposing that those minor differences between cultivated varieties which give them varying commercial

value should be correlated with seed production. Inasmuch as there was a considerable difference in the amount of seed produced by the various selected-head strains under trial in 1914, it seemed worth while to correlate the weight of seed secured from each of these in 1913 with the percentage of yellows shown in 1914. This is done in the following table.

TABLE XIV.—RELATION OF SEED PRODUCTION TO DISEASE RESISTANCE

	Weight of Seed in grams	Amount of yellows in per cent
X 135—19	122	35
X 135— 8	85	46
X 135—17	76	39
X 135—21	64	63
X 135—18	63	69
X 143— 3	75	51
X 143—21	72	55
X 143—10	69	74
X 143—38	68	89
X 143—25	65	66
X 143—18	52	48
X 143—29	47	20
X 143— 9	43	83
X 143— 2	40	63
X 143—28	40	63
VIIIa —16	58	11
VIIIa —13	54	9
VIIIa —22	39	22
VIIIa —25	38	3
VIIIa —15	35	8
VIIIa —34	35	8
VIIIa —35	33	5
VIIIa — 7	32	9
VIIIa —17	30	23
VIIIa —27	30	6
VIIIb —14	53	17
VIIIb —13	50	10
VIIIb — 9	39	6
VIIIb — 3	34	4
VIIIb —18	32	22

Since the test of disease resistance was more severe in the Broesch field and the percentages of yellows are therefore more significant, the following tabular summary is based primarily upon the results from the strains as tested in the Broesch field. The figures from the Hansche field are, however, added for the X 143 and VIIIa strains which were tested in both fields.

Comparison of first and second generations as to disease resistance. This involves a question of much practical as well as fundamental interest. Is this character of disease resistance a fixed thing or is it variable? In the latter case it would seem that without continued selec-

tion it might tend to be lost in succeeding generations and that on the other hand by repeated trials and selection there might be further improvement in these selected head strains. It seems, therefore, worth while to present such evidence bearing upon this as we have to date although it is recognized that this is not adequate for final decision upon the matter.

In the trials of 1914 (see summary, Table XII) seed of the 1911 crop of the parent head strains X 135 and X 143, i. e. the first selected generation, was used alongside of the second generation selections of the same strains. Table XV gives the outcome.

TABLE XV.—COMPARISON OF DISEASE RESISTANCE OF CABBAGE OF FIRST AND SECOND GENERATIONS*

	Yellows Per cent	Living per cent	Heads per cent	Yield per acre in tons
X 143, 1911 seed	19	91	86	12.7
X 143, 1913 seed, average 10	21	88	80	10.5
X 143–29 (best 1913 strain)	4	100	100	13.
X 135, 1911 seed	20	91	81	12.3
X 135, 1913 seed, average 5	16	91	83	12.6
X 135–3 (best 1913 strain)	1	100	99	13.6
Average 1st generations	19.5	91	83.5	12.5
Average all strains 2nd generations	18.5	89.5	81.5	11.5
Average best strains 2nd generations	2.5	100	99.5	13.3

* Seed grown 1st generation 1911, 2d generation 1913, trial 1914.

From these figures it will be seen that the average of all the second generation seed, gave results practically equal to those from the first generation. On the other hand the best selected head strains of the second generation in each case show decided improvement over the parent stock. The conclusion from the above data is in general accord with our experience and justifies the hope that by continued care and selection the present standards may be gradually raised.

Will disease resistance remain constant in different localities? Another question of immediate practical importance is as to whether the disease resistant quality shown by a strain of cabbage as grown in one locality will be shown in like degree, or in any degree at all, in another locality when environmental factors are quite different. In certain cases with other crops, Orton and Barrus (1911) have found that it may not hold. It will require several years of coöpera-

tive experiment to determine this with exactness. Such limited evidence bearing upon this as is available is encouraging. Thus the Houser and the Volga which with us have made the best showing of all commercial varieties, have a reputation in other sections for disease resistance as already noted. The variety of cabbage selected by Close and White (1909) of the Maryland Experiment Station, as disease resistant in that state has proved highly resistant to yellows in the Wisconsin trials of 1914, as has been explained on a preceding page. The most conclusive evidence on this point must come from trials of Wisconsin-grown seed in other sections. Small trial samples of such seed were sent to three states: Iowa, North Carolina, and Delaware, in 1914. The results, while not definite, are altogether encouraging. These trials are being continued.[23]

Locality of seed-growing in relation to disease resistance. It is also a question of much practical importance as to whether the environment under which this cabbage seed is grown will affect its disease-resistant quality. Thus it is evident that there might be gain from the commercial standpoint in sending our selected seed to the Puget Sound region as "mother-seed" to be grown for one generation, providing it does not thereby lose in disease-resisting quality. This also is a matter which can be determined only by trial. Such trial has already been started in coöperation with the Washington State Experiment Station. Until this matter is decided experimentally we shall encourage seed-growing of the disease-resistant Wisconsin Hollander, in Wisconsin or in the locality where it is to be used.

GENERAL CONCLUSIONS

The results of the experimental work of these five summers seem to justify the following conclusions: On the one hand, no specific method of soil, seed, or crop treatment offers any hope for the control of the Fusarium or yellows disease of

[23]These cooperative trials were continued in 1915 and we are now fortunately able to furnish further very encouraging evidence. Trials were made this year of Wisconsin grown selected strains in Delaware under the direction of T. F. Manns, in Ohio under the direction of A. D. Selby and J. G. Humbert, and in Iowa under the direction of C. L. Fitch. In all cases yellows occurred and the evidence was convincing. In all these places the Wisconsin selected strains stood up well, whereas the commercial strains showed much disease. There seems full justification, therefore, for the conclusion that the Wisconsin grown selected strains will maintain the disease-resistant character at least in large degree when planted elsewhere.

cabbage. On the other hand, the development of disease-resistant varieties by selection has already given such promising results that full reliance can be placed in it as the feasible method for the practical control of this malady. With the variety to which chief attention has been given, the Hollander, it seems assured that we have secured by selection a head strain, VIIIa 25, which not only represents the best commercial type but has also a high degree of resistance to yellows. This variety will be distributed for commercial use under the name Wisconsin Hollander No. 8. While it should be clearly understood that the trials upon which these conclusions are based have been limited in the main to three seasons and a restricted region, nevertheless sufficient additional evidence has been secured to show that this disease-resistant quality is inheritable and that it holds, at least in considerable degree, when the variety is grown in widely different regions. Some degree of variation from generation to generation and with changed environment must, however, be expected and further trials to determine this are already under way. As to variation from generation to generation, it seems probable that if no attention is given to this there may be a gradual reversion or loss of resistance. On the other hand, by continued trial on Fusarium-infested or cabbage-sick soil and selection repeated annually, it seems probable that we can not only maintain the present degree of resistance but improve upon it.

Although the experience with other varieties has been comparatively limited it has been sufficient to give confidence that strains highly resistant to yellows can be secured without serious difficulty from certain varieties, e. g., Volga and Houser, and that with a little more time and attention disease-resistant strains of the standard kraut and summer cabbages can be selected and fixed. It is, however, quite probable that for the best results local selections will need to be made in the leading cabbage-growing districts of the country to secure the best adaptation to local needs and conditions. With this idea in mind coöperative relations for the continuation of this work have been established between those interested in the United States department of agriculture and in several of the states. While but little progress has been made upon the fundamental problem, the determi-

nation of the nature of disease resistance, and many of the details of more immediate practical interest also require further attention, it is believed that in the main the conclusions as above outlined will hold and that the practical success in the control of the cabbage Fusarium or yellows by the use of disease-resistant strains of seed is assured.

SUMMARY

Cabbage-growing with the winter variety known as Hollander or Danish Ball Head has assumed considerable proportions in various parts of Wisconsin, and with the kraut varieties has become an established local industry in certain sections.

With the reclamation of marsh lands this industry is destined to increase.

A limiting factor to continued success is the disease known as yellows, caused by the parasitic soil fungus *Fusarium conglutinans*. This fungus invades the roots, either in the seed bed or soon after transplanting, and by killing them and working up through the stem so weakens the plant that it yellows, drops its lower leaves, stops growing, and gradually dies or fails to head. As a result on badly infected or cabbage-sick soil the loss ordinarily ranges from 50 to 95 per cent.

This parasite, probably introduced on seed into southeastern Wisconsin some fifteen or more years ago, has rapidly spread in that section and is continually invading new territory elsewhere, so that it seems destined to follow intensive cabbage culture wherever in the state the conditions favor.

The disease is worst when a period of dry hot weather follows soon after transplanting, since a high soil temperature favors its development.

Once introduced into the soil, it persists indefinitely, so that ordinary crop rotations are of little avail for its control.

Various methods of treatment of seed, seedlings, and soil, including the trial of possible disinfectants and fertilizers,were without any practical effect. Steam sterilization of the soil was the only specific remedy used with success and this is, of course, impracticable for field use.

Clean seed, grown in a clean seed bed, and planted in clean soil will give a sound crop, but any contamination of seed bed or field with diseased cabbage refuse or with infected soil, blown, washed, or otherwise carried from a cabbage sick field, will destroy hope of continued success.

The only practical method of control, therefore, seemed to lie in the possibility of securing disease resisting varieties or strains.

Trial of various commercial varieties shows that there are marked differences in susceptibility among them, the Volga and Houser showing the highest degree of resistance against yellows. Neither of these is, however, suited to commercial culture in Wisconsin. The standard winter varieties of the Hollander or Ball Head type are especially susceptible and the practical problem therefore, became that of securing a Fusarium-resistant strain of this type.

The method employed has been based on the observation that even in the worst diseased fields in the autumn there are occasional sound heads. These have been selected, seed raised from them, this grown in turn on the sickest soil available and those plants which remain sound again saved as mother-heads for seed-growing,

By repeated selection we have thus secured strains of winter cabbage of the Hollander type which have proved in a high degree disease resistant against the Fusarium and have at the same time the best commercial qualities. In 1914, the best selected head strain, VIIIa 25, gave a practically perfect stand, the heads averaging about $5\frac{1}{2}$ pounds each, with a total yield of over 18 tons per acre, on thoroughly cabbage-sick soil, whereas the best commercial strain immediately alongside it showed over 80 per cent of yellows, the heads averaging about $2\frac{1}{2}$ pounds each, and yielding about 2 tons per acre. A series of farmers' trials in 1914 showed gratifying practical results. Where selected head strains were grown alongside of the best commercial strains on badly diseased soil, the yields from the selected strain were in some cases ten times that from the commercial strains.

The second generation selections grown in this way have proved even more highly resistant than the first. Thus the first generation of the selected head strain VIIIa showed at the end of the season of 1912, 96 per cent living and 80 per

Four successive cabbage crops on the same field. T
sin Hollander. Yellows destroyed the crop on t
commercial variet

FIG. 20.—EXPERIMENTAL TRIALS, COMMERCIAL VARIETIES, 1911

One row of each variety was planted on "cabbage sick" soil. All succumbed to yellows about alike except Houser, shown at the right center. (See Table II).

FIG. 21.—EXPERIMENTAL TRIALS, RESISTANT STRAINS, 1912

These are resistant head strains, Wisconsin Hollander, first selection.

otographs show strikingly the resistance of the Wiscon-
d in 1910, and the disease has been severe on
ry year since.

FIG. 22.—EXPERIMENTAL TRIALS, COMMERCIAL VARIETIES, 1913

Most plants succumbed badly to yellows showing that the soil continued to be "cabbage sick". (For more details, see Table V and Figure 11.)

Fig. 23.—EXPERIMENTAL TRIALS, RESISTANT STRAINS, 1914

Wisconsin Hollander, second generation selection. Compare with Figure 22. This is the fifth successive cabbage crop, without manure, on this "cabbage sick" land and yet the better resistant strains yielded over 18 tons per acre.

cent headed, whereas the second generation seed from the heads selected from this crop in 1914, under a more severe test, averaged about 99 per cent living and 94 per cent headed, and the best of this series of head strains showed in turn 100 per cent living and 98 per cent headed. It is believed, therefore, that the degree of disease resistance can, by further selection, be at least maintained and probably somewhat increased.

Seed is being grown from some 2000 heads of these selected head strains in 1915, which will permit distribution for planting on a commercial scale in 1916. This will be distributed under the name Wisconsin Hollander No. 8.

Chief attention has been given in this work to the winter or Hollander type of cabbage. Selections have, however, been made from certain standard kraut and early summer varieties and this work is being continued in coöperation with the representatives of certain other state institutions and the United States department of agriculture. It seems reasonable to hope from the experience to date that Fusarium-resistant strains of the various important commercial types may thus be secured.

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Early Blight of Potato and Related Plants

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MADISON

CONTENTS

Early Blight of Potato and Related Plants*

R. D. Rands

The potato is commonly subject to two blights, late and early. In Europe the former is the better known of the two; while throughout the rest of the world, the latter is more generally distributed. It was shown many years ago that early blight is caused by a fungus now known as *Alternaria solani* (E. & M.) J. & G.; but many points in connection with the life history of this organism, its host range, climatic relations, means of overwintering, and control measures required investigation. In this bulletin are presented the more important results of an intensive study of the disease as it has occurred in central Wisconsin during the past three years.

History and Occurrence of the Disease

The early blight of potato was not early recognized as a distinct disease, due perhaps to the general confusion of all the leaf troubles under the term "blight (*Phytophthora infestans*). As soon as attention was concentrated upon these in America, blighting of the foliage not accompanied by tuber rot was noted. Subsequent study led to the differentiation of tip-burn, arsenical poisoning, and early blight.

The causal organism of the latter was first described as a Macrosporium by Ellis and Martin (1882) from the dying leaves of potato near Newfield, New Jersey. The first reference to the fungus as a parasite and its association with potato leaf blight is that by Galloway (1891). He later (1893) states that it was first collected in Missouri in 1885 and in 1890 "complaints of its ravages" came to the United States Department of Agri-

*The writer is indebted to Prof. L. R. Jones for many helpful suggestions during the progress of the study, and the preparation of the manuscript.

culture from widely separated regions in the United States. In this paper he gives an accurate and detailed description of the disease. The fungus was grown in culture, but from the brief description it is uncertain whether these were pure. However, inoculations produced the characteristic spots in from 8 to 10 days. Following this the trouble was reported by workers in most of the middle west and eastern states. For some time there was much disagreement concerning the true cause of the disease. Some believed the Macrosporium only a secondary invader and the disease primarily of nonparasitic origin, while others considered the fungus a parasite but not the cause of all the trouble.

Jones (1893) writing of the disease reports injuries quite similar produced by paris green. Here for the first time, appears a drawing of a diseased leaf, affected unquestionably with the disease as we know it to-day. At this time he suggested the names early blight and late blight to separate the two diseases. It was not until some time later when Jones (1895, 1896) published the results of further studies that the relation of the Macrosporium to the various troubles entirely cleared up. His field and laboratory studies led him to the conclusion that the fungus was a true parasite and the primary cause of early blight. Here also he clearly differentiates the three other forms of disorder which had been confused up to that time under the name "blight," namely, late blight, arsenical poisoning, and tip-burn. Even after this the troubles were not always separated.* Since the work of Jones, very little has been added to our knowledge of the early blight disease. However, during the past two decades much valuable data have accumulated bearing upon the control of the trouble by spraying. During these twenty years, early blight has been reported from practically every state in the union. Outside the United States it has been recorded from Canada, Mexico, South America, Europe, Africa, Australia, India, New Zealand, New South Wales, and Java. Thus it probably occurs wherever the potato is an important crop. As

*As illustrating the confusion at this time, reference may be made to the Cornell Agricultural Experiment Station Bulletin 113, 1896, by E. G. Lodeman. Accompanying a description (p. 254–261) of what is called "early blight" is a colored plate of a potato leaf affected, not with early blight, but with a clear case of tip burn. In the text book, "The Spraying of Plants," by the same author, the illustration on page 346, labeled "early blight" represents a typical form of arsenical poisoning.

to whether the parasite is native to the potato and has spread with it from its original home in South America to the various countries into which the potato has been introduced is largely a matter of speculation. However, Jones (1903) reports finding it on specimens of wild potato from Mexico.

Economic Importance

It is practically impossible to determine the actual loss caused by early blight, owing to the fact that the situation is usually complicated by the presence of tip-burn, arsenical poisoning, flea beetle injury, or late blight. Results from spraying experiments furnish no accurate basis for estimating the loss since bordeaux mixture reduces at the same time the influence of all the other troubles on the vines, and may in itself furnish a stimulus to greater vigor. All reports show that the disease is of greater consequence in the United States than elsewhere, with the possible exceptions of Australia, Rhodesia and New Zealand. Jones (1903) states that in certain seasons *Alternaria solani* causes more loss in many parts of New England than does the mildew. Several cases are on record of unusual attacks, but more important, however, is the smaller but yearly toll of the disease. Coons (1914) averages the annual loss in Michigan as about 25 per cent. In Wisconsin Jones (1912) states that it may reduce the yield 10 to 25 per cent. The writer considers these figures a conservative estimate.

In the southern states, early blight has been reported to attack seriously leaves, stems, and fruit of the tomato. Edgerton and Moreland (1913), in Louisiania, state that it is a close second to "wilt" in destructiveness and in many regions the "all important disease." In one tomato district they estimated a loss of 50 per cent.* Though the disease transfers readily to the tomato and may be found almost every year in the northern states, yet it appears to do little damage. The writer has, however, found it in both the Chicago and Madison local markets as the cause of a severe rotting of tomato fruits from the south. The evidence here indicated that the disease had developed during transit. In the summer of 1916 it was isolated along with *Gleosporium phomoides* Sacc. from decaying tomato fruits at

*Isolations of the fungus from fresh material received from Dr. Edgerton in July 1916 confirmed his diagnosis of the trouble.

Waupaca, Wis., but which fungus was primarily responsible for the trouble was not determined. Inoculation studies reported later in this bulletin show that *A. solani* is capable of producing a spotting no wise different in appearance from that on naturally infected fruit.

The disease has been found the past two seasons on eggplants in Wisconsin; it seems, however, to be of little consequence especially as compared with the leaf spot caused by *Phomopsis vexans* (Sacc. & Syd.) Harter.* In June, 1916, it was found to be causing a serious blight in seed beds of this host at Eau Claire, Wisconsin. It was learned that the hot beds had remained for a number of years in the same place and that it was the practice to sprinkle them frequently with a hose. These factors operating on the crowded, more or less etiolated seedlings may account for the rapid spread and severity of the trouble.

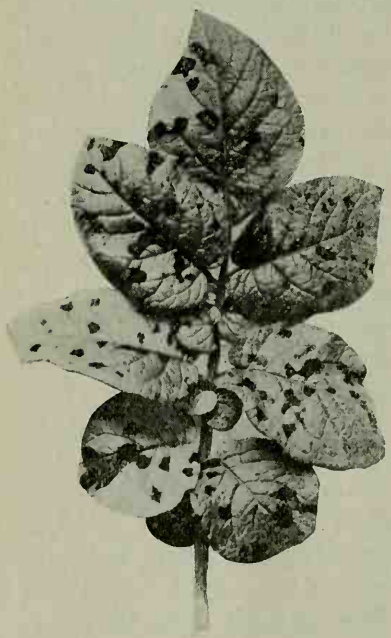

FIG. 1.—EARLY BLIGHT OF POTATO

The leaf is soon weakened from the enlargement of the spots. (Photograph by L. R. Jones.)

SYMPTOMS

The appearance of the spots on the leaves of each of the three common hosts is very similar. They are dark brown or black and show usually a series of concentric ridges which produce a "target board" effect. (Fig. 3) There is often a narrow marginal faded zone which spreads outward as the spot enlarges. The spots are usually oval in shape but under unfavorable conditions, especially on a vigorous leaf, may remain small and angular conforming to the spaces between several small veins. (Fig. 1.) The spots usually enlarge after the death of the leaf. On the tomato the disease may be

* *Alternaria solani* was first recorded on egg-plant by Chester (1893) in Delaware. Later it was listed by Clinton (1904) in Connecticut.

easily mistaken for the leaf spot (*Septoria lycopersici*) which has been much more common on Wisconsin tomatoes during the past two seasons. Without the aid of a hand lens the spots on the egg-plant are almost indistinguishable from those caused by *Phomopsis vexans*. Early blight on the potato is readily distinguished from arsenical poisoning by the darker color of its spots. With tip-burn the leaflet usually shows apical or marginal burning and the concentric rings are absent. There is still less resemblance to the late blight because of the whitish fructification of the ventral surface of leaves affected with the latter trouble.

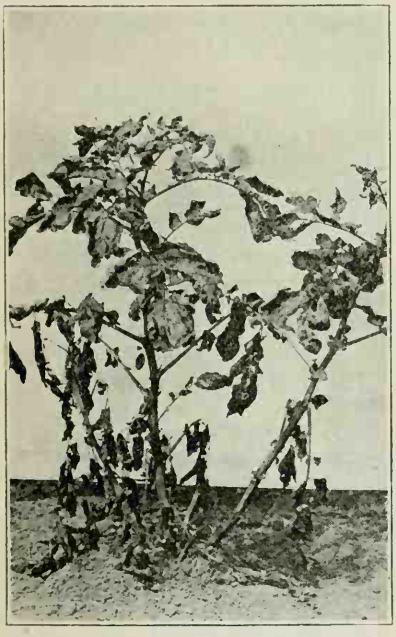

FIG. 2.—A SINGLE HILL OF POTATO DYING FROM EARLY BLIGHT

Early Ohio planted April 28, photographed August 12, 1915. Note the progressive curling and drying of the leaves from the ground upward.

Potato plants may be attacked by early blight at almost any stage of their existence, but, under ordinary conditions, the disease is not able to gain a foothold until the vines have passed their period of greatest vigor and are directing their energy to tuber formation.

Before this time, close scrutiny will generally reveal an occasional spot on the lower, older, and more shaded leaves of the plant. Such leaves have frequently been covered and uncovered (with soil) a time or two during the process of cultivation and are consequently yellowed and weakened. Under favorable conditions the spots increase rapidly in number, and the leaves beginning with the lower ones gradually die until only a few green, spotted leaves remain at the top of the plant. (Fig. 2.) In severe cases spots develop on the petioles and upper stems of the plant.

STUDIES ON THE HOST RANGE OF ALTERNARIA SOLANI

The primary object of these studies was to determine whether the leaf spots of potato, tomato, egg-plant, and Jimson weed (*Datura stramonium*), which have been ascribed to this fungus, were produced by one and the same species of Alternaria. Jones (1896) proved beyond doubt the parasitic relationship of *Alternaria solani* to the early blight of potato, but its connection with the other plants has never been conclusively shown by inoculation tests. The failure of inoculations on Datura and the comparative studies of *Alternaria solani* and the Datura fungus show that the latter is a distinct species, bearing no similarity to *A. solani* in its host relationship. The results are published elsewhere (Phytopathology 7: 327–337, 1917)* The secondary object was to determine within what limits the parasitism of *Alternaria solani* is confined.

During the summer of 1915, pure cultures from single spores were obtained for inoculation purposes from potato, tomato, and egg-plant growing at Waupaca, Wis. They were later grown comparatively on fifteen kinds of agar media and in appearance were practically identical. Abundant spores for inoculations were obtained from each by a method later referred to.

GREENHOUSE INOCULATIONS

The following inoculation methods were used with more or less success in greenhouse experiments made from February to May, 1916; temperature 19 to 23° C.

(1) Drop of heavy spore suspension placed on flat portion of leaf inclosed by round cover slip. Plant placed in glass moist chamber for 48 to 72 hours.

(2) Spores or mycelium introduced into needle punctures. Plant placed under bell jar and atomized frequently with water for 48 hours.

(3) Leaves atomized with spore suspension and for 48 hours kept moist by fine spray from nozzle.

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* This Datura leaf spot which has been widely attributed to *Alternaria solani* is shown to be due to the fungus named *Cercospora crassa* by Saccardo in 1877. Examination of type specimens collected by Saccardo and of exsiccati from various parts of the United States show that the fungus was named from immature material and is really an Alternaria. The new combination, *Alternaria crassa*, with technical discription is given in the article in Pytopathology referred to above.

TABLE 1.—GREENHOUSE INOCULATIONS, MADISON, 1916

Date	Source of inoculum	Plant inoculated; condition, etc.	Method of inoculation	Results
Feb. 25	Potato strain, *A. solani*; mycel on bits of agar	Potato-2 plants 8-10 in. high; vig. 10 leaflets inoc.	No. 2	March 3, 80% infection; spots 8-20 mm. diam on both plants
		Tomato-1 plant vig. 10 leaflets inoc.	No. 2	March 3, 75% infection; spots 4-6 mm. diam.
Apr. 13	Potato strain, *A. solani*; spores from culture	*Solanum nigrum*-1 plant 4in. high; vig.	No. 1	April 20, 90% with spots 1-4 mm. diam.
		Eggplant-1 plant 3 in. high; vig.	No 1	April 20, 100% infection; spots 1-2 cm. diam.
		White Burley tobacco-2 plants 6 in. high; vig.	No. 1	April 20, few spots 1 mm. diam. no further enlargement
Apr. 13	Potato strain, *A. solani*; spores from culture	Potato-1 plant 10 in. high; vig.	No. 3	April 20, minute spots on every leaf; wet continuously
		Tomato-1 plant 8 in. high; vig.	No. 3	April 20, few spots; not wet continuously
		Eggplant-1 plant 4 in. high; vig.	No. 3	April 20, many spots on every leaf; wet continuously
May 7	Potato strain, *A. solani*; spores from culture	Potato-1 plant 14 in. high; vig.	No. 3	May 14, many spots 1-3 mm. diam.
		Tomato-2 plants 16 in. high; vig.	No. 3	May 14, few spots on lower leaves, 2-3 mm. diam.
		Solanum nigrum -2 large plants; fairly vig.	No 3	May 14, few spots on lower leaves, 1-5 mm. diam.
Apr. 14	Eggplant strain; spores from culture	Eggplant-1 plant 5 in. high; very vig.	No. 1	April 20, 100% with spots 3-4 mm. diam.
		Potato-1 plant 12 in. high; vig.	No. 1	April 20, 90% with spots 1-3 mm. diam.
		Tomato-1 plant 12 in. high; very vig.	No. 1	April 20, 80% with spots 2-3 mm. diam; enlarge very slowly
Apr. 14	Tomato strain; spores from culture	Tomato-1 plant 12 in. high; very vig.	No. 1	April 20, 100% with spots 2-3 mm. diam.
		Potato-1 plant 15 in. high; vig.	No. 1	April 20, 100% with spots 3-4 mm. diam.
		Eggplant-1 plant 8 in. high; vig.	No. 1	April 20, 100% with spots 6-8 mm. diam.

These experiments are briefly summarized in Table I. In most cases reisolations from the infected plants were successful. The results show that in the majority of cases *Alternaria solani* from potato crossed readily to tomato and egg-plant, to some extent to nightshade (*Solanum nigrum*), and to cultivated tobacco. In the latter case, penetration occurred, but the mycelium seemed to be unable to spread in the tissues of these vigorous seedlings.

The strains isolated from tomato and egg-plant reciprocally crossed quite readily and both in turn produced a spotting of potato in no wise different from that of ordinary early blight on potato. Aside from a few explainable exceptions the uninoculated needle punctures healed, and in method 3, the plants exposed beside the inoculated plants never developed spots. Therefore it seems justifiable to conclude that the early blight of potato, tomato, and egg-plant are caused by one and the same organism, viz., *Alternaria solani.*

Owing to the difficulty of working with mature plants in the greenhouse it was decided to continue the tests under field conditions.

FIELD INOCULATIONS

Field tests were carried out at Waupaca in central Wisconsin during the summer of 1916. In order to determine within what limits the parasitism of this fungus is confined, it seemed desirable to obtain a wide range of plants, especially as to genera, of the potato family. The effort was successful only to a limited extent because it was impossible, on a few months notice to get seed, particularly of the wild members of the family.*

Eight to ten plants of each species and variety were properly spaced in rows three feet apart, with every third row in potatoes to furnish a basis for comparison. The potatoes were planted May 11 and the other plants were transferred from the greenhouse in early June. The severe and prolonged drought during July and August proved a serious setback, but by artificial watering most of the plants made normal growth. Prior to Sep-

*The writer is indebted to Messrs. Peter Bisset, Plant Introducer U. S. Dept. of Agriculture, Geo. T. Moore, Missouri Botanical Garden, St. Louis, and W. S. Oswald, Minnesota Seed Labratory for seeds or plants furnished for this work.

tember 4 conditions for natural infection were very unfavorable and spots which appeared earlier on the potato did not spread. On account of the extreme heat, artificial conditions for infection could not be maintained with the means at hand. After August 15, several plants of each species were atomized occasionally with spores in order to have the plants ready for rainy weather when such a favorable condition for infection should arrive. Spores from pure culture of the potato strain were used. September 8, several leaves on selected plants of each species were inoculated by the needle prick method, i. e. by placing a drop of heavy spore suspension on each puncture but always leaving an equal number uninoculated for control. The drought was broken on September 4 when a period of moist weather with heavy dews and rains set in, furnishing ideal conditions for infection by *Alternaria solani*.

The main results from these field inoculations are presented in Table II which shows: (1) size and condition of the plants on September 19 and (2) the progress of the disease two weeks after and one month after the beginning of the rainy period.

In most cases an attempt was made to reisolate the fungus from the smaller spots even where sporulation occurred on large spots of the same plant. In several instances, it will be seen that the fungus was not reisolated though spores are recorded for the larger spots. This was probably due to the presence in the plates of the saprophytic fungus, *Alternaria fasciculata*, which is the more rapid grower and is difficult to eliminate. Inoculations on Nos. 3, 4, 5, 9, 13, 14, 24, and 26 were repeated in the pathological garden at Madison, Wis., in September and October, 1916. As the results agree in all essentials with those tabulated, for the sake of brevity they are not listed here.

The table shows that the fungus was able to penetrate almost every plant inoculated. Even the leathery, succulent leaves of *S. grandiflorum* and *S. guttata* were infected as was the potato. Leaves of the former inoculated September 2 were found to be thickly peppered with tiny infection spots September 9. These spots, which measured less than two millimeters in diameter, had made no enlargement when the leaves were again examined a month later. Yet when cultures were made from such spots, October 14, almost every one developed the fungus. What checks the advance of the fungus in the tissues of these plants is not

TABLE II—RESULTS OF FIELD INOCULATIONS WITH ALTERNARIA SOLANI ON VARIOUS GOLANACEOUS PLANTS

Plants tested	Size and condition of plants September 19	RESULTS ON SEPTEMBER 19		RESULTS ON OCTOBER 9			Reisolation of the fungus
		Atomized leaves	Needle punctured leaves	Atomized leaves	Needle punctured leaves	Spore production	
1. *Solanum aviculare* Forst.	12-14 in. high; mature, healthy	Spots 3-12 mm. diam.	90% infection; spots 3-4 mm. diam.	Lower leaves dying from the disease	100% infection; spots 4-6 mm. diam.	Abundant	Yes
2. *S. burbankii* Bittel. small wonderberry	8 in. high 24 in. wide; much weakened by flea beetle	Many specks .5-1 mm. diam.	Leaves dropped off	No enlargement		0	Yes
3. *S. pseudocapsicum* Linn. Jerusalem cherry	6-8 in. high; fairly vigorous	Peppered as above	No evidence of infection	No enlargement	No infection	0	Yes
4. *S. carolinense* Linn. horse nettle	18-24 in. wide; in fruit, vigorous	Spots 1-2 mm. diam.	80% infection; spots 2-3 mm. diam.	Spots on green leaves, 3-4 mm., on yellow leaves 6-8 mm. diam.	Leaves dying from the disease	Abundant	Yes
5. *S. giganteum* Jacq.	2-3 ft. high; very vigorous	Thickly spotted 1-3 mm. diam.	100% infection; spots 5-8 mm. diam.	Spots on old leaves 8-15 mm. in diam., on young leaves 1-4 mm. diam.	Spots 8-10 mm. diam.	Abundant	Yes
6. *S. grandiflorum* Ruiz. and Pav.	6-8 in. high; small, succulent, vigorous, leathery leaved shrub	Spots .5-2 mm. diam.	No evidence of infection	No enlargement	No evidence of infection	0	Yes
7. *S. guttata*	as above	Spots, including yellowish zone, 1-3 mm. diam.	No inoculation	No enlargement even on yellowing leaves		0	Yes
8. *S. jasminoides* Paxt. potato vine	2 ft. high; vigorous woody vine	Spots 1-1.5 mm. diam.	Margins of punctures brownish	Slight increase in size	20% infection; spots 1-2 mm. diam.	0	Yes

9. *S. melongena* Linn. egg plant	1-2 ft. high; weakened by drought	Spots .5-10 mm. diam.	100% infection; spots 8-10 mm diam.	Most of the lower leaves dead from the disease	Abundant	Yes
10. *S. nigrum* Linn. black nightshade	6-10 in. high; upper leaves fairly vigorous	Leaves atomized at Madison Oct. 21; Oct. 30, numerous spots 1 mm. diam.		Nov. 4, many spots 3-5 mm. diam.	0	Yes
11. *S. nigrum var. guinennse* Linn. garden wonderberry	3-4 ft. high; weakened by drought and red spider	Thickly peppered .5-1 mm. diam.	Leaves dropped	Yellowed leaves, spots 3-15 mm. diam.	Few	No
12. *S. rostratum* Dunal. buffalo burr	18-24 in. high; fairly vigorous	10-15 spots per leaf 3-5 mm. diam.	100% infection; spots 8-10 mm. diam.	Lower leaves yellowing and dying from abundance of spots	Abundant	Yes
13. *S. tuberosum* Linn. early Ohio	12 in. high; young upper leaves vigorous	Many spots, 3-5 mm. diam., coalescing	100% infection; spots 3-6 mm. diam.	Leaves dying from disease	Few	Yes
14. *S. warszewiczii* Hort.	1-3 ft. high; very vigorous	Peppered with spots 1-4 mm. diam.	100% infection; spots 3-4 mm. diam	Leaf yellowing; spots 3-8 mm. diam. / Lower, older leaves; spots 10-15 mm. diam.	Abundant	Yes
15. *S. wendlandii* Hook.	2 ft. vigorous leathery leaved woody vine	Thickly spotted 1-4 mm. diam.	100% infection; spots 1-4 mm. diam.	Leaves on the ground; no increase in size of spots	0	Yes
16. *Physalis francheti* Mast. Chinese lantern plant	8-10 in. high; very vigorous	Few specks, .5-1 mm diam.	Faint browning about punctures	Few minute elevated dots / No further development	0	No
17. *P. pubescens* Linn. husk tomato	12-16 in. high; much weakened by drought and red spider	Few spots on yellowed leaves 2-3 mm. diam.	Leaf dropped	No enlargement; leaves on ground	0	Yes
18. *P. virginiana* Mill. wild ground cherry	12-16 in. high; weakened by drought	Few small spots	No inoculation	No further development	0	
19. *Nicandra physalodes* (L.) Pers. apple of Peru	2-3 ft. high; fairly vigorous	Many spots 1-10 mm. diam.	90% infection; spots 5-12 mm. diam.	Leaves yellowing and dying; spots 3-10 mm. diam. / Leaves dying from the disease	Abundant	Yes

TABLE II—RESULTS OF FIELD INOCULATIONS WITH ALTERNARIA SOLANI ON VARIOUS SOLANACEOUS PLANTS—Continued

Plants tested	Size and condition of plants September 19	Results on September 19		Results on October 9			Reisolation of the fungus
		Atomized leaves	Needle punctured leaves	Atomized leaves	Needle punctured leaves	Spore production	
20. *Lycopersicon esculentum* Mill. Yellow peach	18-24 in. high; fairly vigorous	Spots abundant 1-5 mm. diam.	No inoculation	Lower leaves dying from abundant spots		Few	Yes
Red currant	16-18 in. high; weakened by drought	Spots abundant 1-5 mm. diam.	No inoculation	Most of leaves dead due to the disease			
Dwarf stone	10-20 in. high; very vigorous	Abundant spots on lower, faded leaves, 1-4 mm. diam.	No inoculation	Some enlargement		Few	
Stone	18-24 in. high; fairly vigorous	Spots on all mature leaves 2-6 mm. diam.	40% of punctures with browned margins, leaf young	Some enlargement	Spots 1-3 mm. diam.	Few	Yes
Earliana	As above	As above	No inoculation	Some enlargement		Few	
21. *Capsicum annuum var. grossum* Linn. green pepper	12-14 in. high; vigorous, in fruit	Peppered with spots, .5-1 mm. diam.	Margins of punctures brownish	No increase; raised margins of spots indicate healing	No change	0	Yes
22. *Nicotiana alata var. grandiflora* Comes, ornamental tobacco.	18-24 in. high; fairly vigorous	Peppered as above; spots on old leaves, 2-4 mm. diam.	As above	No enlargement; dying leaf at base with few dead areas 8-10 mm. diam.	No change	Few	No
23. *N. tobacum* Linn. White Burley	4-5 ft. high; lower mature leaves healthy	No evidence of infection	No change	No change	Margins of punctures healed	0	

24. *Hyocyamus niger* Linn. black henbane	8-12 in. high; vigorous	Numerous spots 1-5 mm. diam.	60% infection; spots 3-8 mm. diam.	Spots 3-10 mm. diam.	Spots further enlarged	Abundant	Yes
25. *Petunia hybrida* Hort. white petunia	10-20 in. high; vigorous	Numerous elevated specks	40% infection; spots 1-4 mm. diam. light colored	Spots barely visible	No change: leaf yellow	0	Yes
26. *Lyceum vulgare* Dun. matrimony vine	2-3 ft. long; leaves weakened by red spider	Many small specks, .5-1 mm. diam.	Slight browning about punctures	No change	No change	0	No

known. It is believed that such plants, i. e., those on which the spots do not enlarge, should not be considered as hosts, since on them the fungus does not produce spores and therefore can-

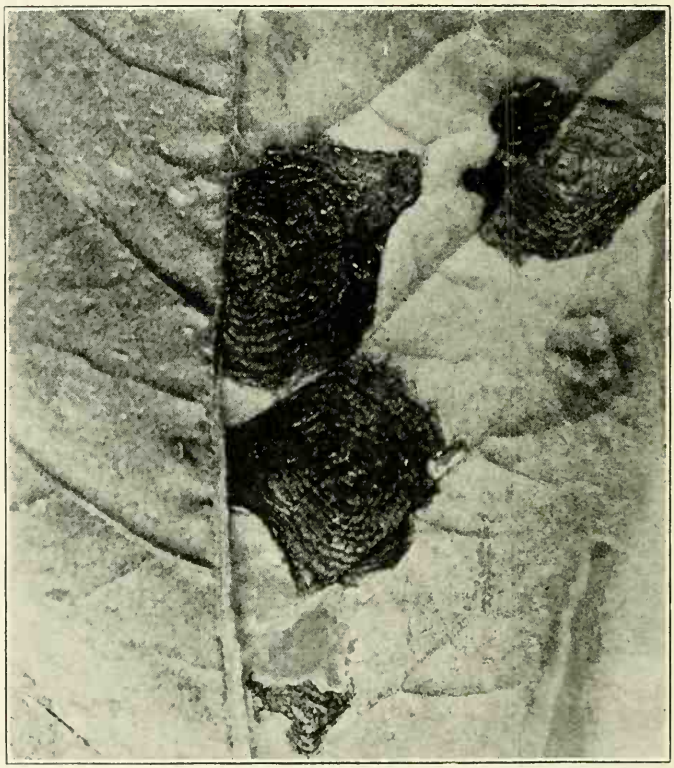

FIG. 3.—POTATO EARLY BLIGHT SPOTS ENLARGED X 3

These show the typical black target board appearance. (Photograph by H. H. Whetzel.)

not complete its life cycle. On this basis the hosts of *Alternaria solani* determined by these studies are listed below.*

1. *Solanum aviculare* Forst.
2. *Solanum carolinensis* Linn.—Horse nettle
3. *Solanum giganteum* Jacq.
4. *Solanum melongena* Linn.—Egg-plant
5. *Solanum nigrum* Linn.—Nightshade
6. *Solanum nigrum guinennse* Linn.—Garden wonderberry

*Though not included in these studies it is probable that the two following species are also hosts of *A. solani*.

Solanum commersoni Dun. listed by Nusslin (1905) and Stuart (1914).

Hyocyamus albus Linn. White Henbane, according to Ferraris (1913).

7. *Solonum rostratum* Dun.—Buffalo burr
8. *Solanum tuberosum* Linn.—Potato
9. *Solanum warscewiczii* Hort.
10. *Hyocyamus niger* Linn.—Black henbane
11. *Lycopersicon esculentum* Mill.—Tomato
12. *Nicandra physalcides* Gaertn.—Apple of Peru

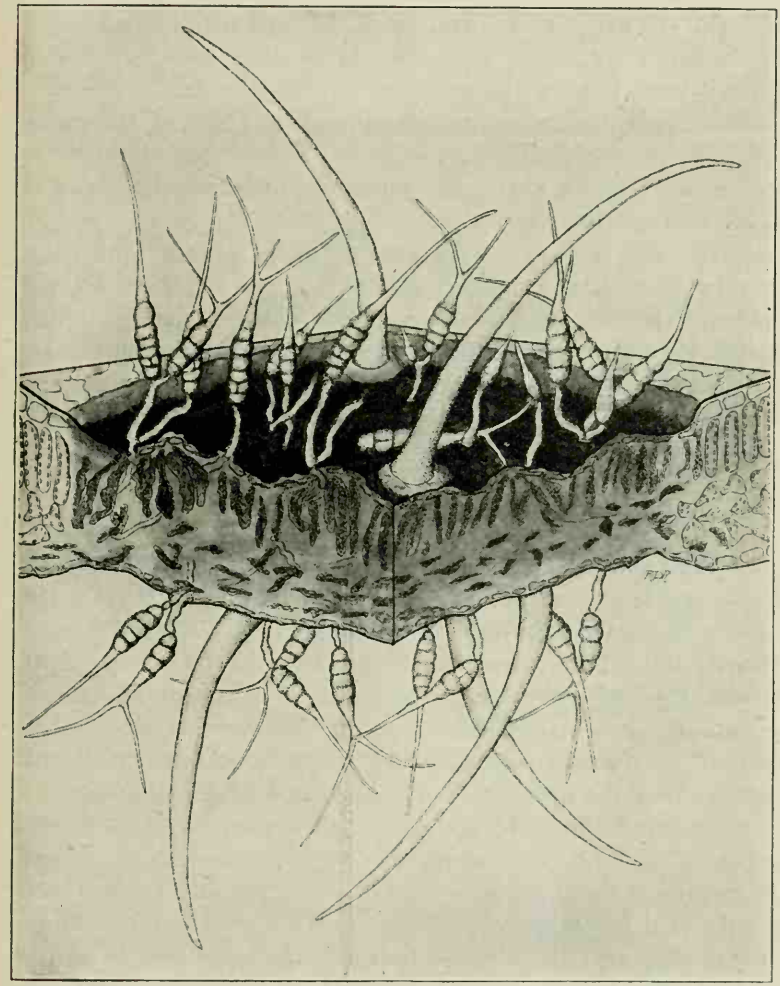

FIG. 4.—DIAGRAMMATIC REPRESENTATION OF A PORTION OF SPOT ENLARGED

The invaded tissue shrinks to about one half the original thickness of the leaf, and the surface is thrown into concentric ridges. The cells are darkened. Spores are produced on both surfaces as shown above intermingled with the hairs.

During May, 1916, inoculations were made on tomato fruits of various ages freshly picked from greenhouse plants. In two

trials spores atomized on the surface failed to give infection after 10 days even though the fruits were moistened frequently and kept in a damp chamber. Under the same conditions needle puncture inoculations invariably resulted in infection. After 15 days there was only slight invasion about the points of inoculation on the green fruits while with the ripe fruits almost complete rotting resulted. McCubbin (1916) in Ontario, reports similar results from inoculations of tomato fruits. Needle puncture inoculations were made on mature fruits of egg plant and green pepper during August, 1916. In each case slight invasion of the tissues about the punctures occurred but no enlarged spots or decay resulted.

Of the nine genera of the potato family tested, four only were found able to perpetuate the fungus, viz., Solanum, Lycopersicon, Nicandra and Hyocyamus. Of these the Solanums though showing considerable variation appear as a group to be the most susceptible. From these experiments it is evident that *A. solani* is not restricted within very narrow limits in its host relationship.

PATHOLOGICAL ANATOMY

An explanation of the "target board effect" (Figs. 3 and 4) characteristic of this disease is suggested by Jones (1896). He believes that such a condition is produced by the more complete collapse and rapid contraction of the interior cells or mesophyll as compared with the epidermal cells. A study of microtome sections of spots in various stages of development shows that greatest contraction occurs in the spongy tissue which would tend to throw the upper part of the leaf into concentric folds.

In the spot the cells are collapsed, shrunken, and deeply stained (Fig. 5). No evidence has been obtained to show that the failure of small spots to enlarge on vigorous leaves was due to suberized layers or other mechanical hindrance to invasion. On the contrary, all evidence indicates the resistance to be directly related to the vigor of the leaf. Though the fungus has never been actually observed inside the cells of the host, there seems no reason to suppose that it cannot enter them. Penetration of the leaf usually occurs directly through the epidermis, and in pure culture the fungus can utilize cellulose when this is offered as its only source of carbon.

THE CAUSAL ORGANISM

TAXONOMY

In the literature on early blight the fungus is commonly referred to under the following names—*Macrosporium solani* Ellis and Martin, *Alternaria solani* (E. & M.) Jones and Grout, and *Alternaria solani* Sorauer. In foreign references the latter is in more general use while the second occurs most frequently in accounts of the disease in America. Sorauer (1896) published

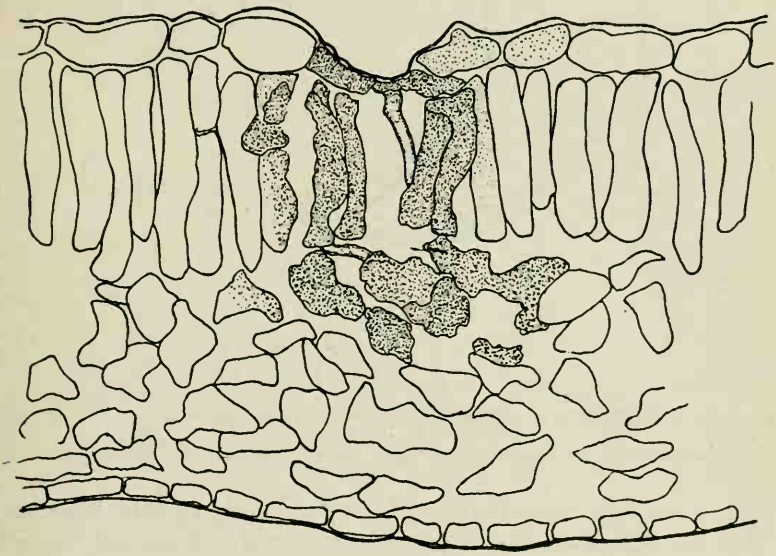

FIG. 5.—CROSS SECTION OF LEAF SHOWING INCIPIENT INFECTION OF ALTERNARIA SOLANI

Penetration usually occurs directly through the cuticle. Shrinkage follows the death of the cells. X4 0.

on the fungus a few months in advance of Jones (1896), but his observations and illustrations of spore chains, as he found them in crude hanging drop cultures, show plainly that his description was based on *Alternaria fasciculata*. From type material received from Sorauer, Jones separated the two fungi, the one a typical Alternaria and a saprophyte which he subsequently named *Alternaria fasciculata*, and the other the true parasite (*Macrosporium solani*). Jones reports frequent cases where spores in cultures of the Macrosporium were joined in catenulate pairs after the fashion of the Alternarias. He then writes a

technical description and gives Sorauer the credit for the new combination. Seymour (correspondence), however, later ruled that inasmuch as Sorauer had applied the binominal confusedly, authority for the new combination should rest with Jones and his assistant (Jones and Grout 1897). This is the usage of Farlow (1905) and of most recent American authors. McAlpine (1903) and Duggar (1909) have objected to calling the fungus an Alternaria on the ground that the catenulation of spores does not occur in nature. The author has examined many spots and

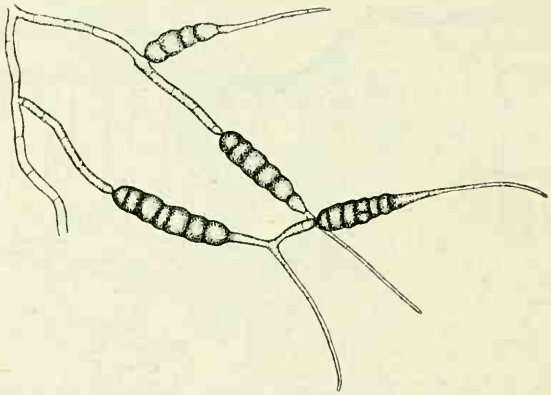

FIG. 6.—MATURE SPORES OF ALTERNARIA SOLANI

Spores drawn from pure culture where catenulation has been noted. X200.

has never seen catenulation on the leaves. It is true, however, that on oat meal agar cultures, spore pairs frequently occur (Fig. 6). In view of the chaotic condition of the literature dealing with Macrosporium and Alternaria and the slight and uncertain distinctions between the genera, the author considers it inadvisable to break away from the well established usage and go back to Mascrosporium.

The following is the probable synonomy of the fungus with citations to the literature:

Alternaria solani (E. & M.) Jones and Grout.
Bull. Torrey Bot. Club **23**:353. Sept. 1896.
Vt. Agr. Exp. Sta. Rept. **10**:45. 1896.
Macrosporium solani E. & M.
American Naturalist **16**:1003. 1882.
Macrosporium solani Cooke. (in part)
Grevillia **12**:32. 1883.
Macrosporium cookei Sacc. (in part). (following Cooke)
Sacc. Sylloge Fungorum **4**:530. 1896.

Alternaria solani Sorauer (in part).
Zeitschr. für Pflanzenkrankheiten **6**:6. 1896.
Sporidesmium solani var. *varians* Vanha.
Naturw. Ztschr. Land- u. Forstw. **2**:113–127. 1904.

MORPHOLOGY

The mycelium at the margin of the spot can be seen, using *in toto* fixations, as slender, radiating, sparsely branched filaments. Later it becomes closely branched, irregular, and deeply stained. Conidiophores have never been found arising nearer than one-

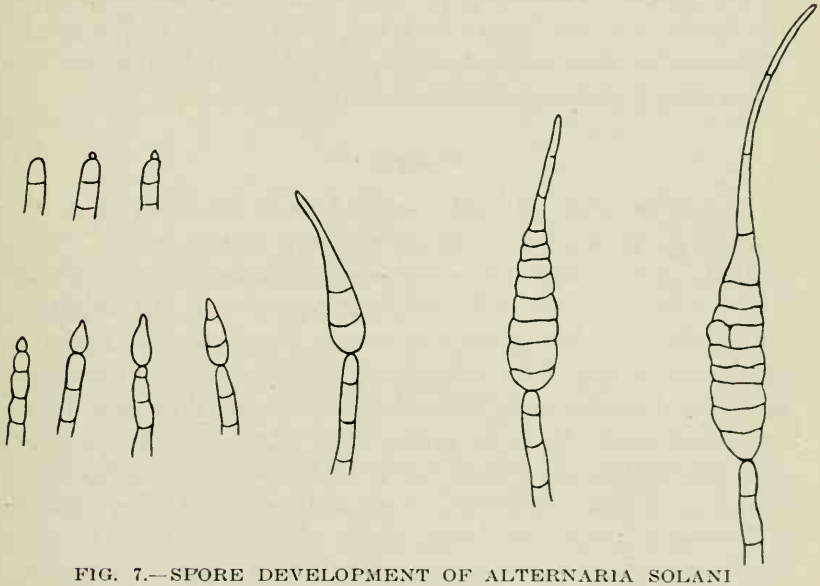

FIG. 7.—SPORE DEVELOPMENT OF ALTERNARIA SOLANI

Progressive stages commencing in the upper left hand corner. Note that the spore starts by budding from the tip of the apical cell of the conidophore as shown in the second and third stages. Drawn from culture X400.

half a millimeter from the boundary of green tissue. Usually one spore is produced on a conidiophore. The conidia arise from the conidiophore, not by the constriction and subsequent enlargement of a terminal cell, but from a bud which forms on that cell. (Fig. 7.) The first indication of the bud is a faint hyaline area on the wall. Soon (often within a few minutes), the wall at this place pushes out and forms a minute projection which has an extremely thin wall and is less than one-fifth the diameter of the conidiophore. This bud grows very rapidly at first and, on this account, the early stages are not easily followed.

A method for obtaining abundant sporulation in pure cultures of this fungus is described elsewhere.* Spores thus produced show greater uniformity in size than those from the spots. Measurements of 100 spores from large, typical early blight spots on potato leaves gave a range in size of 120–296 x 12–20 microns, an average size of 200 x 17 microns. The same number taken from several pure cultures on potato agar gave a range of 104–184 x 14–18 microns, an average of 141 x 16 microns.

Nothing to date has indicated the existence of a perfect stage of this fungus. Overwintered material has been examined and the fungus has been grown on many kinds of media of varying degrees of acidity and exposed to various temperatures but no indications of another stage have developed.

PHYSIOLOGY

Alternaria solani is easily isolated from the spots or from spores, and grows well on all the ordinary culture media. Perhaps the most striking physiological characteristic of the fungus is the intense discoloration which it produces in the medium. On potato agar, young colonies cause a clear yellow pigmentation which, as the colony enlarges, spreads in advance of the mycelium and is eventually succeeded beneath the older part by a deep wine color. In media made +20 Fuller's scale, the coloration approaches a deep brick red in some cases. On slightly acid media the yellow pigmentation predominates and it is practically absent in alkaline media, where also little growth occurs. There is likewise no discoloration when the fungus is grown on +10 to +15 casein agar, nutrient gelatin containing dextrose, starch-nitrate agar, and cellulose agar. After 7 to 10 generations in pure culture the pigmentation is much diminished and in some cases has been observed to almost disappear.

The fungus readily liquefies the above gelatin medium and shows great proteoclastic activity in the utilization of casein as indicated by the clear zone surrounding the colony when lactic acid is added to a casein agar plate. Nitrates are quickly reduced to nitrites and even to ammonia when tested on starch-nitrate agar.

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*Phytopathology 7: 316–317. 1917. This method consists first, in severely wounding the mycelium by shredding a ten day old culture of the fungus on potato agar, and second, for 24 hours, controlling the moisture relation so that the surface does not become dry.

Temperature relations.—Both spore germination and colony growth of *A. solani* are greatly influenced by temperature. At 20°C., ordinarily five to ten germ-tubes arise from the different cells of a single spore, while at 1–3°C., germination will finally occur, but with no more than 2 or 3 germ-tubes. (Fig. 8.) Spores germinated at a low temperature generally produce several

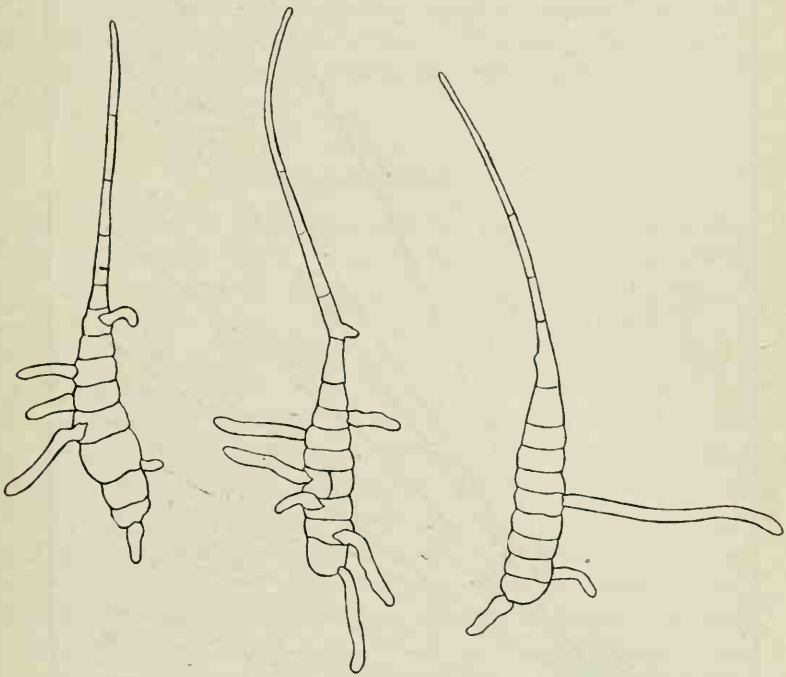

FIG. 8.—GERMINATION OF SPORES OF ALTERNARIA SOLANI

Temperature is an important factor in determining the number of germ tubes and the rate of germination: the two spores to left with the greater number of germ tubes after 1½ hours at 35° C.; the spore to the right with fewer germ tubes after 46 hours at 1-2° C.

more germ-tubes when removed to a higher temperature. Extended studies have been made of spore germination in agar under seventeen different temperatures ranging from 2 to 45°C., in which at intervals the approximate length and number of germ-tubes were determined. These results are plotted in Fig. 9. At all temperatures from 6 to 34°C., the spores germinated within one and one-half hours. Germination took place most rapidly at 28–30°, requiring at those temperatures but 35 to 45

minutes. The germ-tubes formed at 37° were irregular and knotted with bladder-like swellings at the tip. Growth entirely ceased after six hours and subsequent transferenee to a lower temperature showed that they were dead. At 45° the spores were killed before any indication of germination appeared.

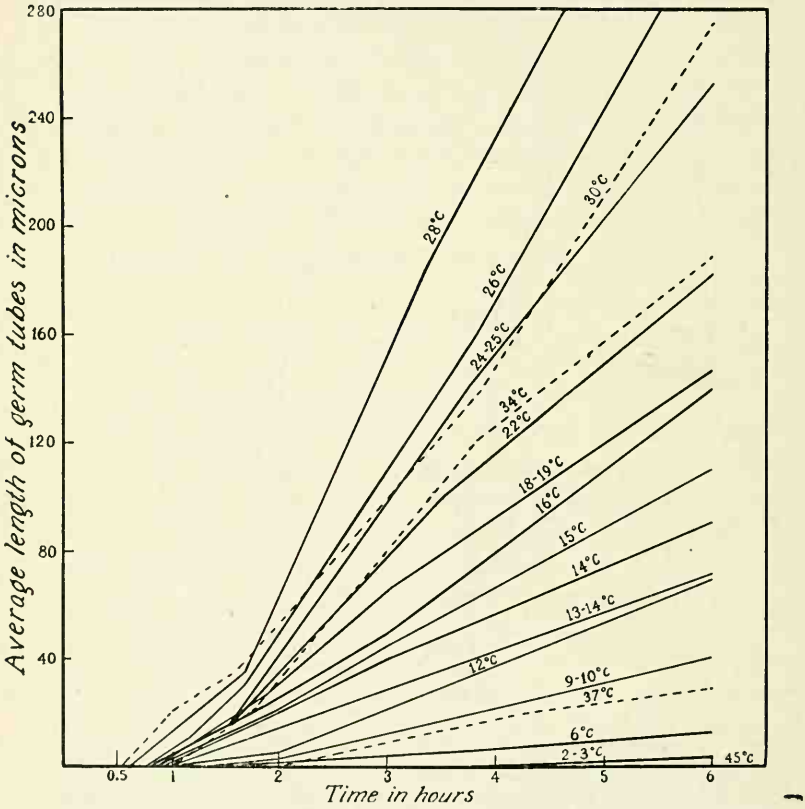

FIG. 9.—THE EFFECT OF VARIOUS TEMPERATURES ON SPORE GERMINATION AND GROWTH OF ALTERNARIA SOLANI

At most temperatures germination commenced during the first hour. The optimum is 26-28° C. At 45° C the spores were killed.

Measurements of colonies grown at these different temperatures give a graph similar to that obtained for spore germination. However, no growth visible to the naked eye took place at 3° or at 45°C., while at 37° there was a slight amount of aerial mycelium. The cardinal temperatures of the fungus are therefore approximately as follows: minimum 1°–2°C., optimum 26°–28°, and maximum 37°–45°.

LIFE HISTORY OF A. SOLANI IN RELATION TO EARLY BLIGHT

SEASONAL DEVELOPMENT OF THE DISEASE

The time at which this disease makes its appearance each year seems to depend largely upon the date at which the crop was planted and upon its subsequent development as influenced by soil and climate. It may be safely concluded that as soon as the crop has passed its stage of greatest vigor and tuber formation has begun, early blight may develop. Whether or not the attack becomes severe depends almost entirely on influencing factors later enumerated.

SPORE PRODUCTION

Spore production is usually delayed until after the death of the host tissues. Very rarely are spores found on spots less than four millimeters in diameter. Both upper and lower surfaces of a spot produce spores, the upper much more abundantly, however. They are very easily dislodged, especially by rainfall. While considerable variation has been noted in the relative abundance of spores on spots of different sizes, it was desired to get some idea of the actual numbers which may occur. For this purpose, spots developed under as favorable natural conditions for sporulation as possible were obtained and counts made. Each spot cut carefully from the leaf was rinsed in a given volume of water which, with a small amount of leached agar, was poured into a level petri dish. One-tenth areas were marked off with a bacteriological counting card. After germination had begun the spores in two such areas were counted by means of the low power of the microscope. The following results were obtained:

Diameter of spot	Distribution as noted on spot	Number of spores
10mm.	Apparently equally abund. on both surfaces	1475
7mm.	Few below, abundant above	930
10mm.	About one-half as many below as above	785
5mm.	Few below, abundant in center above	415
8mm.	Scattered on both surfaces	140
6mm.	Few on both surfaces	115

These figures may give some idea of the abundance of spores which may be produced on a badly diseased plant with, for instance, ten to fifteen spots on every leaflet. The total number

TABLE III.—DEVELOPMENT OF EARLY BLIGHT SPOTS AND SPORULATION OF ALTERNARIA SOLANI ON EARLY OHIO, WAUPACA, WIS., 1916

Leaf and Spot Nos.	Spot Histories in Relation to Climatic Conditions July 13-28																
	13th. Light dew, clear, warm	14th. Rain .2, heavy dew, clear, warm	15th. Rain .23, lvs wet until 9 a. m., clear, warm	16th. Heavy dew, clear, warm	17th. Heavy dew, cloudy, rain .03 (Showers a. m.) p. m. clear, warm	18th. Medium heavy dew, clear, warm	19th. Light dew, clear, warm p. m. Cloudy, rain (.15). (Notes made before rain)	20th. Light dew, clear, warm	21st. Very heavy dew, clear and warm	22nd. Very light dew, clear, warm	23rd. Light dew, clear, warm	24th. Light dew, cloudy, rain (.05) 10-12 a. m.	25th. Heavy dew, cloudy, windy	26th. No dew, clear, warm; plants watered heavily with hose 5 p. m.	27th. Very light dew, clear, warm	28th. No dew, clear, warm
1a	5x8 mm. lf. vig; sp. + ab. — bl.				8x12 mm. sp. o ab; ++ bl.	8x14 mm. sp. +++ ab. bl; lf. yellowing	9x15 mm. sp. —over center ab; scattered bl. (not removed)		10x15 mm. sp. —scattered ab. bl; lflt. dead	No change						
1b					5 mm. sp. o. lf. vig.	6 mm. sp. +++ ab. bl.	7 mm. sp. —ab; ++ bl.		7x8 mm. sp. ++ ab. bl. esp. along veins; lf. vig.	7x8 mm. sp. o		14 mm. sp. o ab; ++ bl. esp. on veins and later growth rings		14 mm. sp. +++ ab.; — bl; lf. partly dead		
2a						8x13 mm. sp. +++ both surfaces	11x19 mm. sp. — scattered ab; ++ in places bl.		12x19 mm. sp. ++ in outer rings ab. —bl; lf. dead	Unchanged, sp. o; lf. dropped						
2b							4x6 mm. sp. o		7x9 mm. sp. ++ ab. bl; more ab.	7x9 mm sp. o; lf. dying		No change; lf. dead				
3a						7x12 mm. sp. ++ both surfaces	7x15 mm. sp. o		9x15 mm. sp. +++ ab. bl.	12x18 mm. sp. o		12x18 mm. sp. o; lf. dead		No change; sp. ++ ab. bl. esp. in later growth rings		15x18 mm. sp. +++ ab. in belt 8-10mm. from center of spot; +++ bl. upon veins

3b						3x 4 mm. Sp. o	3x5 mm. sp. — in center ab; o bl.		4x6 mm. sp. ++ in ctr. ab: — bl.	6x8 mm. sp. o		7x9 mm. sp. ++ ab. bl in center		12x13 mm. sp. ++ ab. bl. esp. on veinlets		
3c									4x5 mm. sp. +++ ab. bl.			8x9 mm. sp. ++ ab. bl. in center		14 mm. sp. +++ ab: places bl; lf. dead		
4a						4x9 mm. Sp. o	6x10 mm. sp o		8x11 mm. sp. o	8x13 mm. sp. o		10x14 mm. sp. o		No change: sp. ++ ab. near ctr: — bl; lf. dead		Sp. o.
4b						4x5 mm. —sp. ab.			5x8 mm, sp. +++ ab. bl; lf. dead			6x9 mm. sp. o but ++ A. fasciculata				
5a						8x10 mm. sp. ++ more bl. than ab.	10x12 mm. sp. — young, ab. bl.		10x12 mm. sp. o; leaf dead			No change				
6a						9x10 mm. Sp. +++ both surfaces	10x12 mm. sp. o		11x14 mm. sp ++ entire spot ab. bl; dead			11x14 mm. sp. o				
7a						4x6 mm, sp. o ab., — bl.	5x7 mm. sp. — ab. ++ outer rings bl.		8x10 mm. sp. ++ ab. bl.			10x11 mm. sp. ++ ab. bl. esp. on veinlets		10x13 mm. sp +++ ab. less ++ bl.		Sp. o
7b						3x7 mm. Sp. o	4x7 mm. sp. o		4x7 mm. sp. ++ ab bl; lf. dead			4x7 mm. sp. o		14 mm. sp. +++ ab. places bl. lf. dead		

Abbreviations: lf., leaf: lflt., leaflet: sp., spore, or sporulation: ab., above; bl., below; +++, very abundant; ++, abundant: +, many; —, few; o, none.

may be further increased during favorable weather by the maturity of a second or even a third crop of spores.

Effect of various factors on spore production; spot histories.—By following the history of a number of spots, a better idea was obtained of the effect of various environmental conditions on the progress of the disease. For this study, Early Ohio plants were selected showing a few scattered spots on the mature leaves. Each spot was measured with a millimeter scale and both surfaces were examined for the presence of spores with a small low power microscope. Meanwhile great care was taken not to injure the leaves in any way. If spores were present their relative abundance was noted after which they were rinsed and brushed off by use of a pipette of distilled water and a soft camel's hair brush. The spot tissue became somewhat wet but the heat of the day caused it to dry out again in a few minutes. The results of these observations are shown in Table III. The effect of light rainfalls, especially those of July 17 and 24, in removing the spores from the upper surface of the exposed spots is seen. The most important evidence obtained relates to the ability of the spot for continued spore production. It shows that the same area on some of the spots produced three and four abundant crops of spores. There is also some evidence on the relation of rain and dew to spore production. It seems quite certain that the unusually heavy sporulation noted on July 18 was largely the result of the moist period beginning with the heavy dew of the night of the 16th and continuing through the forenoon of the 17th; then a fairly heavy dew that night was sufficient to stimulate the fungus to unusual spore formation. Another instance seeming to corroborate this evidence is that of July 21 when spores were found generally abundant. Here the relatively cool weather on July 20 following the rain of the 19th and the very heavy dew that night furnished the proper conditions for spore production.

To obtain further evidence on the effect of rain and dew on spore formation, another series of spots was studied. These observations extended from August 25 to September 6, 1916. Three large plants of the Green Mountain variety, bearing many spots on most of the leaves, were selected. Plant A was protected from dew at night, and from rains, when imminent, by placing over it a dew-proof cage. Plants B and C

were not protected. The spots were examined as in the previous study, but instead of removing the spores with water and camel's hair brush, the brush alone was used. This method was equally effective while being easier of manipulation. The results from this series are shown in Table IV. Fortunately a rather dry period was selected for this study which made it possible to determine the effect of the single factor, dew, on spore formation. Prior to this experiment, the writer had believed that moderately heavy dews were sufficient to induce abundant sporulation of the fungus. The observations recorded in Table IV show that even very heavy dews each night were, with few exceptions, insufficient. The period of the experiment was marked, as a whole, by rather cool weather (see Fig. 10) and where heavy dews are recorded it is positive that the plant surface was wet from 8 p. m. until 7–8:30 a. m. Dews alone were not sufficient but they, when aided by .9 in. rainfall (Sept. 5), caused abundant sporulation on all the spots exposed. Plant A, protected, showed none or only a few spores on the spots. Therefore, concluding from both experiments, it appears that frequent rains aided by heavy dews furnish the essential moisture conditions for optimum spore production of *A. solani* in nature.

VIABILITY AND LONGEVITY OF MYCELIUM AND CONIDIA

Jones (1896) states that the mycelium in the spot retains its life for a year or more. The writer's results in the main corroborate this. Leaves dried between layers of cotton yielded the fungus from both small and large spots when isolations were made after 12 and 18 months. Material 29 months old, apparently as well preserved, gave no growth of the fungus in several attempts at isolation. There is no evidence of the existence of any differentiated or resistant form of mycelium in the spots. In pure culture, mycelium in prune agar was found viable after seven months. Potato agar plates, tested for viability after 15 and 17 months gave negative results. The recent work of Bartram (1916) shows conclusively the great resistance of the mycelium of this fungus in pure culture to very low temperatures.

The condia are also very resistant. Jones (1896) succeeded in germinating conidia one year old but obtained no growth from those two years old. In one instance the writer got 10 per cent germination after 17 months at room temperature.

TABLE IV.—SPOT HISTORIES ON GREEN MOUNTAIN VARIETY: AUG. 25–SEPT. 6, 1916

DATE AND WEATHER CONDITIONS	PLANT A. PROTECTED FROM RAIN AND DEW							PLANT B. EXPOSED TO RAIN AND DEW				PLANT C. EXPOSED TO RAIN AND DEW		
	Leaf 1a	Leaf 2a	Leaf 3a	Leaf 4a	Leaf 5a	Leaf 6a	Leaf 7a	Leaf 1b	Leaf 2b	Leaf 3b	Leaf 4b	Leaf 1c	Leaf 2c	Leaf 3c
Aug. 25. No dew; cloudy, windy, cool	3 spots 4x5mm. no spores	2 spots 5x8mm. few spores both surfaces	4mm. few spores both surfaces	8mm. no spores	6mm. no spores	2 spots enormous sporulation	12mm. few spores center both surfaces	6mm. 2 spots none above, few below	Large marginal spot. enormous sporulation both surfaces	4mm. few spores above, none below	12x14mm. enormous sporulation both surfaces	15mm. abundant spores above, few below	8x12mm. few spores above, none below	8mm. 2 spots, no spores
Aug. 26. No dew; cloudy, windy, cool														
Aug. 27. Med. dew; partly cloudy, cool														
Aug. 28. Very heavy dew; partly cloudy, warm	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores
Aug. 29. Heavy dew; clear, warm														
Aug. 30. Very heavy dew; clear, windy, warm	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores

Aug. 31. No dew; clear, windy, cool														
Sept. 1. Heavy dew; cloudy, cool; mist in A. M.														
Sept. 2. Heavy dew; cloudy, cool	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	No spores	Spores abundant in center above, few below	No spores	Spores scattered over upper surface, none below
Sept. 3. No dew; clear, windy, warm														
Sept. 4. No dew; rain (.27) 6 A. M. vines wet until 10 A. M. cloudy, warm														
Sept. 5. Light dew; rain (.9) 8 A. M.-5 P. M.	Leaves dying from tip-burn & early blight							Leaves mostly dead at this time from tip-burn and early blight						
Sept. 6. Very heavy dew; foliage wet until 10 A. M. clear, warm P. M.	No spores	No spores	Very few spores scattered above	No spores	Few scattered spores both surfaces	Few scattered spores	No spores	Spores very abundant both surfaces	Enormous sporulation both surfaces of entire leaflet	Abundant sporulation both surfaces	Very abundant sporulation both surfaces	Leaf fallen	Very abundant sporulation both surfaces	Very abundant sporulation both surfaces

DISSEMINATION OF CONIDIA

The suddenness of appearance of a general and severe infection of early blight following a period of favorable weather has been noted by various workers. Observational data accumulated during the summers of 1915 and 1916 seem to indicate that the wind is the chief agent of dissemination in such cases. For instance, a field of early potatoes at Waupaca, Wisconsin, was noted to be suffering severely from early blight and tip-burn to the extent that on September 11, 1916, the majority of the vines were dead while an adjacent field of Rurals on the south was green and showed but relatively few spots. However, on a strip of the latter about 80 feet wide, adjacent to the early field, the disease was much more prevalent, but the number of spots was noted to decrease as one proceeded from the boundary line. To determine the relative occurrence of spots, typical leaves were picked from the first two or three rows next to the early field and an equal number 75 feet back. The spots were counted, including all the leaflets on each compound leaf. 12 leaves of lot 1 each bore 45 to 356 spots, average 175 spots per leaf; 12 leaves of lot 2 each bore 20 to 141 spots average 71 spots per leaf. Since potato beetles were practically absent from this field and strong north winds with favorable conditions for spore production and infection had occurred the preceding week, all evidence pointed to the wind as responsible for the general dissemination over this adjacent area.

There seems to be little doubt that the Colorado beetle is another agent of distribution for Alternaria spores. Twice during July, 1916, the examinations of washings from the beetles were made. Fifty adult beetles collected from diseased potato vines were dipped and shaken for a moment in ten cubic centimeters of sterile water from which microscopic examination and poured plates showed abundant spores. Numerous contaminating saprophytes prevented the actual number of spores from being determined.

METHOD OF INFECTION

According to Jones (1896) penetration may occur either through the stomates or directly through the cuticle. With proper conditions the young leaves of a plant can be infected as

readily as the older ones but the rate of enlargement of the spot is distinctly slower in the young leaves.

Though infections in nature frequently occur about flea beetle holes, the observations of several earlier investigators as well as those of the writer indicate no necessary relation between the two. It is not improbable, however, that these little beetles may carry the spores, as is shown for the Colorado beetle and as a result inoculate the wounds they make.

PERIOD OF INCUBATION

In the greenhouse where the cover slip method was used the incubation period both for potato and tomato, varied from 28 to 50 hours. Under field conditions, relying entirely upon heavy dews for the necessary moisture, incipient spots were usually noticeable within 48 to 72 hours after the spores had been atomized upon the plant. Under favorable conditions, within three or four days these spots may enlarge and produce spores which can cause secondary infection on adjacent leaves or plants.

TIME OF NATURAL INFECTION

As observed in central Wisconsin, natural infection is generally first visible from June 20 to July 10 on the crop planted April 25 to May 15. On the late crop, spots may be observed from the middle of August on, depending apparently upon three factors: age, vigor of plant, and weather conditions.

SOURCE OF NATURAL INFECTION

The source of inoculum for the early crop is probably from the overwintered spores and possibly from new conidia produced by overwintered mycelium which has been harbored in the soil in the debris of former crops. It is quite likely that an additional source of infection of the late potatoes is from nearby early fields in the form of spores carried by the wind or by potato beetles seeking the younger and more tender plants.

OVERWINTERING OF THE FUNGUS

The problem of the overwintering of *Alternaria solani* is concerned with but two possibilities, i. e., conidia and mycelium.

It has already been shown that both these structures possess remarkable resistance toward unfavorable conditions.

The writer has no evidence to substantiate, and sees no reason for accepting, the hypothesis offered by Massee (1906) and endorsed by McAlpine (1911) that the disease is transmitted from one generation to another by latent mycelium in the tubers.

To determine definitely under what conditions the fungus can overwinter in Wisconsin, the following experiment was made. On July 22, 1915, some very good material showing abundant sporulation was collected and the leaves dried quickly in the open air. In October, a 6 x 10 foot plot in the plant disease garden at Madison was marked off into four strips and used as follows:

In No. 1—Diseased leaves on the surface
In No. 2—Diseased leaves buried two inches deep
In No. 3—Diseased leaves buried four inches deep
In No. 4—Diseased leaves buried eight inches deep

The leaves were protected by being placed between one thickness of cheese cloth and this in turn was placed between two layers of galvanized iron wire netting. At intervals throughout the winter material was removed from each strip and attempts were made to isolate the fungus from it. The bulbs of soil thermographs were buried four and eight inches in the plot to furnish a continuous record of the soil temperatures, while an air thermograph nearby registered for the air. The records from November 12 to April 20 showed a variation in temperature from +13 to −25°C., for the air, +10 to −6°C. at four inches depth, and +8 to −6°C. at eight inches depth. On 93 out of the total of 160 days for the period the ground was covered with snow. The extremely low temperatures in each case followed periods of snowfall so that it is probable that even the material on the surface was not exposed to as low temperatures as were recorded.

Before burying the leaves in the fall, viability tests gave over 95 per cent germination of the spores. Several attempts failed entirely to isolate the fungus from the spot tissues where the mycelium appeared to be dead. This was an unexpected result, which was not fully understood until the following summer. Then it was found that the mycelium could frequently be killed by drying freshly collected leaves quickly in the sun. Thus unfortunately this test was limited to the conidia alone. Little

difficulty was experienced in isolating the spores for germination tests during the early winter, but later, as the cheese cloth and leaf tissue disintegrated, the conidia were more difficult to find. On December 11, 1915, tests of 40 to 50 spores from each level gave 80 to 90 per cent germination. At no time was there any evidence of the formation of new spores and cultures from the spot tissue developed only saprophytic invaders as Mucor, Fusarium, Penicillium, and *Alternaria fasciculata.*

On April 17, 1916, the final examinations were made with the following results:

(1) Spores overwintered on the surface—2–3 per cent germination
(2) Spores overwintered at 2-inch depth—40 per cent germination
(3) Spores overwintered at 4-inch depth—50 per cent germination
(4) Spores overwintered at 8-inch depth—65–70 per cent germination

The low figure for the surface germination would probably have been higher had not the location for the plot been selected on low ground where excessive water and ice made conditions unusually severe.

From this experiment it seems justifiable to conclude that a relatively large proportion of the abundant spores produced during the moist weather of late autumn remain viable throughout the winter. The primary infections of the next year doubtless come from such spores which have overwintered in the soil. It is easy for these to reach the lower leaves which are indeed often in immediate contact with the soil, and it is noteworthy that the primary infections always occur on such low lying leaves. This theory is in further accord with the observed fact that early blight starts earliest and is worst on old garden soils and suggests the conclusion that crop rotation is a factor in its control.

THE RELATION OF CLIMATE AND SOIL TO THE DISEASE

Climatic factors undoubtedly exert a great influence upon the dissemination and destructiveness of early blight. As to the climatic conditions best favoring an attack of this disease, Jones (1895) finds that hot, dry weather followed by a moist period is best. Rolfs (1898), in Florida, reports that the disease on tomatoes spread with "alarming rapidity" during moist, warm seasons, while dry, cool weather retarded its progress. Lutman

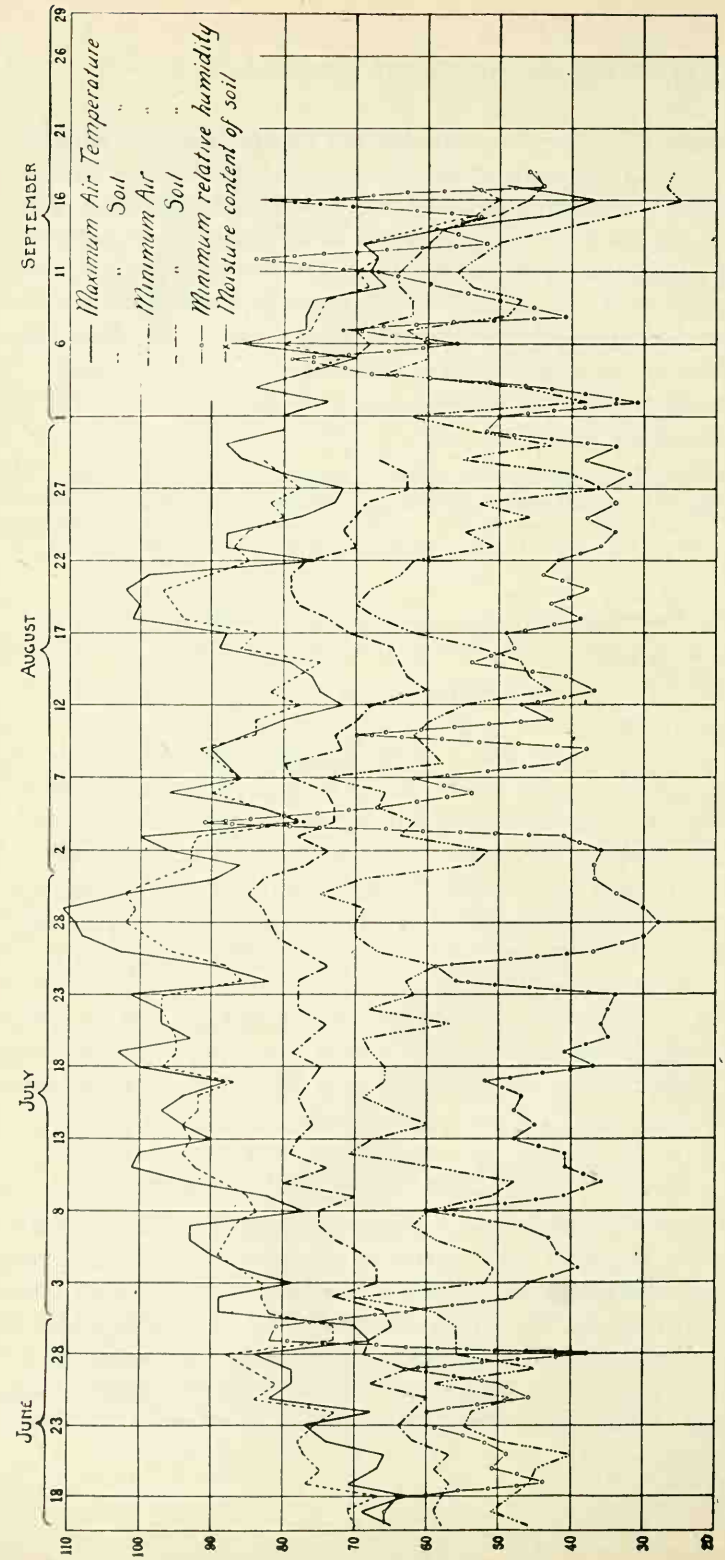
Maximum Air Temperature
" Soil "
Minimum Air "
" Soil "
Minimum relative humidity
Moisture content of soil
JUNE
JULY
AUGUST
SEPTEMBER
18
23
28
3
8
13
18
23
28
2
7
12
17
22
27
6
11
16
21
26
29
110
100
90
80
70
60
50
40
30
20

FIG. 10.—CORRELATION OF ENVIRONMENTAL FACTORS WITH EARLY BLIGHT AT WAUPACA, WISCONSIN. 1916

The curves at the top of the chart represent continuous meteorological records taken in the exeprimental field, the air temperature and relative humidity being obtained in a standard Weather Bureau shelter at the same height as the plants and the soil temperature at 3 inches depth among the roots.

As is indicated by the graphs the season was characterized by abnormally high temperatures low humidities, and a deficiency of rainfall. There is evident a close correlation between these various physical factors, e. g., precipitation, available soil moisture, temperature, and humidity. Their influence upon the host plant and the parasite is indicated.

(1911) summarizes twenty years' observation (1891–1910) made at the Vermont station mainly on the relation of the weather to late blight but including data on early blight and tip-burn as well. A careful study of the twenty diagrams and notes presented shows so much contradictory evidence on the occurrence of early blight that few conclusions are possible. His statement that it is a disease of the drier seasons is fairly well corroborated by the diagrams.

Various writers have called attention to the greater destructiveness of this disease on the lighter, sandy soils as compared with the damage it does on the heavy ones. On account of the very generalized nature of our knowledge of this subject, the author has attempted to get evidence which would more clearly show the influence of climatic and soil factors on the severity of the disease. For this purpose continuous meteorological records (air humidity and temperature) were obtained in a standard Weather Bureau shelter at the same height as the potato vines for the seasons of 1915 and 1916. Soil temperatures among the roots and soil moisture determinations were obtained only for the summer of 1916. The light, sandy soil on which the experimental plot was located both seasons, proved ideal for such studies on account of the more decisive response of the plants to changes in environmental conditions. Fortunately for this study the two summers represented extremes in opposite directions from the normal in regard to the conditions favorable for the disease. The season of 1915 was characterized by much wet, cloudy weather during July and early August and by relatively high temperatures. The remainder of August and the first week of September were dry and clear, and normal in temperature. The disease was first noted July 3 on the early crop planted April 25 to May 10, but did little damage prior to the third week in July when the plants began to set tubers. From this time on through August it spread with great rapidity and together with tip-burn resulted in an estimated loss of 35 to 50 per cent. A heavy frost on August 27 and a subsequent severe attack of late blight resulted in considerable loss to the late crop, which had shown but little early blight.

The season of 1916 began with a very wet, cold June with excessive rainfall making conditions unfavorable for the planting and growth of the crop. The first ten days in July were marked

by mild favorable weather after which a period of dry weather with extremely high temperature began and continued almost unbroken throughout the summer until September 5. On twenty days of this period the temperature at the height of the plants reached or exceeded 90°F., and on fifteen days the thermometer registered 100°F or more. On June 26, spots could be found on occasional lower leaves of most of the early fields examined but the vines were very vigorous and were just beginning to flower. By the time the hot weather began, the second week in July, the disease had made but little headway. The high temperature of air and soil and consequent reduction in the available soil moisture now quickly weakened the plants, thus making ideal conditions, so far as host susceptibility was concerned, for the rapid spread of early blight. By July 30, the vines were mostly dead from tip-burn and but very few early blight spots could be found.

The late crop, planted between June 1–15, escaped very largely the severe drought. The rainy weather of September and early October, however, enabled early blight to spread so that 30 to 40 per cent of the foliage was badly diseased. This injury, in connection with two light frosts, operating on the already much retarded plants, it is believed, was an important factor in reducing the tuber yield.

The meteorological records for 1915, being incomplete, are not given. The data summarized in Figure 10* cover, therefore, only the season of 1916. When these are considered in connection with the spot history records (Tables III and IV), made during the same period, there is evident a very close correlation between the various environmental factors and the occurrence and development of early blight. The evidence shows, (1) that in order to have the optimum conditions for an epidemic there must be relatively high temperatures in combination with a more or less weakened condition of the plant so that the fungus can make its greatest spread; (2) that such development will not occur unless the above conditions are prefaced by relatively moist periods of high humidity and abundant dew or rainy weather when spore production and infection can readily take place. The season of 1915 represented just such a correlation of conditions for the early crop. In 1916, on the contrary, no such optimum climatic combination prevailed, so that, although the

*The writer is indebted to Prof. H. W. Stewart of the University of Wisconsin, who determined the moisture equivalents from which the hygroscopic coefficients (approximate nonavailable moisture) were calculated.

plants were in a most susceptible condition, there was no general occurrence of early blight until late autumn.

These studies suggest a possible explanation of the severity of this disease in some countries and its practical absence in others. While the writer has had no opportunity personally to observe it in other countries it is noteworthy, according to reports in literature, that the organism occurs in practically all important potato growing regions of the world. The difference in destructiveness, therefore, must be due, not to the lack of introduction, but to a difference in climatic conditions. As already noted it is reported more severe in the United States, Australia, New Zealand, and South Africa than in Europe. Conclusions from studies in Wisconsin seem to indicate the following interpretation: the disease is more destructive in the first countries named because in general the average summer temperatures of these regions are not only higher but probably subject to greater fluctuations and extremes which, combined with variations in rainfall, make conditions less favorable for the growth of the plant. In central Europe, on the contrary, where early blight as a serious disease is practically unknown, the moderately low summer temperatures and the uniformally distributed rainfall furnish highly favorable conditions for the host plant, while less favorable for the best development of the parasite.

CONTROL MEASURES

RESISTANT VARIETIES

Stuart (1914) summarizes the results of five years' observations of the relative resistance to early blight of 153 American and foreign varieties of potatoes. Four of the ten varieties found most resistant to early blight are also found among the ten most resistant to late blight. But one of the ten was of American origin and it was of no commercial importance. The European varieties, though quite resistant, did so poorly under our climatic and soil conditions as to be practically worthless from a commercial standpoint. He concludes: "the value of the disease resistant varieties is problematical rather than actual. The plant breeder, by mating them with the most desirable commercial types, may develop commercial types of resistant varieties." Green and Waid (1906) of the Ohio station,

however, believe that much can be done in building up resistant varieties by selecting seed from resistant hills.

The McCormick variety is said by Norton (1906) to show decided resistance to early blight. Prof. T. H. White of the Maryland station furnished the writer with seed of this variety which was tried out in 1915 and 1916 in Wisconsin. The unusually large coarse vines showed by far the greatest resistance compared with the fifteen other varieties grown. However, in late September, 1915, when the stage of greatest vigor had passed, they also showed 20 to 30 per cent of the foliage badly diseased. The poor quality of the tuber will probably prevent it from becoming of much commercial importance where more desirable varieties can be profitably grown.

SPRAYING

The early spraying trials by Jones, aimed particularly at early blight (see Jones and Morse 1905), as well as the long series of potato spraying experiments at the New York and Connecticut stations, have shown the practical control of this disease with bordeaux mixture. Lutman (1911), summarizing the twenty years' spraying in Vermont, states that three to four applications of the 5–5–50 bordeaux "efficiently protects the plants from the attacks of the early and of the late blight." Milward (1909) states that increased yields result from spraying in Wisconsin when not less than four applications are given and the spraying commenced not later than August 15.

Stewart (1914) states that in the ten year series at Geneva there was an average increase from spraying for both blights of 97.5 bu. per acre. The 4–4–50 formula is recommended for the first two applications with an increase to 6–4–50 in the late sprayings. On the other hand Clinton (1916) obtained an average increase of 38 bushels per acre in Connecticut with three applications of the 4–4–50. Additional evidence bearing directly on the control of early blight is given by Jack (1913 and 1916) for Rhodesia in South Africa. There early blight appears to be by far the most important disease of the potato. Several years results showed an increase in yield, due to spraying with bordeaux mixture, ranging from 16 to 57 per cent.

Wherever this disease causes practical injury on the tomato, spraying with bordeaux mixture has also been recommended.

Edgerton and Moreland (1913) advise one application in the cold frame and one every ten days thereafter in the field if the disease is prevalent.

The spraying experiments conducted by the writer were designed primarily to furnish evidence on control correlated with his life history studies of the fungus, and secondarily to test out under Wisconsin conditions the recommendations of workers in other states.

SPRAYING EXPERIMENTS AT WAUPACA

The season of 1915 was unusually favorable for the development of early blight. Spraying was done, however, only on the late crop which, in the experimental plot, was completely killed by frost on August 26 before much differentiation between sprayed and unsprayed was noticed.

In 1916 both early and late potatoes were sprayed, but unfortunately for the experiments on early potatoes, little disease occurred this season. In spite of this the results obtained seem worthy of record. On the late crop, planted between June 5 and 15, it operated in weakening the already much retarded vines and was undoubtedly responsible for a large part of the shortage in yield.

On early potatoes—Experiments were undertaken in two gardens which had grown several successive crops of potatoes and in which early blight had been noted as severe in 1915. The plots were sprayed by hand with a modified Hudson and Thurber compressed air sprayer. This pump proved quite satisfactory for plots of small size and, with high pressure, gave a very fine spray. Great care was taken to cover all leaves thoroughly with the mixture. The amount applied each time was determined by the differences in gross weight of the container before and after spraying. As a rule about 150 gallons per acre were used for each of the first two applications, and 175 to 200 gallons per acre for the later sprayings. Since early blight was a negligible factor on account of the extreme drought, the beneficial results obtained are attributable primarily to the lessening of tip-burn and flea beetle injury. However, it is noteworthy that, whereas a dozen or more spots developed on each control plant in Plots 1 and 3 (Experiment B), only rarely

could an infection be found on Plot 2, which received weekly applications (9 in all), beginning when the plants were 6 inches high. The results are combined in Table V.

TABLE V.—SPRAYING EXPERIMENTS ON EARLY POTATOES

Experiment A, Van Patten Garden; Six Weeks Variety

Plot	Treatment	Yield				Increase	
		Actual number lbs.			Bu. per A.		
		Large	Small	Total		Bu.	Per cent
1	Bordeaux 5-5-50 June 16, 24; July 1, 8, 15, and 29	42.5	15.0	57.5	87.0	4.5	5.2
2 Control	Paris green and lime	38.0	11.0	49.0	82.5		
3	Bordeaux 5-5-50 July 1 and 15	45.5	8.5	54.0	90.8	5.3	5.8
4 Control	Paris green and lime	47.5	7.5	55.0	84.4		
5	Bordeaux 5-5-50 July 1, 10, and 29	56.0	11.0	67.0	107.6	19.3	17.9
6 Control	Paris green and lime	23.75	3.75	27.5	88.3		
7	Bordeaux 5-5-50 July 8, 18, and 28	77.0	12.0	89.0	144.0	38.8	26.9
8 Control	Paris green and lime	55.5	10.0	65.5	105.2		
9	Bordeaux 5-5-50 June 24; July 8 and 22	53.0	12.5	65.5	104.4	17.1	16.3
10 Control	Paris green and lime	43.5	12.5	56.0	87.3		
11	Bordeaux 2-4-50 June 24; July 8 and 22	43.5	17.0	60.5	92.3	—2.3	—2.4
12 Control	Paris green and lime	46.5	15.5	62.0	94.6		

Experiment B, Taylor Garden; Early Denver Variety

1 Control	Paris green and lime	38	27.5	65.5	112.2		
2	Bordeaux 5-5-50 June 16, 24; July 1, 8, 15, 22, 29; Aug. 5 and 14	41	29.5	70.5	120.7	17.5	14.5
3 Control	Paris green and lime	15.5	12.0	27.5	94.2		

On late potatoes—In experiments A and C (Table VI) the spraying was done on selected rows in one tenth acre plots which had been cropped successively to potatoes for several years. These were sprayed in the same manner as the early potatoes. The other trials were carried out on various farms near Wau-

paca, where the fields had been subjected to a four year rotation. Here an upright barrel outfit on a cart was employed.

Though much retarded in development the late potatoes escaped to a large extent the severe drought during July and August. Revived by the heavy rains in September they made good growth, and, had frost held off until late October, a fair yield could have been obtained. The entire plot in Experiment A was heavily watered with a hose several times during the early part of the season, which fact accounts partly for the greater amount of disease and the consequent greater difference in yield as compared with the other experiments. This plot also received the greater number of sprayings. Prior to September 13, early blight was practically absent in any of the fields except Experiment A. The rains and favorable weather following this date permitted rapid spread of the disease on the already weakened plants. Thus during a month of favorable growing weather for the plants a good portion of the leaf area in most cases became badly diseased. Flea beetles and tip-burn were practically absent and no late blight was found. In all these experiments those rows which received two or more applications of the 5–5–50 bordeaux contained in every case larger and more vigorous plants even before any disease occurred. This seemed to be due entirely to the stimulative action of the spray. The disease was not absolutely controlled in any case, not even plot 1 of Experiment A, which received 7 applications. Several light frosts in September complicated the situation in Experiment B where the sprayed plants on this sandy type of soil showed a striking resistance to frost injury. Aside from this, however, the uniform and consistent increase from the spraying is attributable to but two factors, viz., (1) the practical control of early blight and (2) the stimulative action of the bordeaux mixture on the plants. The results are presented in summary form in Table VI.

RECOMMENDATIONS FOR SPRAYING

While the period during which the foregoing experiments were conducted was not typical of the average year in many respects, yet the intensive study made of the disease in connection with them seems to warrant the following deductions:

For the early crop under Wisconsin conditions the disease can be profitably controlled by four to six applications of the

TABLE VI.—SPRAYING EXPERIMENTS ON LATE POTATOES

Experiment A, Turrell field; Rural New Yorker No. 2

Plot	Treatment	Yield				Increase	
		Actual Number lbs.			Bus. per A.		
		Large	Small	Total		Bus.	Per cent
1	Bordeaux 5-5-50 June 28; July 8, 18, 28; Aug. 7, 17; Sept. 6	123.0	10.5	133.5	331.9	144.2	43.4
2 Control	Paris green and lime	60.5	15.0	75.5	187.7		
3	Bordeaux 5-5-50 June 28; July 12, 26; Aug. 9, 24; Sept. 6	107.0	16.0	123.5	301.4	113.7	37.7

Experiment B, Constance field; Rural New Yorker No. 2

1	Bordeaux 5-5-50 Aug. 12; Sept. 7	188.0	35.0	223.0	111.5	23.0	20.6
2 Control	Paris green and lime	149.0	28.0	177.0	88.5		
3	Bordeaux 5 5-50 Aug. 12, 22; Sept. 7	190 0	31.0	221.0	110.5	22.0	19.9
4 Control	Paris green and lime	133.0	27.0	160.0	80.0		
5	Bordeaux 5-5-50 Aug. 12, 22	167 0	35.0	202.0	101.0	21.0	20.8
6 Control	Paris green and lime	133.0	26.0	159.0	79.0		
7	Bordeaux 5-5-50 Aug. 12, 22; Sept. 7	163.0	38.0	201.0	100.5	21.5	21 4
8 Control	Paris green and lime	140.0	24.0	164.0	82.0		
9	Bordeaux 5-5-50 Aug. 12; Sept. 7	149.0	34.0	183.0	91.5	9.5	10.4
10	Bordeaux 5-5-50 Aug. 12, 22; Sept. 7	152.0	31.0	183.0	91.5	9.5	10.4

Experiment C, Taylor field; Rural New Yorker No. 2

1	Bordeaux 5-5-50 July 8, 22; Aug. 5, 17; Sept. 13	85.0	18.9	104.9	190.5	74.7	39.2
2 Control	Paris green and lime	46.9	16.9	63.8	115.8		
3	Bordeaux 2-4-50 July 8, 22; Aug. 5, 17; Sept. 13	38.5	18.1	56.6	102.7	18.5	18.0
4 Control	Paris green and lime	28.3	18.1	46.4	84.2		

Experiment D, Pinkerton field; Rural New Yorker No. 2

1	Bordeaux 5-5-50 Aug. 12; Sept. 7	250.5	95.0	345.5	93.9	13.9	13.7
2 Control	Paris green and lime	198.5	98.0	296.5	80.0		
3	Bordeaux 5-5-50 Aug. 22; Sept. 7	299.5	81.0	380.5	103.4	27.7	26.7
4 Control	Paris green and lime	197.0	66.0	263.0	71.5		
5	Bordeaux 5-5-50 Aug. 22; Sept. 7	229 5	80.5	310.0	84.3	8.6	10.2

standard 5–5–50 bordeaux mixture. Complete control can only be attained by weekly sprayings begun when the plants are six to eight inches high and continued through the remaining period of growth.

For the late crop, the results indicate that the three to four applications ordinarily recommended for the control of late blight will also largely control early blight.

Thoroughness of application cannot be overemphasized in spraying for early blight.

SANITATION

From the evidence already presented that primary infection results from spores overwintering in the soil, and from observational data on the persistence of the fungus in dead vines, it is clear that in certain cases sanitation becomes an important factor to be considered. Crop rotation is of course the rational measure and in those cases where it is desired to crop the land continuously to potatoes, all dead vines should be raked together and burned immediately after harvest. Such measures will tend to reduce the number of primary infections but they should be regarded merely as contributing to the success of the more certain method of control, viz., spraying.

SUMMARY

Early blight, *Alternaria solani* (E. & M.) J. & G., of potato and related plants is a characteristic leaf spot disease distinguished by the concentric markings or "target-board" appearance of the spot.

This disease is practically world wide being found wherever the potato is an important crop, but it is of economic importance in but few countries, especially the United States, Australia, New Zealand, and South Africa.

The damage from this disease is indirect, i. e., it causes the premature death of the foliage and this results in decreased yields. During some years early blight does more damage than late blight but it is the annual small loss which makes it a serious obstacle to successful potato culture. On the tomato, where it causes spotting of both leaves and fruit, Edgerton and Moreland, 1913, place it next to wilt in importance.

Early blight, in Wisconsin, occurs commonly on potato, tomato and eggplant. The identity of the fungus on these hosts has been established by morphological and cultural studies and by reciprocal cross inoculations from single spore cultures. The

leaf spot of Jimson weed (Datura) which has been widely attributed to the same fungus, is shown to be due to a similar but distinct species of Alternaria which was early described by Saccardo as *Cercospora crassa*. For this the author has given the new combination *Alternaria crassa*.

To determine the host range of the fungus, inoculations were made under field conditions on 30 species and varieties of the family Solanaceae. On 29 of these penetration and incipient infection occurred. However, the fungus was able to complete its life cycle on but 12 of the plants, which in addition to two others not included in the tests, make its known host range 14 species and varieties representing the genera Solanum, Lycopersicon, Nicandra, and Hyocyamus.

The early blight fungus was first described in 1882 and named *Macrosporium solani* Ellis and Martin. Jones and Grout, 1896, and Sorauer, 1896, changed the name (the latter on insufficient evidence) to *Alternari solani*. Though the writer has never observed conidia in chains in nature and they occur but rarely in culture, the present uncertain taxonomic relationship of the two genera, Alternaria and Macrosporium, and the established usage leads him to provisionally retain the latter binominal, *Alternaria solani* (E. & M.) J. and G.

The important diagnostic characteristic of the fungus is the long, single or forked, terminal beak of the conidium.

On potato and other vegetable and fruit extract agar, the colony produces a brilliant yellow pigmentation of the medium, later becoming reddish.

After repeated trials to obtain spores in culture, it was found that by stirring or shredding the agar and mycelium in the petri dish and carefully regulating the moisture for 24 hours abundant sporulation could be secured. This served as the source of material for spore germination and inoculation studies.

The cardinal temperatures for spore germination and mycelial growth on favorable media fall within the following limits: minimum 1–3°, maximum 37–45°, optimum 26–28°C. Five to ten germ tubes emerge at the optimum while at the minimum usually not more than half this number develop.

Spore production in nature may begin when the spot has reached a diameter of 3 to 4 millimeters. A given spot may produce 1500 to 3000 spores in two to three successive crops during a season.

The conidia are readily dislodged from their conidiophores, and local dissemination appears to be chiefly effected by wind and rain. Colorado potato beetles may also spread the disease.

Infection may occur via the stomates or directly through the cuticle.

The period of incubation varies from 48 to 72 hours.

Primary infection may result from overwintered conidia or possibly from new conidia produced by overwintered mycelium.

Though conidia were found to overwinter on leaves on the surface of the ground, the proportion surviving the winter was greater on those buried at 2, 4, and 8 inch depths.

Early blight ordinarily makes little development until the host has passed its period of greatest vigor and is being weakened by external conditions or by the drain of tuber formation. Optimum spore production is dependent upon frequent rains aided by heavy dews. Climate and soil exert a controlling influence upon the development of the disease. In general it becomes most serious when the season begins with abundant moisture which is followed by high temperatures unfavorable to the host plant but with sufficient moisture to insure maximum sporulation. Periods of continued drought check its spread completely. The conclusion is, therefore, reached that the optimum conditions for an epidemic of early blight require relatively high temperatures alternating with moist periods in combination with a more or less weakened condition of the plant.

The unusual resistance of the McCormick potato to early blight, reported by Norton, 1906, has also been observed by the writer, but unfortunately this variety is a poor commercial type. The possibility of securing resistant varieties with the best commercial qualities has been shown by Stuart, 1914, to offer little immediate encouragement, but he is continuing breeding experiments with this in mind.

Sanitary measures are recommended based on the evidence as to the overwintering and origin of primary infections. These include crop rotation and the destruction of the dead potato tops in gardens where continuous cropping is practised.

Spraying experiments conducted by the writer confirm the results of others and show that timely and thorough spraying with home made bordeaux mixture profitably controls early blight. (See summarized recommendations for spraying, p. 42).

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Frost Necrosis of Potato Tubers

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CONTENTS

Frost Necrosis[1] of Potato Tubers

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The late or main crop of potatoes as grown and handled in the northern tier of states is likely to be exposed to freezing temperatures from the last month preceding digging through all the stages of harvest, transportation, storage, and delivery to the ultimate consumer. The danger of freezing injuries is one of the most serious risks of commercial potato growers and dealers and the problems of the transportation companies are also seriously complicated thereby.

In 1917, when freezing temperatures occurred very generally through the northern states before or during potato harvest, the resultant losses probably constituted a greater toll upon the Wisconsin crop than all other disease factors combined, and even in 1918, when the climatic conditions were especially favorable, freezing injuries were common and serious. These consisted not only in the immediate loss of tubers frozen in the field or warehouse, but also in the later appearance in storage of potatoes exhibiting the more obscure freezing injuries.

Frost necrosis distinguished from other injuries. It is important at the outset to point out the general characters of frost necrosis that it may be distinguished from other types of injury. It is well known that when once frozen solid the potato tuber is killed and collapses immediately upon thawing. If, however, the exposure to freezing temperatures is moderate or of short duration, it often happens that only a portion of the tubers are thus frozen solid and collapse, the rest remaining unaffected as far as external appearances indicate. If, however, such superficially sound tubers are cut open, various evidences of internal injury will be found in at least some of them. In

[1] The term frost necrosis is synonymous with the term freezing necrosis used by Link and Gardner in an unpublished manuscript. (See footnote 1, page 20.) The writers agree with them that frost necrosis is a local or restricted freezing injury which results from exposure to temperature sufficiently low to cause ice formation in the tissues and is thus distinct from chilling injury which results at temperatures not low enough to induce ice formation in the plant tissues. The writers use the term frost necrosis rather than freezing necrosis since frost necrosis has been used in a previous publication (Jones, L. R. and Bailey, E., Frost necrosis of potato tubers. Phytopath. 7. 71-72. 1917).

most cases such injuries remain strictly internal and hence, if the potatoes are marketed their defects are not detected until the potatoes reach the ultimate retailer or consumer.

The irregular occurence and distribution of tubers which show such internal lesions of frost necrosis makes them difficult to sort out in storage lots. Naturally, potatoes frozen during harvest or transportation become mixed with the sound ones, but it is a surprising fact that when storage chambers are subjected to the same freezing temperatures and uniform conditions of ventilation, certain scattered individual tubers will be injured and others not. This individual susceptibility of potatoes to freezing injuries, combined with the still more confusing fact that frost necrosis is often mistaken for pathological conditions arising from other causes, makes it important that there be a further understanding both of the conditions and nature of freezing injury to potato tubers. This is especially needed at this time because of two recent coordinated developments involving critical consideration of potato tuber maladies. On the one hand is the movement for the state inspection and certification of potato seed stocks, on the other is the development of the national market inspection service. In both cases it is necessary to differentiate frost necrosis from other types of tuber injury or disease, especially the non-parasitic "net-necrosis" and the Fusarium "ring necrosis." Indeed, it was because of the evident confusion of frost necrosis with certain of these other types of injury that the senior author's attention was first directed to this problem. Frequently within the past four years potatoes showing distinct symptoms of ring or net necrosis have been found in storage cellars where it was definitely known that they were generally sound when stored and had been subjected to freezing temperatures while in the cellar. One striking example of typical net necrosis occurred in a certain lot of selected exhibition potatoes shown at the meeting of the Wisconsin Potato Growers' Association in 1914. The exhibitor was confident that the tubers were normal when he started from home but they had been subjected to freezing temperatures in transit. Similar conditions were found in several lots of potatoes in the exhibition of 1918. The matter presented so much of practical as well as scientific interest that further observations have been supplemented by careful

experiments to determine the effects of various freezing temperatures upon potatoes.

Previous publications. Several previous publications have embodied the results of more or less extensive investigations upon freezing injury to potatoes. The most valuable of these is that of Müller-Thurgau (4, 5, 6), who undertook to determine the temperatures at which plant tissues freeze. His first concern was with the phenomena of supercooling and the determination of the ultimate freezing point, but in connection with this (5) he investigated the turning sweet of chilled potatoes.

Since then, Apelt (1) in Europe, 1907, has approached these questions by somewhat different methods, while in America Appleman (2) published his observations in 1912. In general, where their conclusions have not been in agreement, our own results have confirmed those of Müller-Thurgau. In none of these earlier publications, however, was critical attention focused upon the internal lesions or symptoms of frost nécrosis and it is chiefly here that our own efforts have aimed to supplement those of previous workers.

While the details must be left for later consideration it will be helpful at the outset to summarize the conclusions upon which there is general agreement.

Plant tissues, in general, must be cooled to some degree below the freezing point of water before ice crystallization begins. With the potato it is the consensus of judgment that there is no killing of tissue or other permanent or injurious effect short of ice crystallization. Where tubers are held at temperatures near or slightly below the freezing point of water, but above the freezing point of the potato tissue, they turn sweet owing to the accumulation of sugar produced by the gradual starch conversion. It is commonly believed by potato handlers and has even been stated in literature by Norton (7, p. 70) that this is due to their having been slightly frozen. Müller-Thurgau (5, p. 753), and others since, particularly Apelt (1, pp. 12–27) and Appleman (2, p. 330), have disproved this. By storing potatoes for long periods as low as –1.66°C. (29°F.) Appleman (2, p. 333) determined that sugar accumulated most rapidly at 0°C. or below, and that freezing with potato tubers began between –2.2° and –3.3°C. (26° and 28°F.). Müller-Thurgau (5, p. 753) stored potatoes at temperatures ranging

from 0° to –3°C. for two weeks and found them still unfrozen after that period. Our own results as will appear later, confirm their conclusions that there is a considerable range possible in this critically low temperature at which tubers may turn sweet before they begin to freeze. Furthermore, none of these men has ever found potatoes to become sweet as a result of freezing consequent upon rapid cooling. Instead they determined the rate of sugar accumulation to be very slow even under most favorable temperatures. Our own experience is in accord with this in that we have regularly tasted tubers frozen experimentally without discovering evidence of increased sugar content in the potatoes which we have subjected to freezing temperatures enduring from 2 hours to 2 days. Hence, while sweetness indicates that tubers have been held for some time dangerously near their freezing point, it does not indicate that they have been frozen.

Müller-Thurgau (4, p. 147) showed that living plant tissues in general require supercooling to some degree below their true freezing point before ice crystallization begins. He found that the freezing point of the expressed sap of a potato tuber was –0.65°C. while the living potato tuber tissues in his experiments required supercooling to –3.2° to –6.5°C. before they began to freeze. Apelt's results with the potato, using a less reliable method we believe, are not in full accord with this, but our own trials confirm Müller-Thurgau's conclusions that supercooling is the normal course when potato tissues freeze. The earlier workers were led to define rather exact temperature limits for these phenomena with potato, generalizing, perhaps, from work upon a few tubers of uniform type, although they do not agree among themselves upon these limits. On the other hand, our work shows that there is considerable range in variation between individual tubers, even in the same lot of potatoes. The most interesting point and one of considerable practical importance in relation to symptomatology, is that there may also be a considerable range in susceptibility to frost necrosis between the different tissues in the same tuber. Here again Müller-Thurgau records the greater sensitiveness of the "cambial" as compared with parenchymatous tissues, and of the stem end as compared with the eye end of the tuber, but Apelt failed to confirm these differences. Our own results

not only show the correctness of Müller-Thurgau's general observations but enable us to go considerably farther than did he in defining such local differentiation. It is, indeed, because of these differences as to tissue susceptibility that potato tubers when subjected to the higher freezing temperatures may exhibit various types of internal symptoms.

EXPERIMENTAL MATERIALS AND METHODS

Potato tubers which showed necrotic areas internally and which were known to have been subjected to freezing tem-

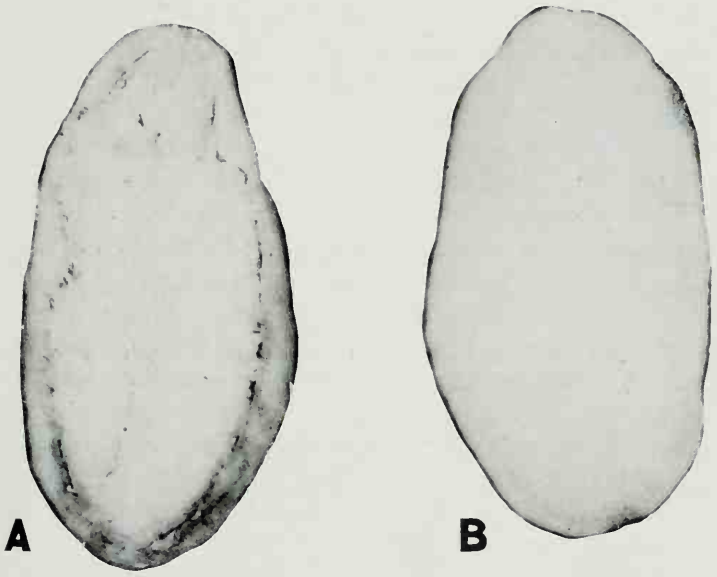

FIG. 1.—FROST NECROSIS PRODUCED EXPERIMENTALLY

A and A′ are longitudinal halves of a potato tuber. A was exposed to temperatures ranging from +10° to —5° C. for 24 hours and shows vascular discoloration of the net type of freezing injury. Notice more severe injury to stem-end (below). A′, control half, was not subjected to freezing temperatures.

peratures were found so frequently that, as already explained, it seemed advisable to determine experimentally the symptoms of freezing injury as compared with those of other maladies. The results of such work during the years 1915–1916 show conclusively that potato tubers when slightly frozen are often internally discolored while externally unharmed. Later, in 1918, when a freezing machine became available from which accurate temperature data could be obtained, a more critical study was

made of the temperatures at which this injury becomes apparent. Most of the temperature data herein tabulated were secured from these later experiments but they accord in general with those obtained in the earlier trials.

Earlier work, 1915–1916. In 1915 we obtained a quantity of potato tubers of the variety Rural New Yorker which had been grown, harvested, and stored under conditions as nearly uniform as practicable. In addition to these, potatoes were used in 1916 which were harvested at different stages of maturity so that data were obtained on the susceptibility of potatoes of different ages. At first each tuber used was cut in half longitudinally, one half kept for a control and the other frozen. In no case did the necrotic symptoms (fig. 1), which appeared so frequently in the frozen halves, develop in the controls. Potatoes which showed internal spotting of any kind were rejected for experimental work, and where potatoes were not cut in halves the stem end was cut off in advance to determine whether or not any internal spotting was present.

The tubers were either exposed out-of-doors or in a simple freezing chamber. In the out-of-door experiments great numbers of potatoes could be kept under like conditions, from 30 to 50 tubers often being used in a single experiment. This afforded a better opportunity for studying individual variation in susceptibility than was possible in the freezing chamber, where, at most, only 12 to 15 tubers could be tested at one time. In the out-of-door experiments a thermograph was used for recording temperatures; in the freezing chamber thermometer readings were made. The apparatus used in these experiments was of the simple ice cream freezer type of construction, easily understood from figure 2, which shows the insulating box surrounding the three cylindrical tin cans, each fitted with a tight cover and completely enclosing the one next inside. The tubers were held at the level of the mercury bulb of a long-stemmed thermometer, the scale of which was well above the cover of the freezing chamber so that it was not necessary to change its position to read the temperatures.

In setting up an experiment the ice and salt were first packed about the container, the potatoes next inserted in the inner chamber and the can covers and the thermometer then put in position. Using this method a half hour or more was necessary

for the temperature of the freezing chamber to drop to the desired degree below 0°C. Attempts were made to reduce materially this preliminary cooling period by packing the freez-

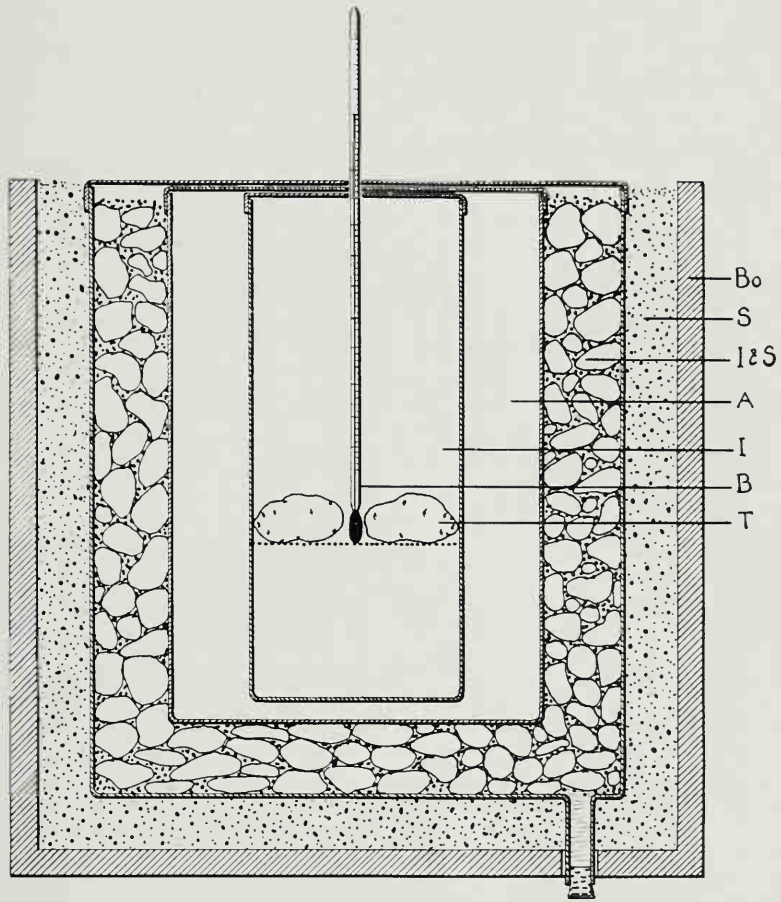

FIG. 2.—FREEZING APPARATUS USED IN 1915-1916

Diagram of freezing chamber in which the containers are all cylindrical tin cans fitted with tight covers, except the outermost, which is of wood. Tubers (T) are placed in inner chamber (I) supported by a wire gauze which is held at the level of the mercury bulb of the thermometer (B). This inner chamber is insulated by air space (A) and cooled by the freezing mixture of ice and salt (I and S). Sawdust (S) is packed between the box (Bo) and freezing mixture.

ing mixture about the chamber an hour before the insertion of the tubers that chamber and container air might be fully chilled in advance. It was found to make little difference, however, since the air disturbance consequent upon opening

the chamber and inserting the tubers was such that the preliminary period needed to bring the chamber to 0°C. was practically as long as by the first method. Müller-Thurgau used a freezing machine not unlike that described above and he re-

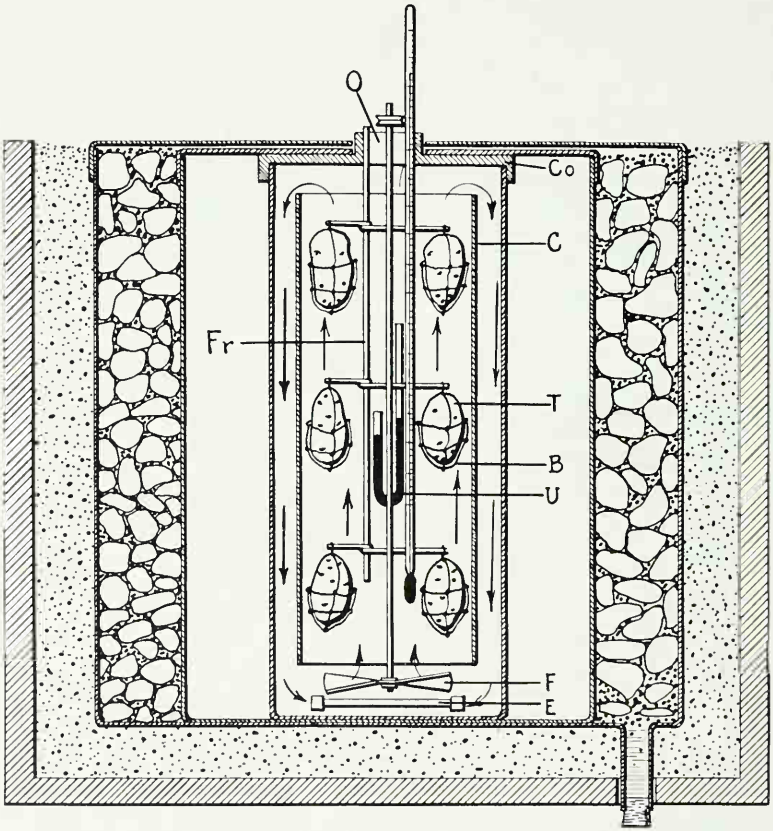

FIG. 3.—THE POTTER FREEZING APPARATUS

The general structure of this machine is like that used in the earlier work (fig. 1). The inner freezing chamber, however, has several new features. Heat is furnished by electric coil (E) which is regulated by electrical connections with the thermostat, the U-tube of which is represented by U. These electrical connections (not shown in diagram) were made through opening (O) in the heavy iron cover (Co) which also supports the frame (Fr) for the wire baskets (B). The arrows indicate the general direction of air currents which are produced by revolving fan (F). It is to be noted that the inner cylinder (C) is open at both ends, above and below, this permitting free air circulation.

cords constant temperatures throughout an experiment. Apparently he did not take into consideration the rate of fall nor

[1] For the use of this apparatus we are indebted to Geo. F. Potter, of he Department of Horticulture. Mr. Potter designed it primarily for study of the effects of freezing temperatures on the roots of nursery stock. He will publish the full details in relation to its construction and operation soon, but we are permitted through his courtesy to indicate the general features and unusual advantages of this apparatus.

the fluctuations which must have occurred where experiments were continued for several hours.

Later work, 1918. In our recent experiments (1918), we have used the Potter freezing apparatus[1] which has furnished more accurate data with ability to satisfactorily control the temperatures. The general construction of the freezing chamber (fig. 3) is similar to that described above but special devices are added for accurately controlling the rate and degree of cooling the freezing chamber. This is accomplished through the insertion of an electric heating coil with a regulating device such that the temperature can be made to fall at an exactly controlled rate and stopped and held constant at any desired point short of the extreme temperature procurable by the ice-salt mixture. Since this latter point is much below the temperatures with which we were concerned in our potato freezing trials the apparatus proved highly efficient and satisfactory. In most of these trials the apparatus was so adjusted as to drop the temperature in the experimental chamber to 0°C. at the end of the first half hour and to lower it 3½ degrees each hour thereafter until the desired minimum was reached.

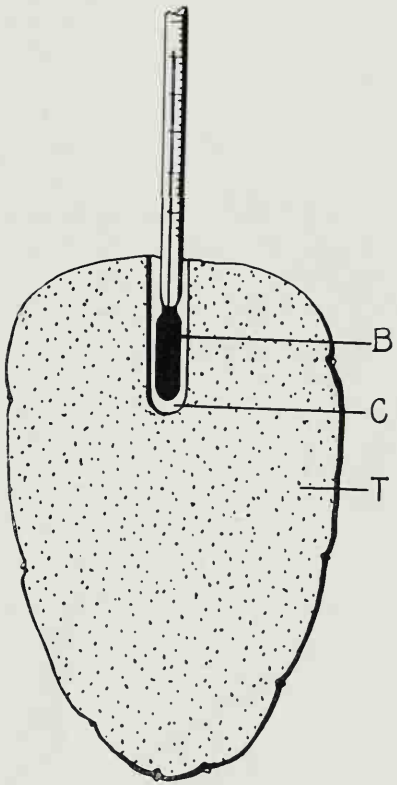

FIG. 4.—LONGITUDINAL SECTION OF TUBER AS USED IN SUPERCOOLING EXPERIMENTS

Thermometer bulb (B) is inserted in cavity (C) made in stem end of tuber (T).

For determining the internal temperatures of freezing potatoes, Müller-Thurgau's method was employed as described by him (1, p. 168). Two thermometers were used, one of which was suspended in the air of the freezing chamber, the other in a cavity made in the end of a tuber as shown in figure 4.

In order to preclude any undue pressure or the freezing of sap from the cut surface of the tuber upon the mercury bulb of the thermometer, the thermometer was so suspended that it did not press against the bottom of the pit and the cavity was made about twice as great in diameter as the thermometer and was carefully dried out with filter paper to rid it of free surface sap. No doubt the mercury bulb touched the walls of this cavity but the data (Table IX) indicate that the temperature readings were not influenced perceptibly by pressure or the freezing of water upon the bulb. While the temperatures obtained in this may not indicate the temperatures of the whole tuber, they do markedly differ from the air temperatures and give some indication of what may be taking place inside the tuber.

In the 1918 experiments carefully selected tubers were used chiefly of the Rural New Yorker variety. These had in all cases been harvested and stored without risk of freezing and sufficient numbers of untreated tubers were cut open to prove them to be generally free from internal lesions. This enabled us to proceed confidently in their use without previous cutting of each experimental tuber since this exposure of freshly cut tissue introduces a disturbing factor. The later trials were conducted during the latter part of the normal storage period, February–July. In some cases the tubers were kept previous to trial in the warm, dry laboratory long enough to secure partial wilting in order to compare normally turgid with wilted specimens. In the latter part of the period (March–July) Triumph potatoes were introduced into the trials. These had been previously stored at temperatures approaching 0°C so that there had resulted a considerable sugar accumulation. In June and July recently dug, immature southern samples of Triumphs were available for comparison with this old stock. Some Early Ohios and Irish Cobblers were also tested at this time. In previous years trials had been made involving different varieties, degrees of turgidity, and stages of maturity. The details regarding these are given later in this article so that it will here suffice to state that in general neither variety, size, relative turgidity nor stage of development nor maturity of the tuber influenced in any marked degree the liability to frost necrosis or the type of resultant injury.

THE SYMPTOMS OF FROST NECROSIS IN POTATO TUBERS

Effect of freezing upon the potato. A potato tuber that has been completely frozen will upon thawing be soft and watery and will quickly collapse or decay. If the tuber is cut open water drips freely from it and even before cutting the sap freed by freezing oozes through the skin so that the surface is soon wet. This soft, wet condition immediately indicates the trouble to one experienced in handling potatoes exposed to frost. Very often potatoes are thus frozen and collapse on one side only (Pl., fig. C), owing to one-sided contact with a frosty cellar wall if in storage or to a cold car floor if in transit, or it may occur through partial exposure at or near the surface of the ground before harvest. If such a frozen potato is cut across soon after thawing the cut surface of the interior flesh, although watery, is not at first discolored. Upon exposure to the air it will, however, very soon pass promptly through pink, red, and brown discolorations to a uniform inky blackness. This, according to Bartholomew (3, p. 631), is due to the oxidation of certain elements in the freed sap upon their contact with the air. Evidently the absence of discoloration before the tuber is cut is due to the fact that in the process of freezing and thawing the sap passes from the interior of the cells to the intercellular spaces thus driving out the free air and making its reabsorption almost impossible until the tuber is cut. It is often the case in nature that the exposure to freezing temperature stops short of the time or degree necessary to the uniform or complete freezing of the tubers. In this case few or none of them may show the softening or the wet surface characteristic of the frozen tuber yet, when they are cut open, various types and patterns of internal discoloration may be found. Since such frost necrosis may bear close resemblance to other types of internal discoloration of the potato tuber, and indeed necrotic lesions of different types may occur in the same lot of potatoes, we have undertaken to induce frost necrosis by experimental methods in order to determine the various forms of lesions.

Symptoms of frost necrosis as developed experimentally. As a rule, potatoes from the experimental freezing chamber which do not immediately show evidences of complete freezing, i. e.,

become soft and watery, will thereafter develop no external evidences of injury even though extensive internal necrosis has resulted. In exceptional cases, however, upon tubers having a clean, smooth, white skin, locally darkened areas may gradually appear where the interior discolored areas lie in the cortex close under the skin (fig. 7, B). This is not, however, a uniformly reliable symptom and even where detected requires confirmation through cutting of the tuber.

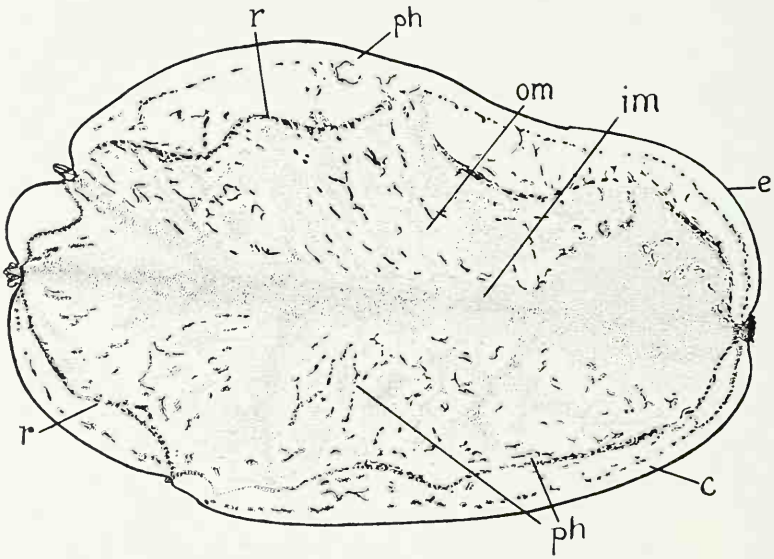

FIG. 5.—DIAGRAM OF LONGITUDINAL SECTION OF A POTATO TUBER

The heavier black portions represent vascular elements, the stippling indicates translucent tissue of high water content. The vascular ring (r) connects the stem end of the tuber at the right with the eyes scattered over the surface. The other gross structures are as follows: Corky epidermis (e), cortex (c) with scattered phloem elements (ph), outer medulla (om) with scattered phloem elements (ph), and inner medulla (im).

The internal lesions of frost necrosis appear as discolored areas in the flesh. These may not show marked discoloration in tubers cut immediately after their removal from the freezing chamber, but, as will be discussed later, color differentiation is completed after five or six hours. In many cases this discoloration is quite definitely limited to the vascular ring or follows the finer network of vascular elements which branch from this through the outer cortex or interior pith regions. Frequently where the injury is more severe or of longer stand-

ing the discolored lesions appear as blotches or diffused areas scattered less regularly through the flesh. Even in such cases critical examination shows that the discolorations are limited to well-defined areas. This can best be determined by examining a thin razor section of a necrotic tuber by transmitted light. In such sections the central core of pith and the vascular elements are highly transparent in contrast with the starch-filled parenchyma cells of the cortex and outer pith, and the darkened cells killed in the process of freezing are almost without exception those of the vascular elements and the cells bordering upon them.

As is shown in figure 5 the arrangement of the vascular system of a potato tuber is unlike that ordinarily met with in modified stems for in addition to the vascular ring there are throughout the cortex and pith—except in the inner core mentioned above—a network of small branching conductive elements largely composed of phloem elements, and when these vascular elements are all blackened we have a typical net necrosis (fig. 2, A and Pl., fig. D). This symptom, however, is less common in potatoes frozen in field, pits, etc., than are the blotches which appear in the cortex, vascular ring and outer pith, and which have as centers vascular elements (fig. 3). Müller-Thurgau (6, p. 455) noted this distribution of lesions and figured it in 1886. He says in regard to the tubers in which ice crystals have been formed, "These tubers showed externally in no way the appearance of frozen potatoes, but when they were cut, soft places were evident which, upon exposure to the air, turned red and later brown. As the observations showed, these dead tissue areas were the parts where the first crystal formation had occurred. These were never uniformly distributed throughout the potato but showed alike in over 100 trials of this kind a very constant relation in that they occur in the cambium-zone and immediately adjacent parts."

He adds, "In addition to the roundish dead spots in such potatoes one finds early-killed cells about the irregularly-running little bundles of vessels which are the places where the ice is formed very early and it is possible that along these paths the freezing process is distributed to new centers." Why these

tissues are more susceptible to low temperatures than others is a question for the plant physiologist to determine. Müller-Thurgau attempted to explain it upon the basis of water or carbohydrate content, but gives no conclusive results based upon experimental evidence.

Types of necrotic lesions. No two frosted potatoes show identical internal lesions but we have found it practicable and convenient to distinguish three types of necrosis which may be

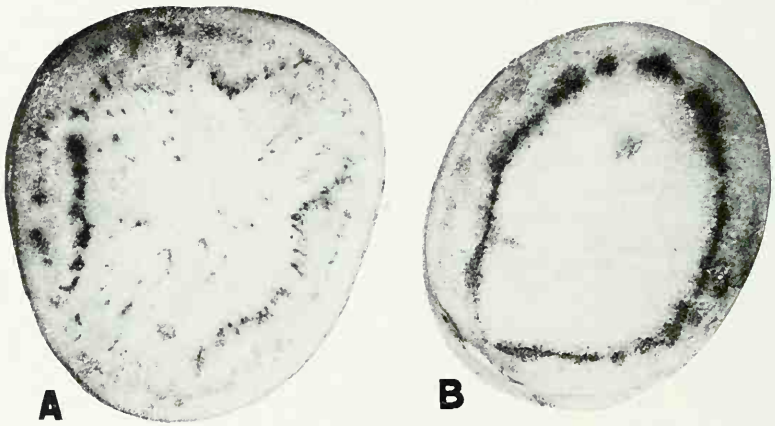

FIG. 6.—NET AND RING TYPES OF FROST NECROSIS EXPERIMENTALLY PRODUCED

Cross section of two tubers which had been exposed before cutting to a temperature of —8.5° C. for two hours. The symptoms are much more intense than those produced at higher temperatures (See fig. 1).

A—Intense net discolorations. Notice blackened vascular elements in both medulla and cortex.

B—Intense ring type somewhat complicated by blotch.

termed net, ring, and blotch. It is, of course, to be understood that any such grouping is somewhat arbitrary, that one type often merges into another, and that of each there are variations.

(1) In the net type there is more or less general blackening of the finer ramifications of the vascular elements extending as a network from the vascular ring internally toward the pith and to a less extent externally into the cortical region (fig. 6, A and Pl., fig. D).

(2) The ring type is characterized by a more pronounced blackening of the tissues in and adjacent to the vascular ring. It may be rather wide and diffuse (fig. 9, B) or narrow and

intensely blackened (fig. 6, B) and is often restricted to the stem end.

(3) The blotch constitutes a less well-defined type where the discoloration appears as small ovoidal or larger irregular patches ranging from an opaque grayish color to sooty black. These occur most commonly in the vascular ring and cortex although they may be located in the pith (fig. 7, A and B, and Pl., fig. A, B, E).

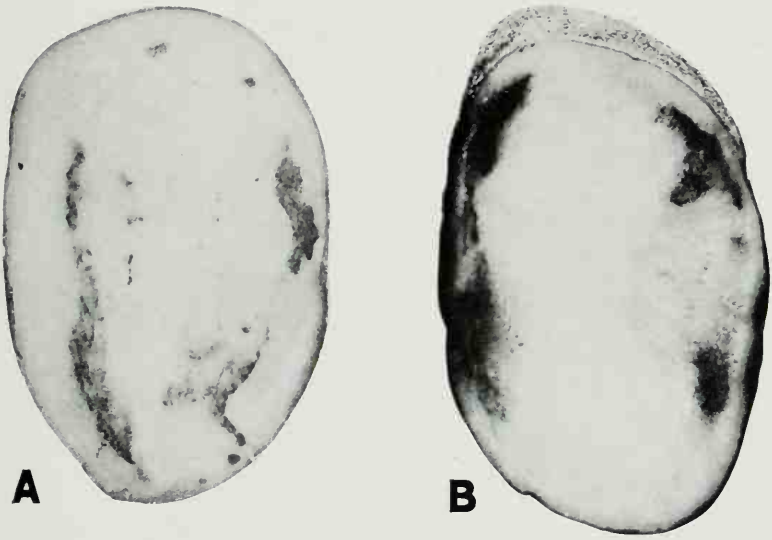

FIG. 7.—BLOTCH TYPE OF FROST NECROSIS

A—Longitudinal section of a tuber exposed to temperatures ranging from 0° to —4° C. for nine hours. Blotches more abundant in stem end.
B—Cross section of the stem end of a necrotic tuber. The intense blotches in the vascular and cortical regions were evidenced by dark areas on the exterior of the tuber.

When any considerable number of tubers are subjected to identical freezing conditions it will be found upon cutting them open that different types of frost necrosis may have resulted so that one cannot with exactness associate these different symptoms with definite temperature exposures. Numerous observations have, however, shown that some conditions of freezing give a preponderance of certain necrotic types. For example, with Rural New Yorker tubers held at –5°C. for two hours a high percentage of net necrosis resulted (Table 3), the symptoms becoming more intense with prolonged exposure. This

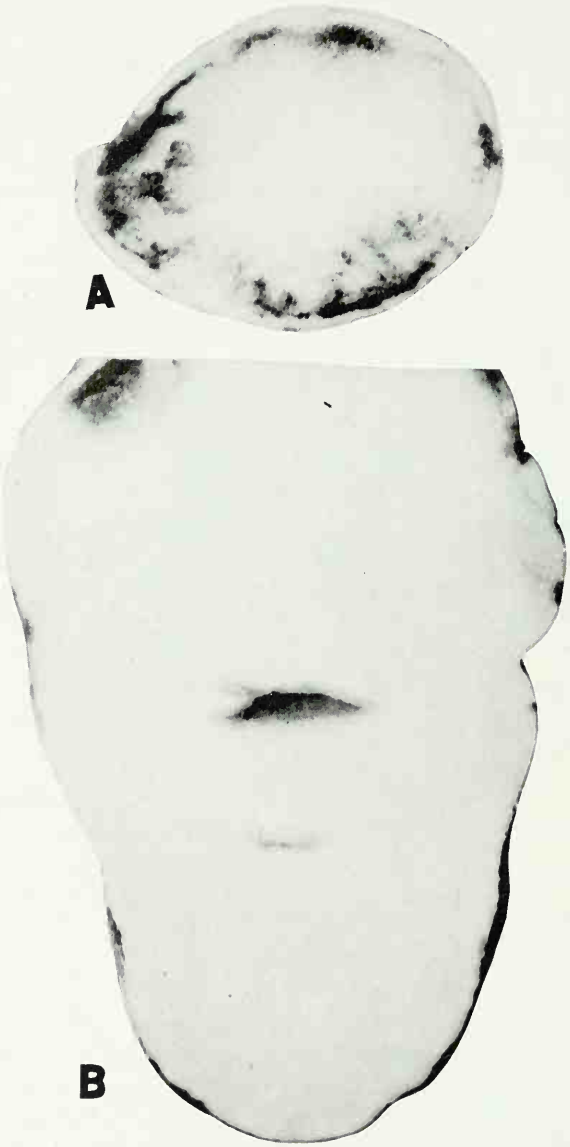

FIG. 8.—BLOTCH TYPE OF FROST NECROSIS FOUND IN STORAGE

A—Cross section of the stem end of a tuber frozen in storage.
B—Longi-section of the remainder of the same tuber. The lesions in this case are confined to a relatively small portion of the stem-end. The growth cracks in the interior flesh have no relation to freezing injury.

same symptom type occurred in the Triumph variety as a result of an exposure of –8°C. for less than two hours and practically never at higher temperatures. The ring type is but slightly less common than the blotch in tubers of all varieties subjected for long periods to high freezing temperatures. Both occur commonly in potatoes which have been frozen in storage. Less definite blotch discolorations of the opaque type predominate in field frozen specimens (fig. 8), frequently being restricted to a sunburned side of the tuber. With Rural New Yorkers this blotching occurs with prolonged exposure, 12 hours or more, at –3°C. Tubers of the Early Ohio variety often in our trials showed a sooty ring, water-soaked and intensely black even when not subjected to extreme exposures. These observations, which are in the main deduced from a series of experiments with well-matured tubers during winter storage, are not presented as final evidence that varietal differences are constant factors. On the contrary, examination of hundreds of samples of several varieties of potatoes which were accidentally frozen do not indicate any such uniformity. They do show, however, that minor varietal differences appear where freezing conditions are accurately controlled.

While we have learned to expect internal darkening of the tissues as a regular symptom of severe frost necrosis, there are mild types in which this may not show much when the tubers are first cut open. In some such cases, even with tubers which had stood for a number of hours after removal from the freezing chamber, the only evidence of frost necrosis upon cutting them open was that the injured areas seemed drier and filled with air, and they showed a grayish-white tint when first exposed but within a short time turned red, then brown, meanwhile shrivelling somewhat. Although kept for a week or more none of these vascular or other injured tissues turned dark except on the cut surface. We have interpreted this as a mild type of local injury in which after certain cells were killed their freed sap was so absorbed by the adjacent tissues as to hasten their collapse and permit the entry of air into the intercellulars.

In addition to the symptoms above described potatoes may begin to freeze on the outside before any internal injury has taken place. This occurs most commonly where potatoes are

touching a freezing surface (Pl., fig. C) but also often happens in the Triumphs which have a very thin corky layer. Rarely it occurs in other varieties and without any apparent cause.

Symptoms of frost necrosis as found in storage. The occurrence of early autumn frosts in northern Wisconsin in both 1917 and 1918 caught potatoes so frequently that there have been numerous opportunities for observing the resultant effects upon such potatoes during winter storage. In general, these

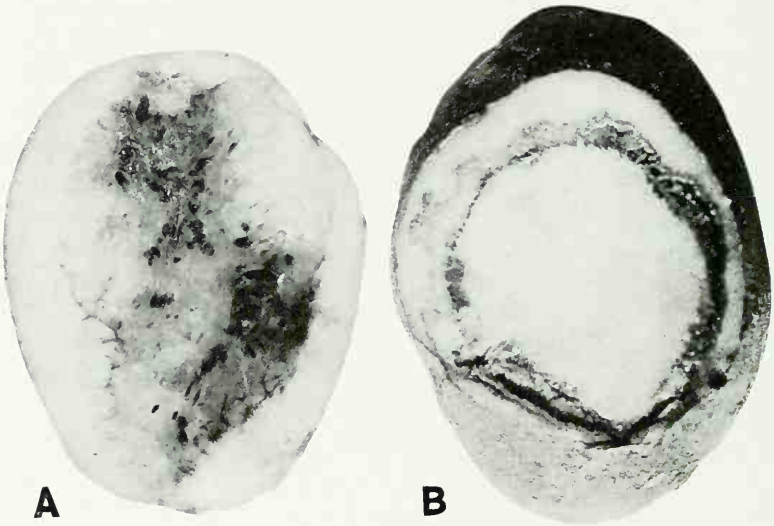

FIG. 9.—DRIED OUT NECROTIC LESIONS

Tubers found in storage in March which appeared perfectly sound externally. A—Net type of frost necrosis in which pitting has resulted from drying out. B—Ring type, very opaque discoloration, also pitted.

observations have shown that under good storage conditions and where only internal necrosis occurs the symptoms do not change much. As a result, tubers showing the milder degrees of internal frost necrosis may lie in the storage bin all winter practically indistinguishable from the normal tubers with which they are intermingled. It is true that if the internal lesions are very extensive such tubers will tend to wilt or shrivel worse than the normal ones and show internal pitting when cut (fig. 9). Also, Fusarium dry rot attacks them rather more frequently, probably following up the dead vascular areas from the stem end tissues. So far as can be judged from general

observations, such Fusarium invasion in its earlier stages merely intensifies the injuries, slowly increasing their area and giving the tissues a darker color, but not essentially changing their type. If this proceeds to the later stages of dry rot the distinguishing symptoms of frost necrosis are soon obliterated.

Black heart symptoms may also complicate those of frost necrosis particularly in storage. While it is probable that in many cases these symptoms may have resulted from other factors than those which condition frost necrosis there is some evidence that they may occur as a result of freezing.[1] In February, 1919, some tubers were found in Rhinelander, Wis., which showed both the net type of frost necrosis and black heart. They had been stored in a well-ventilated room held at temperatures constantly below 60°F., averaging nearer 40°F., and had been subjected to one sudden freezing temperature when a door had been left open on a very cold day.

Rate of discoloration of frozen tubers. Since the lesions of frost necrosis result directly from the oxidation of cells killed during the freezing process, they are not evident in tubers when they are first removed from the freezing chamber but appear only after such tubers have been exposed to warm air for several hours.

In order to determine the color changes which occur during the oxidation process and the time necessary for their completion, experimentally frozen tubers were thawed at different temperatures and slices cut from them at short intervals during several days. It was determined that the color cycle, like that described and pictured by Bartholomew (3, p. 631) for black heart, ranges through pinks, browns, and grays and seems to

[1] The difficulty of learning exactly the causal factors concerned with internal discolorations is well illustrated by recent observations with two lots of seed potatoes. In one case the grower stored his potatoes temporarily in pits in the autumn and found some "wet" tubers indicative of freezing upon transferring later to the winter storage cellar. These were sorted out and the rest of the tubers, some of which were preserved for seed, kept well and began to sprout normally the following May. When cut open during the winter storage period frequent cases of frost necrotic discoloration were detected. Preparatory to planting the tubers were disinfected in May and then left in the open for several days to dry and start new sprouts, being covered with blankets. Upon cutting this seed stock it was found to show much black heart in addition to frost necrosis. The grower suspected frost as responsible for all his injury but E. T. Bartholomew, who examined this with us, diagnosed the black heart as resulting from heat consequent on exposure to the sun following disinfection. This was confirmed by similar exposure of another lot of seed tubers, known to be free of internal discolorations. Leaving these a few hours exposed to hot June sun was enough to induce a considerable amount of black heart. While this heat injury is less likely to occur at digging time it is nevertheless possible, especially with the early or southern crop.

develop simultaneously throughout the injured tissues. The time required for the ultimate dark color to be reached depends in part upon the air temperature; thus, at temperatures of 10° to 15°C. from ten to twelve hours were required, while at 25° to 30°C. only five or six hours were necessary. There was no evidence that the rate of thawing influenced the degree of injury nor that tissues which had received severe freezing injuries blackened more rapidly than did those with lesser injuries.

FROST NECROSIS SYMPTOMS CONTRASTED WITH THOSE OF OTHER TUBER MALADIES[1]

In freshly frozen tubers frost necrosis may, in general, be easily distinguished from other potato tuber diseases by the distribution and color of the lesions. Sometimes it may happen that the lesions shown by a single tuber may be so little characteristic as to leave one in doubt, but if several tubers are available, confident judgment is usually possible. If, however, such tubers have lain for some time following the injury, secondary storage rots may set in and complicate matters. Since the same forms of storage rot may follow secondarily after various other initial injuries the only recourse in such cases is to seek for as clear evidence as is obtainable concerning the character of the initial injuries and base final judgment upon this.[2] It is also helpful in diagnosis of injuries in stored potatoes to know the region from which the tubers came since, to

[1] Since detailed descriptions of the above-mentioned tuber diseases occur in current phytopathological literature no attempt is made here at their full characterization. Should this be desired in any case the following citations will furnish illustrated accounts: Late blight dry rot, Jones, L. R., Giddings, N. J., and Lutman, B. F., *Investigations of the potato fungus Phytophthora infestans*. U. S. D. A., Bur. Pl. Ind. Bul. 245, pl. 2, 1912; Fusarium dry rot, Orton, C. R., *Potato diseases*. Penn. State Agr. Exp. Sta. Bul. 140, p. 26, fig. 13, 1916; Bacterial brown rot, Smith, E. F., *Bacteria in relation to plant disease*, v. 3, p. 174, pl. 23, 1914; Net necrosis, Orton, W. A., *Potato wilt, leaf-roll and related diseases*. U. S. D. A., Bur. Pl. Ind. Bul. 64 (professional paper), p. 8–9, pl. 2, fig. 2, 1914; Black heart, Bartholomew, E. T., *Black heart of potatoes*. Phytopathology, v. 3, pp. 180–182, pl. 19, 1913; Internal brown spot, Horne, A. S., *The symptoms of internal disease and sprain (streak-disease) in potato*. Jour. Agr. Sci., v. 3, pp. 322–333, pl. 19, 1910.

[2] Critical attention has been given to the symptoms of frost necrosis as it appears in the city markets, especially in the markets of Chicago where northern grown potatoes are handled, by Geo. K. K. Link and M. W. Gardner. Their observations were continued over a period of sufficient duration to afford an opportunity to study both initial frost injuries and those complicated by storage rots at different seasons. The writers have had access to their results in an unpublished manuscript which will be issued later by the United States Department of Agriculture as a handbook of diseases of vegetables occurring under market, storage, and transit conditions, prepared under the direction of W. A. Orton of the Bureau of Plant Industry and W. M. Scott of the Bureau of Markets.

one acquainted with conditions, this may give important suggestions as to the probable initial causes. The commonest of such types of tuber injury initiated by factors other than freezing are as follows:

1. Dry rot. Of these, late blight rot caused by *Phytophthora infestans* is distinguished from frost necrosis by the fact that the initial lesions are strictly superficial, the discoloration rarely proceeding deeper than the cambial region and with no tendency to follow the vascular distribution as does frost necrosis.

The common types of Fusarium dry rot, of which examples occur in practically every lot of storage potatoes, as a rule show conspicuous external lesions and when cut open the uninvaded flesh is uniformly bright and normal in appearance whereas freezing injuries show as persistent discolorations.

2. Wet rot, soft rot. Following severe freezing injuries to potatoes all fully frozen tissues collapse immediately upon thawing. Often only part of a tuber is so involved, in which case the remaining flesh if cut open may show the net or blotch lesions characteristic of frost necrosis. As a rule, however, bacterial wet rot immediately follows as a secondary trouble and proceeds to the destruction of the entire tuber. In case of severe attacks by the bacterial blackleg disease the tubers may show a soft rot either while in the soil or soon after harvest. In most cases, however, such rapid wet rot is a secondary development following late blight or some other initial injury to the tuber, especially in heavy wet soils.

3. Ring necrosis. Stem-end bundle blackening occurs in some degree in many potato tubers, showing as a darkening when the stem end is cut across. This may be very shallow (perhaps one-eighth inch or less) in which case it is considered non-parasitic in origin, or it may extend well through the length of the tuber, in which case it is usually attributed to Fusarium invasion. The former type should lead to no confusion with frost necrosis but the latter may. In general, it may be differentiated by its being more strictly limited to the vascular elements of the cambial ring without the attendant net necrosis or blotch lesions of frost necrosis.

4. Brown rot. This name is applied to the bacterial disease, caused by *Bacillus solanacearum*, which may cause a wet, slimy

rot of the vascular ring. It is, however, readily distinguishable, as a rule, by the showing of a typical grayish bacterial exudate from the vascular elements in the earlier stages, by the wetter condition of the tuber in the later stages, and by its restriction to southern stock, whereas frost necrosis is to be expected in northern stock.

5. Net necrosis. This name has been applied to a condition where the vascular elements brown more or less throughout the flesh of the tuber even during the developmental stage, i. e., before digging. This is considered non-parasitic and is inheritable from generation to generation. It seems impossible by appearance alone to distinguish confidently between this inheritable net necrosis and the net type of frost necrosis. In practice, however, where one is dealing with any considerable number of examples of necrotic tubers, there will probably be little difficulty in correct diagnosis. In the case of frost necrosis only a part of such tubers should show lesions of the net necrosis type, others showing ring and blotch discolorations. Probably in most cases some significant evidence may be obtainable also as to the history of the sample, including liability to exposure to freezing temperatures.

6. Black heart. The typical black heart lesions, resulting from high temperature storage or asphyxiation through confinement with insufficient free oxygen, consist of clearly delimited internal discolorations. In certain cases of frost necrosis as already cited (see p. 19) J. P. Bennett has found black heart symptoms where the history of the tubers seemed to preclude the above types of asphyxiation. In any case, this is not likely to be common or seriously confusing.

7. Internal brown spot. This non-parasitic and non-infectious malady is characterized by definite brown spotting of the interior flesh of the tuber. It is readily distinguishable by its brown color from the internal grayish or purplish black frost blotch necrosis. The distinction is made surer by the absence in this brown spot malady of any tendency toward vascular discoloration of the ring or net types so commonly associated with frost necrosis. According to Horne's description internal brown spot lesions may be delimited by cork cells in which case microscopical examination should assure their differentiation from frost necrosis.

THE AMOUNT AND TYPES OF FROST NECROSIS WHICH OCCUR AT DIFFERENT TEMPERATURES

Because later experiments (pp. 35–36) show that the rate of fall of temperature is one of the factors which seem to influence the amount of injury tubers sustain when chilled, the following data are compiled entirely from the 1918 experiments in which the Potter freezing machine was used. They show a certain uniformity in the types of injury which occur at the same temperatures, but also indicate the striking individual resistance of tubers in many cases. Unless otherwise indicated, tubers of the variety Rural New Yorker were used in these tests, and the air temperature was dropped at the rate of 3½°C. per hour after the zero point was reached. The percentage of injury as shown in these tables is not very conclusive since only 10 or 15 tubers at most were exposed at one time. However, they correspond in general with the data obtained in the earlier experiments where larger numbers of potatoes were exposed under uniform conditions out-of-doors and where individual resistance also showed strikingly.

Injury above –3.2°C. Müller-Thurgau (4, p. 147) held that the critical temperature at which potatoes regularly began to freeze was –3.2°C. and Appleman (2, p. 333) stated that this process began at temperatures ranging from –2.2° to –3.3°C. In our experiments, therefore, the attempt was made to reconcile their results. In numerous experiments tubers were held at –2°C. for hours (in some cases for 48 hours) and no injury ever resulted. Similarly, temperatures ranging from –2.0° to –2.5°C. were tested and found to be too high to produce injury. Between –2.5° and –3.0°C., however, although frost necrosis did not always occur, it did in perhaps 50 to 75 per cent of the experiments, depending upon the length of the exposure and the individual susceptibility of the tubers under trial. The following table gives data as to amounts and predominating types of injury from several of the experiments in which temperatures of from –2.5° to –3.2°C. were used.

TYPES OF FROST NECROSIS IN MARKET POTATOES

A.—STEM END INJURY

Cross section of the stem end showing irregular blotches. The whitish areas together with the wilted appearance of the surface of the tuber indicate drying out which often follows freezing injury in storage. The general distribution of lesions in such tubers is well represented in figure E, a longitudinal section of another tuber in which injury is restricted to the stem end.

B.—GENERAL DISCOLORATION OF STEM END TISSUES

Cross section of the stem end in which blotches are accompanied by a general discoloration. Whitish areas in the cortex again indicate drying out.

C.—RING DISCOLORATION AND ONE-SIDED FREEZING INJURY

Cross section of a tuber one side (left) of which was evidently in contact with a freezing surface. The double ring of darkened vascular elements may have resulted from the same exposure as did the one-sided injury or from another exposure to freezing temperatures.

D.—NET TYPE OF FROST NECROSIS

Section of a tuber which shows a very uniform darkening or browning of the vascular elements throughout the tuber. This symptom is very common in turgid tubers which have been exposed to temperatures approaching —5° C. Notice how sharply the injury is limited to the vascular elements.

E.—STEM END INJURY OF THE BLOTCH TYPE

Longitudinal section showing discoloration and drying out of the outer tuber tissues. Note that the injury is confined to the upper portion of this tuber, which is the stem end. See also figures A and B.

Reproduced from hand-colored photographs made under the direction of G. K. K. Link and M. W. Gardner of the Bureau of Plant Industry, U. S. Department of Agriculture.

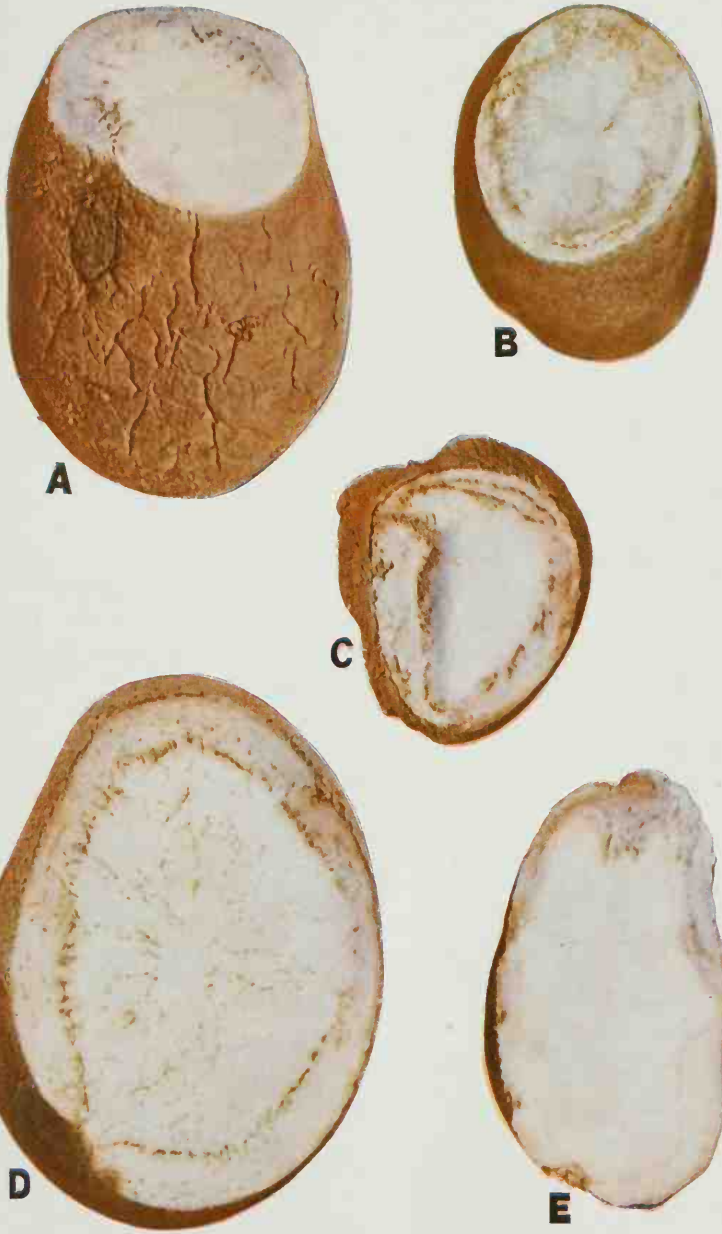
A
B
C
D
E

TABLE I—TYPES AND AMOUNTS OF INJURY AT —2.5° TO —3.2°C. (27.5° TO 26.2°F.)

Exp. No.	EXPOSURE		INJURY	
	Temperature °C.	Period	Frost necrosis	Frozen solid
1	−2.5°	6 hrs.	none	none
2	−2.5°	12 hrs.	blotch (faint), 20%	"
3	−2.8°	12 hrs.	" " , 15%	"
4	−2.5° to −3.0°	18 hrs.	" (sooty), 30%	" [1]
5	−2.6° to −3.2°	18 hrs.	" and ring, 60%	"
6	−3.0° to −3.8°	18 hrs.	" " " , 80%	10%
7	−2.8° to −3.2°	24 hrs.	" , 100%	80%

[1] In experiment No. 4 two tubers which had been peeled were included. These were frozen solid and the unpeeled were not. See further data bearing on this, Table III, Exp. 8, Table IV, Exp. 2, and later discussion of this point.

From Table I it appears that long exposures to critical temperatures are conducive to the production of the blotch type of injury, and that they ultimately result in the tubers freezing solid. The fact that this blotch type of discoloration is found commonly in field frozen specimens and that it occurs so regularly from prolonged exposure at these high temperatures is significant. Occasionally, however, the ring type is produced at these temperatures.

Injury at −3° to −4.5° C. The temperatures below −3°C. were employed in further experiments to determine the time during which such temperatures must be maintained in order to produce frost necrosis and also to furnish material for studying symptoms of tubers frozen at these lower temperatures. In storage and transportation tubers are often accidentally subjected to dangerously low temperatures for short periods. Table II gives the result of several experiments in which these temperatures were employed.

TABLE II—TYPES AND AMOUNTS OF INJURY at —3.2° to —4.4°C. (26.2° to 24°F.)

Exp. No.	Exposure		Injury	
	Temperature °C.	Period	Frost necrosis	Frozen solid
1	—3.2 to —3.7	5 hours	Blotch, 10%	None
2	—3.2 to —4.0	5 hours	Blotch and ring, 20%	None
3	—3.6 to —3.9	3 hours	None	None
4	—3.7 to —3.9	2 hours, 30 min.	None	None
5	—3.6 to —4.2	5 hours	Blotch and ring, 50%	None
6	—3.5 to —4.2	12 hours	None	100%
7	—4.0 to —4.3	3 hours	Net and ring, 60%	None
8	—4.2 to —4.4	2 hours, 30 min	Net and ring, 50%	None

Injury at –5° to –5.6° and at –6° to –8°C. The results of both the 1916 and 1918 experiments show that the highest percentage of net necrotic discoloration occurs after short exposures to temperatures of –5° to –5.6°C. These are not exclusive of other types but they predominate.

TABLE III—TYPES AND AMOUNT OF INJURY AT —5° TO —5.6°C. (23° TO 21.9°F.)

Exp. No.	Exposure		Injury	
	Temp. °C.	Period	Frost necrosis	Frozen solid
1	—5	1 hr., 30 min.	blotch and net, 30%	none
2	—5	1 " 50 "	ring and net, 100%	"
3	—5	2 "	blotch and net, 50%	"
4	—5	3 "	blotch, 70%	30%
5	—5.5	1 ", 30 min.	ring and net, 20%	none
6	—5.4	2 "	" " ", 50%	"
7	—5.5	2 "	blotch and net, 60%	"
8	—5.6	1 "	ring and net, 60%	2 peeled

TABLE IV—TYPES AND AMOUNT OF INJURY AT —6° TO —8°C. (21.2° TO 17.6°F.)

Exp. No.	Exposure		Injury	
	Temp. °C.	Period	Frost necrosis	Frozen solid
1	—6.0	1 hr.	net, 80%	none
2	—6.2	30 min.	" and ring, 20%	(2 peeled)
3	—6.5	45 "	" " blotch, 60%	none
4	—6.8	30 "	" " ring, 40%	"
5	—7.0	1 hr.	" " blotch, 70%	"
6	—7.4	45 min.	" " " , 100%	"
7	—7.8	2 hrs.		100%
8	—8.0	1 "	net and blotch, 100%	0

The net type seems almost as prevalent at these lower temperatures (–6° to –8°C.) as at the next higher (–5° to –6°C.) but in each case where it is recorded as being present the blotch predominated. In experiment No. 8 the net type occurred in Triumph potatoes while the blotch refers to the condition in the Rurals.

Not only do these experiments show that net necrosis develops very commonly as a result of short exposures at rather extreme temperatures, but it has been found as the predominating type in cases of freezing injury to storage potatoes where the temperature has been known to drop suddenly. On the other hand, it has rarely been observed in cases of field injury before digging.

Injury at –10.5° to –11.7°C. Potatoes freeze solid at temperatures below –10°C. if they are exposed for any considerable time. Internal frost necrosis develops promptly in all such tubers with the blotch type predominating over the net. It is also of interest to note that at these extreme temperatures freezing begins more often at the surface and proceeds inward. Thus, in experiments 3, 4, and 5 of Table V the tubers reported as frozen solid were not entirely frozen but had begun thus to freeze from the surface, and in some the peripheral half-inch was thus killed but the interior was intact.

TABLE V—TYPES AND AMOUNT OF INJURY AT —10.5° TO —11.7°C. (13.1° TO 10.9°F.)

EXP. NO.	EXPOSURE		INJURY	
	Temp. °C.	Period	Frost necrosis	Frozen solid
1.....	-10.5.....	45 minutes.....	blotch and net, 70%	none
2.....	-11.0.....	30 minutes.....	" " ", 70%	30%
3.....	-11.2.....	1 hour.....	blotch, 40%.....	60%
4.....	-11.7.....	45 minutes.....	" , 40%.....	60%
5.....	-11.7.....	1 hour.....	" , 10%.....	90%

RELATION OF TUBER CONDITION TO SUSCEPTIBILITY TO FREEZING

Throughout the course of these investigations individual susceptibility of tubers to freezing injury appeared constantly in field and storage as well as in experimentally frozen specimens, and it seemed probable that it might be explained by some internal condition of the tuber which could be produced experimentally if the external factors were controlled. Consequently, potatoes at different stages of growth which had been subjected to varying storage conditions were exposed to similar freezing temperatures and the results compared critically.

Relative resistance of mature and immature tubers. During the season potatoes of different stages of maturity were tested for resistance. Three plantings of the Rural New Yorkers were made on June 1, July 13, and August 10, respectively. All were dug on October 3, at which time the tubers from the first planting were mature, those from the second about half-grown, while those from the third measured from one-half to two-thirds of an inch in diameter. Soon after harvest, when these tubers were still turgid and unmodified by storage, trials were made in which several tubers from each of these plantings were exposed to the same freezing temperatures but no consistent difference in susceptibility appeared. To be sure, in some trials a larger number of mature than immature tubers remained normal, but in others the immature tubers seemed more resistant to freezing temperatures. As is common with turgid Rurals the net symptoms predominated in all of these tubers.

Figure 10 shows three of these potatoes, one from each planting, which were exposed together to –6.5°C. for about two hours.

Influence of relative turgidity of tubers. It is a natural supposition that the relative turgidity of the tuber tissues may influence their susceptibility to freezing injury. In some of the earlier trials partly wilted tubers were exposed along with turgid

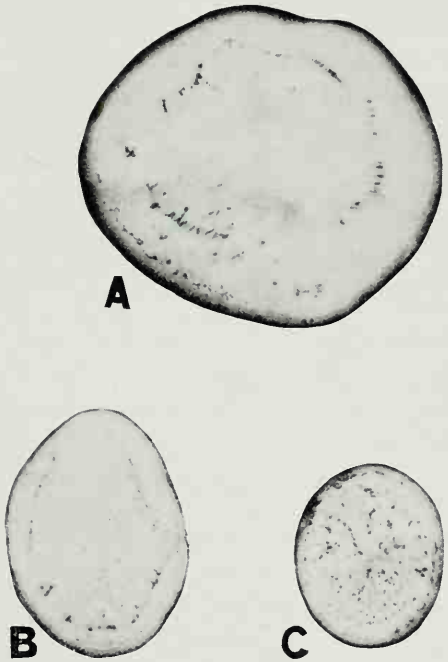

FIG. 10.—INFLUENCE OF MATURITY UPON SUSCEPTIBILITY TO FROST INJURY

Sections of three tubers of different stages of maturity which were exposed together to a temperature of —6.5° C. for two hours. All were harvested on October 10; A from seed planted June 1, B from seed planted July 13, and C from seed planted August 10.

ones, and no consistent differences developed. Such comparisons have been made at various times during three seasons with like results. Owing to the individual variations between tubers it is difficult to make as convincing comparisons as might be desired, and it is impracticable to use a divided tuber for such experimental purposes because of the possible disturbing effect of cut surfaces upon supercooling.

In an effort to establish moisture conditions which were as nearly uniform as possible, in some later experiments turgid

Rurals were carefully paired off as to size and weight, the pairs numbered as 1 and 1′, 2 and 2′, etc. Numbers 1, 2, 3, etc., were placed in a damp chamber and 1′, 2′, 3′, etc., in a desiccator and both stored at a temperature of 10°C. Several pairs of tubers, e. g., 1 and 1′, 2 and 2′, etc., were removed and exposed to freezing temperatures each week for a period of two months and although the tubers used may have gained slightly or lost considerably in weight during storage their susceptibility to freezing was not consistently altered.

Tables VI and VII show the results of two experiments which give an idea of the distribution of injury in the two lots of potatoes.

TABLE VI—SYMPTOMS OF FROST NECROSIS AS SHOWN IN PAIRS OF TUBERS WHICH WERE STORED UNDER DIFFERENT MOISTURE CONDITIONS FOR 6 WEEKS AND THEN EXPOSED TOGETHER TO —4° TO —7° C. (24.8° TO 19.4° F.) FOR 2 HOURS

TUBER WEIGHTS IN GRAMS			PER CENT GAIN (+) OR LOSS (−)		FROST INJURY	
Original[1]	After 6 weeks					
	Damp chamber	Desiccator	Damp chamber	Desiccator	Damp chamber	Desiccator
31	34	29	+9	−16	net (faint).....	blotch (sooty)
55	55	48	0	−13	normal	ring "
34	34	29	0	−15	ring.....	" "
50	50	44	0	−12	"	normal
51	51	48	0	−6	"	"

[1] As indicated above each one of a pair of experimental tubers had the same original weight.

A comparison of these results shows that loss of turgidity does not consistently alter susceptibility. For example, the desiccated tuber of the second pair lost 13 per cent of its original weight (55 grams) and was injured upon exposure to freezing temperatures while its turgid mate remained normal but in the fourth and fifth pairs the opposite condition obtains. There the desiccated tuber of the fourth pair lost 12 per cent of its original weight (50 grams), that of the fifth pair 6 per cent of its original weight (51 grams), and yet both

apparently gained in resistance to freezing. Results of the same type appear in Table VII.

TABLE VII—SYMPTOMS OF FROST NECROSIS AS SHOWN IN PAIRS OF TUBERS WHICH WERE STORED UNDER DIFFERENT MOISTURE CONDITIONS FOR 6 WEEKS AND THEN EXPOSED TO —2.5° TO —7.0°C. (27.5° TO 19.4° F.) FOR 2 HOURS

Tuber weights, grams			Per cent. gain (+) or loss (—)		Frost injury	
Original	After 6 weeks		Damp chamber	Desiccator	Damp chamber	Desiccator
	Damp chamber	Desiccator				
23	23	21	0	—9	normal.....	ring (faint)
43	45	41	+2	—2	ring (opaque)..	net "
28	28	19	0	—32	" "	black heart
40	40	38	0	—5	" (faint)....	net (faint)
61	61	56	0	—8	normal.....	" "

Here the desiccated tubers of the first and fifth pairs seem less resistant and in the other cases the symptoms differ only slightly in the corresponding pairs. However, where the tuber was very much wilted, as was the desiccated one of the third pair, which lost 32 per cent of its water content, intense symptoms were produced which resemble black heart. This is an extreme form but it occurs not uncommonly, and it is indicative of the increase of sootiness of necrotic symptoms with decrease of water.

Relation of sugar content. Müller-Thurgau (6, p. 493) has shown that the relative sugar content in the tuber may influence its freezing point. For example, by preliminary storing at low temperatures he raised the sugar content from 0.53 per cent to 2.21 per cent. His trials then showed that the true freezing point with these tubers was lowered from –1.0°C. for those of the normal sugar content, to –1.5°C. for those of the excessive sugar content. It is to be noted, however, that in his trials he secured extremes of variation far beyond those which are ordinarily met with in normal potato tubers and even so the influence upon the freezing point was not proportionately great. This factor may, however, be influential in determining the relative injury to the different tissue elements in the tuber.

Influence of wounds and bruises upon susceptibility. The presence or absence of a film of moisture on the exposed surface of a wounded or bruised tuber seems to determine the influence of such wounds and bruises upon susceptibility to freezing injury. When wounds or bruises are corked or healed over as in the case of common scab, dry rot, or mechanical injuries, they have no important influence upon the susceptibility of the tubers. Even freshly cut surfaces often seem not to cause freezing to take place at higher temperatures as Müller-Thurgau (4, p. 172) predicted. In his experiments with freshly peeled tubers he found that supercooling was prevented by the presence of the surface film of exuded sap on such tubers. He explained this as being due to the fact that this free sap began to crystallize at the freezing point of sap (about –1.0° C.) and that when the sap throughout the tuber was chilled to this degree the presence of crystals on the outside caused the freezing process to extend from the outside inward, without the usual supercooling phenomenon. In our experiments tubers were freshly cut in different ways, some were peeled and some split in half longitudinally, and from others slices were cut, most often from the stem end. It was found that peeled potatoes usually froze solid at temperatures which produced only minor injuries, if any, in sound tubers. In a few cases, however, typical necrotic symptoms appeared in these peeled tubers just as in the case of tubers with surfaces only partially exposed. In some cases freezing started on these cut surfaces and progressed inward for two or three millimeters while the usual necrotic symptoms appeared in the deeper-lying tuber tissues.

Relative susceptibility of sprout and tuber tissues. Sprouts have in our experiments always proved more resistant to freezing injury than the tissues of the tuber from which they arise. As a result, if a sprouted tuber is exposed to freezing temperatures the parent tuber may show considerable internal necrosis and have its sprouts unaffected (fig. 11). Since this has an important bearing upon the relation of frost necrosis to the value of potato seed stock, numerous trial plantings were made, some in sand in the greenhouse bench and some in the field soil. In certain of these experiments, in order to make closer comparisons, the trial tubers were cut in halves, one half being

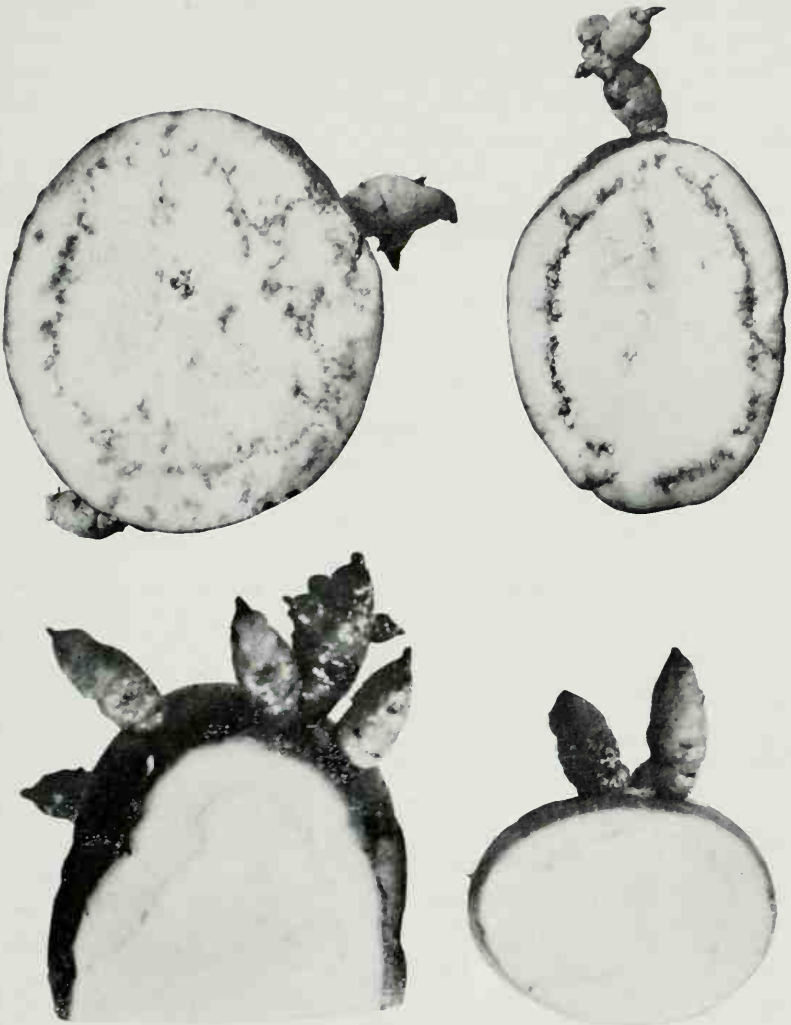

FIG. 11.—EFFECT OF FROST ON VIABILITY OF TUBERS

A and B (Upper)—Sections of two tubers which were stored at 25° C. for three months, chilled at —5° C. for two hours, then returned to the 25° C. temperature.

C and D (Lower)—Control tubers held constantly at 25° C.

Sprouts had developed on all tubers when A and B were frozen. Freezing produced necrotic symptoms in A and B without apparent injury to the sprouts which, however, continued to grow much less vigorously than did those of the control tubers, as is shown in this photograph taken three months after A and B were chilled. The photograph also indicates the lack of storage rots and drying out in necrotic tubers, even where stored at such a relatively high temperature.

held as a control, the other chilled after the surface was well dried off. In practically all cases such exposed tubers retained viability even where there was internal necrosis but the sprouts started more slowly, and where the frost necrosis was very extensive the parent tuber rotted before the sprout developed independent roots. As a result, planting frost-necrotic tubers in the field yielded only about 50 per cent of a stand. Those plants which survived, although they started more slowly, made rapid gains later and were ultimately as vigorous and productive as the normal controls. The tubers thus secured from this frost-necrotic seed were in turn all examined for any traces of vascular necrosis, and found to be free. While this was to be expected, it is worthy of note as again emphasizing the distinction between net necrosis induced by freezing injury and the hereditary net necrosis from which the symptoms may sometimes be indistinguishable.

While, therefore, in general, it is inadvisable to plant tubers showing any large amount of frost necrosis, nevertheless slightly necrotic tubers may safely be used if one cuts them and rejects pieces which show lesions extensive enough to predispose to rot.[1]

SUPERCOOLING AND ICE CRYSTALLIZATION ASSOCIATED WITH FROST NECROSIS

No attempt has been made in connection with these studies to follow the microscopic phenomena associated with the changes in the potato, but it has been the conclusion of previous investigators that the formation of ice crystals in the sap is antecedent to the death of such plant tissues. So far as our evidence bears upon the matter, it is in accord with this idea. In most cases where frost necrosis resulted it was, indeed, possible to detect ice crystals in the tissues either by their macroscopic appearance if the tubers were immediately cut open, or by holding the suspected tuber close to one's ear and pressing

[1] Supplementing Müller-Thurgau's 1882 work (5) Wollny (8) attempted to determine the influence of prolonged cold storage upon the viability of tubers. He took normal tubers, divided them into longitudinal halves and stored one set of halves in a cold chamber at 0° C. and the controls at 10° C. After 35 days he planted each set separately and recorded growth throughout the season. The aerial vegetative parts were quite uniform from both kinds of tubers but at harvest time the hills from seed tubers which had been stored at 10° C. contained more and larger tubers than did those from the parent seed tubers which had been stored at 0° C.

sharply between thumb and finger, when the presence of ice crystals is revealed by a faint crunching sound. This is, however, but a crude test and its unreliability was shown by the fact that frost necrosis appeared in some cases where ice crystals were not so detected. Still more significant is the fact that in other cases ice crystals were heard when no evident injury resulted. So far as any conclusion was justified, therefore, it is that frost necrosis does not necessarily result from a slight amount of ice crystallization but that this must proceed to a certain advanced stage to produce death of the associated tissues.

It is a matter of common experience concerning the effect of freezing upon plant tissues that there are wide variations in susceptibility and various theories have been developed to account for this. Since our experiments give no new evidence bearing on these we will simply record the facts without attempting to relate them to such theories.

attempting to relate them to such theories.

Another interesting phenomenon having relation to ice crystallization is that known as supercooling. On this some evidence was secured. It is a familiar fact that any liquid must be cooled to some temperature below its freezing point before crystallization begins. This range of temperature below the freezing point is supercooling. Following supercooling there is a sharp temporary rise of temperature to the higher degree, this latter constituting the true freezing point of the solution (fig. 12). Since potato sap carries considerable matter in solution its freezing point is lower than that of pure water. Müller-Thurgau determined it to be about –1.0°C. but in our experiments it often more nearly approximated –2.0°C. than –1.0°C. and varied widely with individual tubers (Table IX).

Müller-Thurgau found further that where he made comparative determinations of the supercooling points of living plant tissues and of the expressed sap, the living tissues had a lower supercooling point than did the expressed sap. He also found that when the potato was frozen, then thawed, and frozen again, the extreme supercooling was not required for the second freezing. This lowering of the supercooling point in living tissues he attributed to the resistance of active protoplasm.

Relation of time element to supercooling. Müller-Thurgau held that the supercooling point varied directly with the air temperature to which the tuber was exposed; i. e., was depressed with the fall of air temperature. He justifies this conclusion by such data as are given in Table VIII.

TABLE VIII.—MÜLLER–THURGAU'S RESULTS SHOWING RELATION OF SUPERCOOLING POINT TO AIR TEMPERATURES

Exp. No.	Exposure		Potato Temperatures	
	Temperature °C.	Time	Supercooling point °C.	Freezing point °C.
1.....	- 4.5.....	2 hours.....	-3.2.....	-0.8
2.....	- 5.0.....	not given.....	-3.5.....	-1.2
3.....	- 7.2.....	" "	-4.1.....	-1.4
4.....	-11.0.....	4 hours.....	-5.7.....	-1.0
5.....	- 9 to -12.....	5 "	-6.1.....	-0.98

From our experience it requires some further explanation than is afforded by Müller-Thurgau's figures to understand why freezing should have occurred at the end of 2 hours at -4.5° C. and at the end of 4 or 5 hours at the extremely low temperatures of experiments 4 and 5, Table VIII. Müller-Thurgau, in his experiments, already explained, had no way of regulating the rate of fall of the air temperature in his freezing chamber. Fortunately, with the Potter freezing apparatus we were able to do this. We therefore undertook to repeat Müller-Thurgau's experiment controlling this time factor. The results, as shown in Table IX, indicate that the rate of fall of the air temperature influences the supercooling point.

TABLE IX—RELATION OF SUPERCOOLING TO RATE OF FALL OF FREEZING TEMPERATURES

Exp. No.	Variety	Air Temperature		Potato Temperature		
		Max. temp. °C.	Time to drop from 0° to max. temp.	Supercooling point °C.	Time to supercool	Freezing point °C.
1	Rural.....	—5.5	95 min.....	—4.0	105 min.....	—1.25
2	"	—5.0	90 "	—4.95	174 "	—1.3
3	"	—10.5	80 "	—4.2	73 "	—1.8
4	"	—11.0	40 "	—3.1	49 "	—1.7
5	Irish Cobbler	—5.6	20 "	—3.5	100 "	—1.7
6	" "	—6.0	60 "	—5.5	125 "	—1.7
7	" "	—11.0	55 "	—3.2	58 "	—2.3
8	Early Ohio...	—4.4	120 "	—4.15	112 "	—1.9
9	" " ...	—8.0	40 "	—2.2	75 "	—1.6
10	" " ...	—11.0	45 "	—2.8	55 "	—1.5

From these data it seems evident that the supercooling point does not vary simply with the air temperature but that it is influenced by other factors, including the rate of fall of the temperature. Comparing tubers of the same variety, experiments 3 and 4 show that in 3 a slow drop to –10.5C. gave a lower supercooling point (–4.2°C.) than did a rapid drop in 4 to practically the same point. With another variety, in experiment 6, a slower drop to –6°C. gave a lower supercooling point (–5.5°C.) than did the rapid drop to –11°C. in experiment 7, (supercooling point –3.2°C.). It will be noticed that in general the supercooling points recorded in our trials (Table IX) represent about the same range as Müller-Thurgau's (Table VIII). These are also in accord with our general experience; viz., that potatoes do not begin freezing until exposed to –3°C. or lower. It will be noted, however, that in two cases, experiments 9 and 10, the supercooling point was reached above –3°C. These are to be regarded as exceptional cases requiring explanation. In the first place, in this method (see fig. 4) mutilated tubers are used and where freshly cut surfaces are exposed, even with precautions to dry them, the supercooling point may be raised. In the second place, the supercooling point may be influenced by such external factors as mechanical disturbance, as was indicated in some of our experiments.

The ultimate freezing point. The ultimate freezing temperatures as shown in Table IX are in general somewhat lower than Müller-Thurgau's, Table VIII. In both cases it will be noted that there is a considerable variation. It will be evident that the method employed can give only approximate results at the best, and also that this varies with individual tubers.

Relative temperatures of air and potato. Tables X and XI show in detail the comparative temperatures of air and the interior of the potato tuber and the supercooling range as fol-

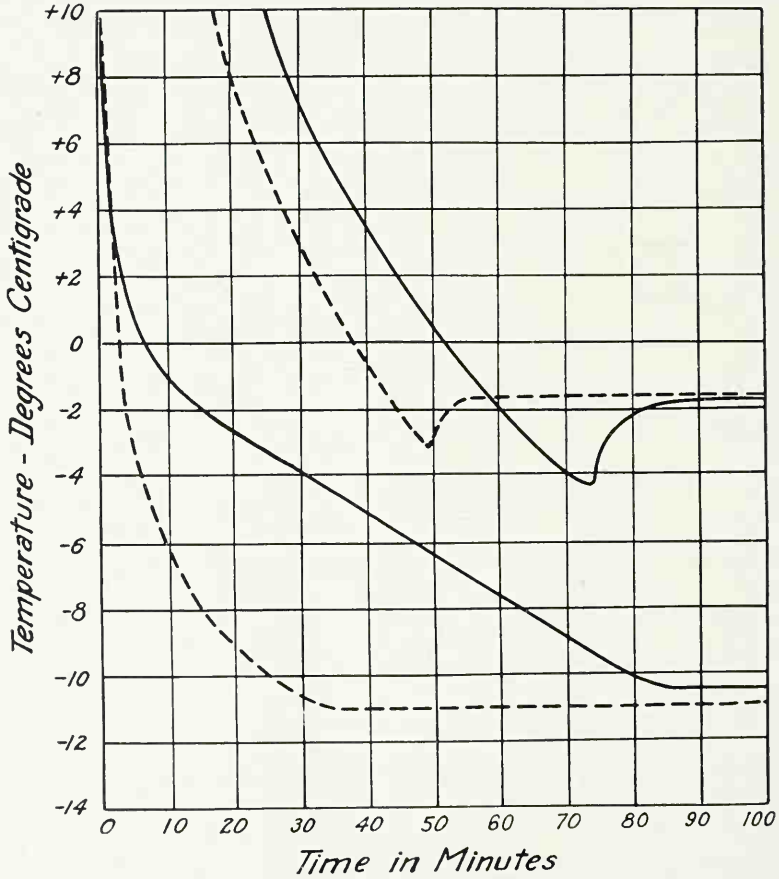

FIG. 12.—GRAPH REPRESENTING THE RELATIVE TEMPERATURES OF AIR AND TUBER IN SUPERCOOLING EXPERIMENTS

The upper curves represent the temperatures of the interior of the tubers and the lower represent corresponding air temperatures. The dotted lines indicate the temperatures in experiment No. 4 (Table 9) while the continuous lines belong to experiment 3 (Table 9). A comparison of these curves shows that where the air temperature dropped rapidly, as in experiment 4, the supercooling point of the tuber occurred more quickly and at a higher temperature than where the air temperature was dropped slowly, experiment 3. See further evidence of this in Tables 10 and 11 and accompanying text.

lowed through two experiments, in one of which the temperature fall was more gradual than the other. In both cases the internal temperatures could not be accurately recorded in the earlier stages owing to the fact that the thermometers were graduated only for lower temperatures. These data are, however, unimportant.

The apparent influence of the rate of fall of air temperature upon the supercooling range is shown graphically in figure 12.

Due to the sudden rise of temperature in the interior of the potato just following the supercooling period, all curves which represent the internal temperature of potato tubers have a profile similar to that represented in this graph (fig. 12).

TABLE X—THE INTERNAL TEMPERATURE VARIATIONS OF A POTATO WHEN THE AIR TEMPERATURE IS DROPPED SLOWLY TO —5°C (TABLE IX, EXP. 2)

Time	Temperature	
	Air °C.	Potato °C.
0	+10	
10 min.	0	
20 "	—1.0	
40 "	—1.8	
50 "	—2.4	
60 "	—2.7	
70 "	—2.7	+2.0
80 "	—4.6	+0.3
90 "	—5.0	—0.9
100 "	—5.4	—2.0
110 "	—4.6	—2.7
115 "	—4.8	—3.2
120 "	—4.8	—3.3
125 "	—4.7	—3.5
130 "	—4.9	—3.8
135 "	—4.6	—3.9
140 "	—4.8	—4.1
145 "	—4.7	—4.2
150 "	—4.7	—4.4
155 "	—5.0	—4.5
160 "	—4.7	—4.7
165 "	—5.0	—4.7
170 "	"	—4.85
171 "	"	—4.9
172 "	"	—4.91
173 "	"	—4.92
174 "	"	—4.95
175 "	"	—2.9
180 "	"	—1.8
185 "	"	—1.5
190 "	"	—1.4
195 "	"	—1.3
200 "	"	—1.3
205 "	"	—1.3

TABLE XI—THE INTERNAL TEMPERATURE VARIATIONS OF A POTATO WHEN THE AIR TEMPERATURE IS DROPPED SLOWLY TO —10.5 C. (TABLE IX, EXP. 3. THE SAME DATA ARE GRAPHED IN FIG. 12.)

TIME	TEMPERATURE	
	Air °C.	Potato °C.
0 min.	+1.0	
5 "	+0.0	
10 "	—1.0	
15 "	—2.0	
20 "	—2.8	
25 "	—3.3	
30 "	—4.0	
35 "	—4.5	
40 "	—5.2	
45 "	—5.5	+2.0
50 "	—5.8	+0.2
55 "	—6.0	—0.3
60 "	—7.8	—1.4
65 "	—8.2	—2.9
70 "	—8.7	—3.2
71 "	—8.8	—3.4
72 "	—9.0	—3.8
73 "	—9.1	—4.2
74 "	—9.2	—4.1
75 "	—9.3	—2.9
76 "	—9.5	—2.5
77 "	—9.7	—2.4
78 "	—10.0	—2.35
79 "	—10.0	—2.3
80 "	—10.2	—2.1
85 "	—10.5	—1.8
90 "	"	"
95 "	"	"
100 "	"	"

SUMMARY

1. The potato crop suffers a considerable damage each year because of freezing injuries.

2. The most serious danger in Wisconsin and the other northern states is in autumn, when the early frosts come before or during the period of digging and handling the crop.

3. Similar danger exists in all the stages of transportation and delivery of the crop during the winter.

4. Where the tubers are frozen solid they immediately collapse upon thawing and because of their wet appearance are easily detected and sorted out.

5. In case of mild exposure only a part of the tubers may be so frozen, the rest appearing normal externally. Such tubers

are commonly held as satisfactory for storage, market or seed purposes.

6. If, however, these tubers are cut open, although all are externally sound, a certain proportion of them will usually show evidences of internal frost necrosis.

7. Such internal freezing injuries are not ordinarily visible externally, even after long storage, but in white-skinned varieties they may show as darkened areas on the skin, and in prolonged dry storage frost-necrotic tubers wilt faster than normal ones.

8. Frost necrosis is, however, at once apparent upon cutting open the tubers because of the darkening of the necrotic tissues.

9. The tissues of the stem end of the tuber are in general more sensitive to freezing injury than those of the eye end and the vascular tissues more sensitive than the parenchymatous.

10. As a result, tubers subjected to freezing temperatures when cut open may show internal discolorations of any of three types: (1) Ring necrosis, discoloration of the vascular ring, especially evident at the stem end when the tuber is cut crosswise; (2) net necrosis, in which the vascular tissue including the small thread-like phloem elements scattered through the pith and cortex are darkened; and (3) blotching, in which discolored tissue in patches, usually having vascular elements as centers, is distributed irregularly throughout the tuber.

11. Frost necrosis, especially of the net and ring types, is frequently confused with other potato tuber maladies, especially with the inheritable (non-parasitic) net necrosis and the Fusarium bundle browning, or "ring disease." It is especially important to differentiate these various types of trouble in potato seed stock.

12. Since the steam end tissues are the more sensitive, internal frost necrosis is most quickly detected by cutting off a little from the stem end of samples of suspected tubers, especially any such as show incipient wilting.

13. The necrotic discolorations develop promptly after the freezing (within a few hours, faster at higher temperatures), passing through pink to dark brown or black and ordinarily undergoing very little further change thereafter, even during long storage.

14. When drying out occurs in storage it is often evidenced internally by whitish air-filled patches or, in the more extreme cases, by small cavities within the blackened areas.

15. The turning sweet of potato tubers is often, but incorrectly, attributed to freezing. It is due to long storage at low temperatures which are, however, above the point of frost injury, and it will disappear if the tubers are again held at higher temperatures. Hence, while sweetness indicates that tubers have been held for some time dangerously near their freezing point, it does not indicate that they have been frozen.

16. There is a considerable difference between individual tubers in susceptibility to frost injury, even in the same lot of potatoes.

17. In general, neither variety, size, maturity, nor relative turgidity of potato tubers influences to any marked degree the liability to injury nor the type of resultant frost necrosis.

18. "Sweet" tubers may be more resistant to freezing than normal tubers. Müller-Thurgau showed experimentally that tubers with excessive sugar content regularly froze at lower temperatures than other tubers, but that the difference between the freezing points of "sweet" and normal tubers was not sufficient to be of economic importance. Our experiments in this case are too limited to be conclusive.

19. When wounds and bruises are healed over they apparently do not influence susceptibility to freezing. However, in tubers with freshly cut moist surfaces, freezing may begin at relatively higher temperatures and in such cases the injuries may consist of a freezing solid of the tissues from the cut surface inward.

20. In general, frost necrosis will appear in at least a portion of tubers which are subjected to a temperature of –10°C. for one hour, to –5°C. for two hours, or to –3°C. or slightly lower temperatures for several hours.

21. Although the actual freezing point of potato sap is about –1°C. the living tuber will endure long exposure to temperature at or near –3°C. without injury. This is because of the fact that the tissue must be supercooled before incipient ice crystallization can occur, but once this begins there is a sharp rise of the internal temperature to about –1°C., the true freezing

point, and the freezing injury continues to develop at this higher temperature.

22. The supercooling range seems to be dependent upon the air temperature and the rate at which this temperature is dropped. Thus, at –3.5°C. the supercooling point approaches the air temperature. If the air temperature is dropped slowly to –5°C. or below, it will approach –5°C. while if dropped rapidly to the same point it will be much higher, i. e., nearer –3°C.

23. Sprouts are more resistant to freezing than the tubers from which they arise, but uninjured sprouts on necrotic tubers often do not outlive the germination period, probably due to extensive vascular injuries of the tuber; hence if chilled tubers are planted they often fail to produce plants.

24. Plants produced by the frost-necrotic halves of experimental tubers grew more slowly than those from the control halves, but ultimately produced as large and healthy plants and as abundant a crop.

25. Necrotic symptoms never appear in the progeny of frost-necrotic seed potatoes.

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Fusarium Resistant Cabbage

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MADISON

CONTENTS

Fusarium Resistant Cabbage[1]

The disease known as cabbage yellows is making impossible the successful culture of the cabbage in large and apparently increasing areas in the United States. Nearly ten years ago the senior author began a study of the disease in the Racine district of southeastern Wisconsin. This soon led to the development of a disease-resistant strain of the Hollander or Ball Head variety which has since been distributed and successfully grown commercially under the name Wisconsin Hollander. The general facts regarding the nature of the disease and the results with control measures, especially through disease resistance, were presented in an earlier bulletin.[2] Since that date the work has been continued with some advances both in regard to the study of the disease and the control measures.

Cause and development of the disease. Cabbage yellows is caused by the soil fungus *Fusarium conglutinans* Wollenw. This has been found by Tisdale[3] to penetrate the root hairs of the cabbage plants as does the similar flax wilt Fusarium, pushing thence back through the cortical tissues until it reaches the vascular system. The invasion of the vessels proceeds rapidly from the fibrous roots through the stem into the leaves. This leads to the progressive browning and death of the vascular elements followed by a slow yellowing of the aerial parts. The invaded plants soon begin to shed their lower leaves while making a weak effort at continued growth above. The disease may appear in the seed bed but is chiefly in evidence in the field after transplanting. In the worst cases in such field attacks, death may result in a week or two after the plants are set out. The

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[1] This work has been much strengthened because of the hearty support of Dr. W. A. Orton, head of the Office of Cotton, Truck, and Forage Crop Disease Investigations, U. S. Department of Agriculture. This office has contributed most of the services of Dr. J. C. Walker and has also met other expenses. It has further cooperated through Dr. J. B. S. Norton, who has successfully grown seed from selected heads in the Washington greenhouses during two winters. Both Dr. Norton and Professor L. L. Harter, also of this office, have offered valuable suggestions.

[2] Jones, L. R., and Gilman, J. C. The control of cabbage yellows through disease resistance. Wis. Agr. Exp. Sta. Res. Bul. 38. 1915.

[3] Tisdale, W. H. Flax wilt: a study of the nature and inheritance of wilt resistance. Jour. Agr. Res. XI: 573. 1917.

majority of "yellows" diseased plants continue their sickly existence for a few weeks, gradually succumbing, while some of those slightly invaded may live through the summer and even form heads.

When the soil is once infested, the fungus seems capable of persisting almost indefinitely, such soils being thereby rendered "cabbage sick." Even in the worst "cabbage sick" soils, however, there is a marked variation in severity of attack from year to year. This results from the fact first demonstrated by Gilman[4] that aggressive host invasion occurs only at relatively high soil temperatures, 17°C. (62°F.) and above. This means that the most serious development of yellows is limited to those seasons having relatively hot weather during the early part of the growing season and especially during the first month following transplanting, which is late June and early July in Wisconsin.

Distribution of the disease. In geographical distribution the disease seems to be rather widespread in its occurrence in the eastern United States[5] but it is not by any means universal in its ravages. It seems to be most serious commercially in the older and more intensive cabbage-growing sections from Iowa and southern Wisconsin across Illinois, Indiana, Ohio, Pennsylvania, Maryland, Delaware, and New Jersey. Northward the disease is certainly less prevalent in central Wisconsin, even in old cabbage growing areas, than it is in the southern part. Such data as are available from Michigan, New York, and lower Canada indicate that in these sections also, although present in the southern areas, it lessens as one goes northward. Farther southward its occurrence has been reported to us, but as soon as one passes to the regions where cabbage is grown as a winter or early spring crop the seriousness of the disease wanes. This is probably explained by the low soil temperature prevailing during the early growth of the crop under these conditions. The facts as to the distribution or minor occurrence of this parasite are especially hard to determine since the only evidence of its presence is the development of the disease in cabbage, and even

[4] Gilman, J. C. Cabbage yellows and the relation of soil temperature to its occurrence. Ann. Mo. Bot. Gard. 2: 25. 1916.

[5] Harter, L. L., and Jones, L. R. Cabbage diseases. U. S. Dept. Agr. Farmers' Bul. 925. 1918.

where this occurs it is often difficult for one not quite familiar with both diseases to distinguish it with certainty from the bacterial black rot.[6] The reported distribution seems to accord with the conception that the cabbage Fusarium is widely distributed in the United States, at least from the Mississippi Valley eastward, and that the serious development of the disease where intensive prolonged cabbage culture occurs is conditioned upon favorably high soil temperatures during the early stages of development of the plant. It is noteworthy in this connection that the disease has not been found[7] in the cabbage fields of Holland and Denmark although these include the oldest and most intensive cabbage districts of the world. It has not developed in the cool soil of the Puget Sound coast. Whatever its present distributional limits, there seems to be good ground for believing that in the United States it is certain to be introduced sooner or later into all parts of the country where cabbage culture is long practiced and that once introduced it will persist and spread wherever soil temperature conditions permit. In the earlier bulletin[8] trials were recorded with various measures aiming at the control of the parasite after once introduced. All of these, save selection for disease resistance, gave negative results. The conclusion was reached, therefore, that it is only through securing Fusarium-resistant strains, suited to local market and climatic conditions, that the cabbage industry can be developed on a sound, permanent basis in most parts of the United States. Work to this end has, therefore, been continued with the cooperation of the United States Department of Agriculture. This has included, (1) further work with the Wisconsin Hollander; (2) the development of resistant strains of other varieties, especially of late summer types used largely for the manufacture of sauerkraut; (3) cooperation with growers and commercial

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[6] Jones and Gilman, Wis. Agr. Exp. Sta. Res. Bul. 38, p. 9.

[7] This statement is based upon the judgments of Dr. F. Kölpin Ravn, of Denmark, Dr. Johanna Westerdijk, of Holland and Dr. Otto Appel of Germany, each of whom some years ago saw the disease as it occurs in Wisconsin. Further evidence of the non-occurrence of the disease in the cooler climates of Europe and Asia has been secured in 1919 during visits to our trial grounds of the following foreign pathologists, none of whom had previously met with it, Messrs. G. H. Pethybridge, Ireland, A. D. Cotton, England, Ivar Jorstad, Norway, and K. Nakata, Japan.

[8] Jones and Gilman, loc. cit.

organizations in the production and distribution of seed of the resistant strains; (4) further studies on the relation of environment to the development of the disease.

RECENT WORK WITH WISCONSIN HOLLANDER

The name Wiseonsin Hollander was given in the earlier bulletin to the Fusarium-resistant strain selected from the commercial Hollander or Danish Ball Head. For the details concerning this, reference may be made to the former publication.[9] The large commercial cabbage growers of Wisconsin are interested only in one or the other of two types of cabbage: (1) the late variety, Hollander or Danish Ball Head, used for winter storage purposes, (2) the earlier varieties for immediate use chiefly in the local kraut factories. The first of these takes the lead in most parts of Wisconsin and has therefore merited such further attention as was necessary to its commercial distribution and use. This has involved during the last five years the critical watching of its growth in commercial fields under different environmental conditions, attempts at possible further improvement, and attention to the growing and distribution of adequate supplies of seed.

WISCONSIN HOLLANDER IN COMMERCIAL FIELDS

During the last five seasons, 1916–1920, the Wisconsin Hollander has been grown commereially on a constantly increasing acreage in the older Racine-Kenosha cabbage soils. The seed has been grown locally either by individual farmers or under the supervision of a growers' committee organized for this purpose. In 1916 there was sufficient seed distributed for widespread planting, though on a limited scale. Since 1917 the supply has been reasonably adequate for local needs. To determine for themselves the relative merits of the yellows-resistant Wiseonsin Hollander as compared with the non-resistant commercial strains, cabbage growers were urged during the first two seasons, 1916 and 1917, to plant at least one or more rows of some commereial type in the same field with the Wiseonsin Hol-

[9] Jones and Gilman, loc. cit.

lander and observe the results. They were very striking. In 1916 during the hot weather of July, the disease was unusually destructive. Figure 1 shows some of the evidences which convinced the cabbage growers that even under these most trying conditions of 1916 they could succeed with the home-grown

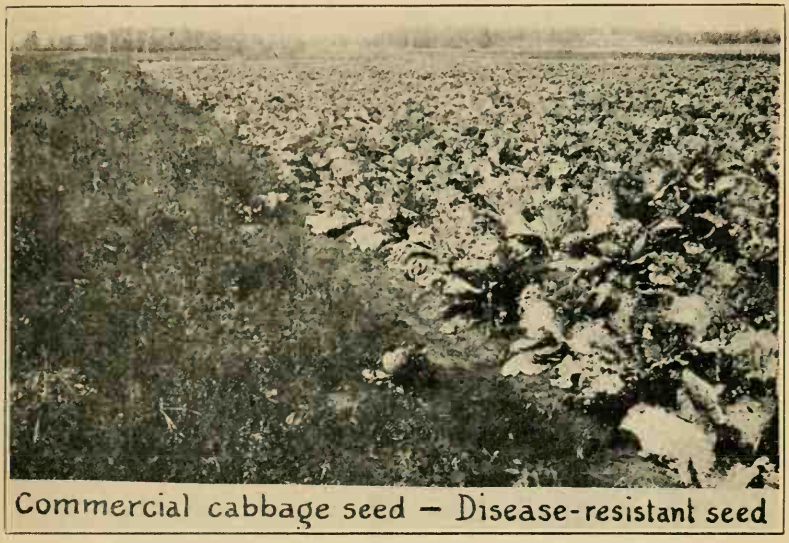

FIG. 1.—WISCONSIN HOLLANDER VS. COMMERCIAL HOLLANDER ON SICK SOIL

A farmer's trial of Wisconsin Hollander in 1916 (Scheckler's third field, Table I). The Commercial Hollander cabbage which was planted on the left was practically destroyed by yellows and the ground was occupied by weeds. The Wisconsin Hollander in balance of field, at the right, gave a highly profitable crop.

seed of Wisconsin Hollander when the non-resistant strains of Hollander were a commercial failure. Counts were made in late August of the percentage of plants showing signs of yellows in each of these fields where the Wisconsin Hollander was planted beside a comparable commercial variety. The results were as follows from the twenty fields.

TABLE I.—RESULTS OF COMMERCIAL TRIALS OF WISCONSIN HOLLANDER RESISTANT COMPARED WITH A SUSCEPTIBLE COMMERCIAL STRAIN. RACINE DISTRICT. 1916. (SEE FIGURE 1 FOR APPEARANCE OF ONE OF THESE FIELDS.)

Name of Grower	Percentage of yellows	
	In Wisconsin Hollander	In commercial Hollander
Bartholomew	22.5	92.0
Hansche, A. & S.	14.3	91.0
Hansche, A & S. (second field)	11.0	86.0
Hansche, Fred	17.0	86.5
Hansche, L. E.	12.8	50.0
Klapproth	43.7	94.7
Piper	22.7	90.3
Drummond	33.7	93.7
Horner	23.9	85.8
Braid	54.6	100.0
Kraus	25.3	73.0
Scheckler	21.7	98.0
Scheckler (second field)	30.0	98.8
Scheckler (third field)	17.8	94.5
Jacobson	15.6	91.2
Broesch, M.	30.2	98.3
Broesch H.	27.2	93.2
Thompson Bros.	33.6	85.2
Lichter	11.7	89.6
Abresch	17.5	88.4
Average of twenty fields	**24.3**	**89.0**

As shown by the foregoing averages, less than one-fourth of the Wisconsin Hollander plants showed Fusarium infection, whereas the commercial strains averaged nearly 90 per cent. These figures are not nearly so striking as was the actual appearance of the fields. This is due to the fact that in most cases where the disease did occur in the resistant strain it was so slight that the plants listed as having yellows usually formed good-sized heads, whereas most of those attacked in the commercial strains either died early in the season or formed no heads if they lived.

Results in 1917. In 1917 several growers continued to plant one or two control rows of commercial cabbage in the field with the Wisconsin Hollander for purposes of comparison. The disease was less severe than in 1916, but prevalent enough to show a large gain where the resistant strain was used. Table II gives the results from four of the "sick" fields where such control rows were included in the planting.

TABLE II.—A COMPARISON OF WISCONSIN HOLLANDER AND COMMERCIAL HOLLANDER IN FARMERS' FIELDS, 1917.

Name of grower	Strain of seed	Per cent of yellows
Thomas	Wisconsin Hollander	2
	Commercial Hollander	50
Lichter	Wisconsin Hollander	5
	Commercial Hollander	88
Horner	Wisconsin Hollander	10
	Commercial Hollander	75
Johnson	Wisconsin Hollander	7
	Commercial Hollander	97

This shows an average of only 6 per cent of yellows in the Wisconsin Hollander as compared with nearly 80 per cent in the commercial strains.

Results in 1918–19. During the two seasons 1918 and 1919 the cabbage growers having "sick" soil accepted the evidence of the superiority of the Wisconsin Hollander and ceased to plant non-resistant controls in their fields. Comparisons that could be made were those in our trial grounds where, under the conditions of 1918, no yellows whatever was evident in the best resistant selections and the average of all Wisconsin Hollander selections under trial showed less than 1 per cent of diseased plants whereas the commercial control showed about 85 per cent.

In 1919, owing to the hot dry weather in July, the disease was much worse than in 1918. The result was that a large percentage of the plants of even the most resistant strains of Wisconsin Hollander showed some indications of infection, the average of all strains being about 70 per cent. Most of these were slightly diseased, however, and 80 per cent lived through the season, whereas of the non-resistant controls every plant showed yellows and only 1 per cent lived through the season.

The results under the most extreme climatic conditions and in the various types of soil have, therefore, continued fully to justify confidence in the practical merits of the Wisconsin Hollander as originally distributed. Efforts have been kept up, however, during this time to improve upon it in any way practicable.

EARLY WISCONSIN HOLLANDER, A NEW STRAIN

The Wisconsin Hollander was selected from the strain of the Hollander or Danish Ball Head. In the subsequent trials of this selection beside the original Ferry[10] Hollander the former has proved to be more vigorous, to have a little longer stem, a more flattened head, and to require a longer season for maturing. (See Fig. 2.) While this makes it a somewhat heavier

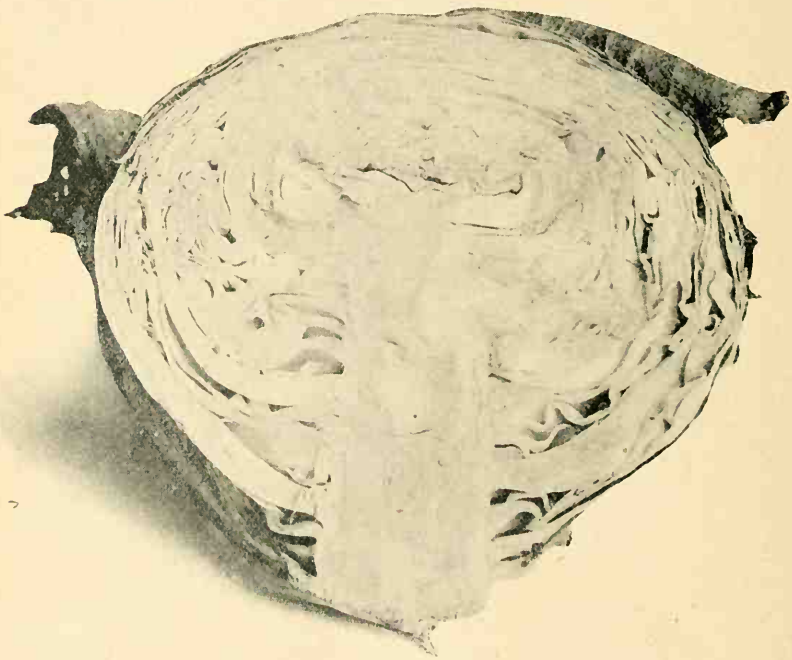

FIG. 2.—LATE WISCONSIN HOLLANDER

Section of a typical head of Late Wisconsin Hollander cabbage. In comparison with the Early Wisconsin Hollander, (Fig. 3), note the coarseness in texture, and tendency toward "flattening."

yielder in seasons having a favorably long autumn, under less favorable conditions, it may fail to mature as large a percentage of heads. In any case the date of harvest and marketing is delayed somewhat. In the judgment of representatives of the seed company and of W. J. Hansche, secretary of the local cabbage growers' committee, it has seemed commercially desirable

[10] In making our recent comparisons with the original Ferry type we have had the helpful cooperation of Mr. Coulter and Mr. MacKinnon.

to try to secure a strain through further selection from the Wisconsin Hollander which would more fully combine with disease resistance the original Hollander characters of earliness, round head, and short stem. Owing to Mr. Hansche's skill, gained through long experience in handling and judging Hollander cabbage, we have in recent years left with him the immediate responsibility for the head selections with this in view. Each season we have included in our trial grounds such head strains as

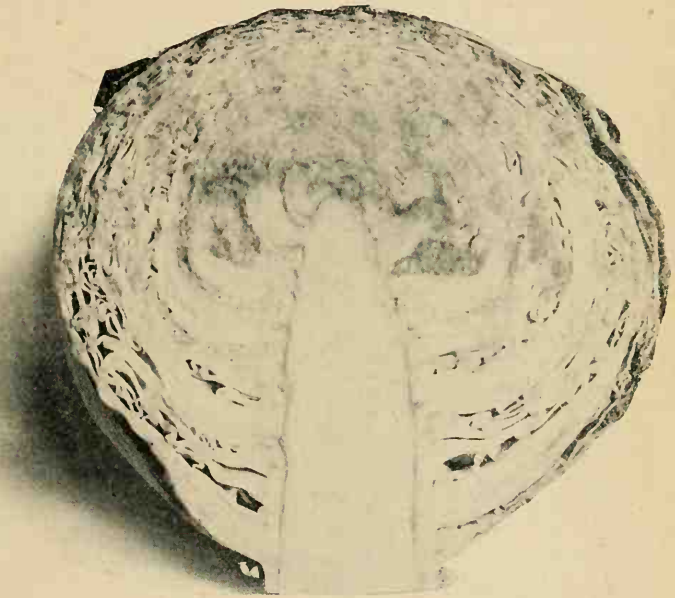

FIG. 3.—EARLY WISCONSIN HOLLANDER

Section of a typical head of Early Wisconsin Hollander cabbage. In comparison with Late Wisconsin Hollander (Fig. 2), note the compactness, close grain and shape approaching the spherical. This is accepted by expert practical growers and representatives of commercial seed houses who have inspected the trial fields as meeting the highest standards as a winter or storage cabbage type. The desired type is combined with a high degree of resistance to yellows.

he has selected in order to determine their relative disease resistance. A strain has thus been secured which combines well the desired characters. This has descended from a single head which Mr. Hansche selected in a field of Wisconsin Hollander in 1916. The seed plant from this head was forced in the greenhouse during the winter of 1916–17 and from a few seeds thus secured by self pollination plants were grown for trial in 1917. Owing to the late maturity of the seed these plants were forced

in a cold frame apart from the other strain, hence they could not be closely compared with the latter as to disease-resistant quality. They made an excellent showing in this respect, however, and also maintained well the round head and short stem of the parent plant, while, considering their late start, they matured somewhat earlier than the other Wisconsin Hollander strains. All of the sound heads of this strain were saved and set out for seed growing in an isolated plantation in 1918. Seed from one of the best of these plants was saved as a separate head strain for the 1919 trial grounds, the balance mixed for field use. The results in all cases were highly satisfactory in that along with a degree of disease resistance fully equal to that of the older strains of Wisconsin Hollander, these plants showed with much uniformity the desired characters for which the parent head was selected—shorter stem, rounder head, and earlier maturity. (See Fig. 3.) In these respects the new type is closely similar to the original Ferry Hollander. Under the conditions of 1919 the field crop of the recent selection matured nearly two weeks earlier than the older type of Wisconsin Hollander. To distinguish the two types, the new one will hereafter be designated as the Early Wisconsin Hollander and the older strain, now in general use, as the Late Wisconsin Hollander. It is hoped that commercial growers and seed dealers who may use these strains will cooperate with us in maintaining them independently since they represent types worthy of such segregation. Apparently one or the other of these types will meet adequately the needs in the various sections where the Hollander cabbage is now grown in a large commercial way. In order to provide for this, the available seed of the Early Wisconsin Hollander has been sent to the Puget Sound region for the production of a seed crop which should be available for commercial distribution in 1921.

RESISTANT SELECTIONS OF OTHER VARIETIES

The Hollander, which is a winter storage or shipping cabbage, is the variety of chief commercial interest in Wisconsin. With the development of this winter cabbage industry, however, has come an increasing number of kraut factories. These use little or no Hollander cabbage as a rule, the needs of this industry being best met by special types of the late summer or "domestic" cabbages of the Flat Dutch group. Of these kraut varieties the

one in most favor in the Racine district—when the present problems were outlined—was the Brunswick. In other kraut-growing sections the All Seasons is generally preferred. Since both of these are rather late fall varieties the All Head is generally grown in addition for early kraut use because it has a reputation for sure heading, desired kraut quality, and matures a week or more in advance of either All Seasons or Brunswick. Accordingly, efforts have been made to secure resistant strains of each of these three kraut types beginning with the Brunswick.

WISCONSIN BRUNSWICK

The first selections were made in a badly diseased field in 1913. The seed from which this field was grown was supplied by Mr. F. W. Gunther, kraut manufacturer of Racine, and was imported from Germany by him. Trials of the original or commercial strain of this seed made in 1912 and 1913 as reported in our earlier publication[11] (pp. 34, 35) showed it to be about as susceptible to yellows as the average commercial Hollander varieties and this accords with the general experience of Racine cabbage growers. Seed was grown from two of these selected heads in 1914 and tested in our 1915 plots. The results showed these selections to be distinctly superior in Fusarium resistance to the parent commercial strains. Fortunately the progeny of one head proved distinctly better than the other and to be of good Brunswick type. Its behavior as compared with the non-resistant control is shown in Table III. Selections of heads for further seed growing were made from this one head strain. It should be noted that 1915 was an unusually cool summer and that consequently the yellows disease was not very bad even in the control plants.

TABLE III.—RESULTS IN 1915 WITH THE BEST FIRST GENERATION SELECTION OF BRUNSWICK CABBAGE.

Strain	Yellows	Living	Headed
	Per cent	*Per cent*	*Per cent*
Selected Brunswick (XI-4-2)	18	100	95.0
Control (Commercial Hollander)	84	85	76.1

[11] Jones and Gilman, loc. cit.

Further trial was therefore made of this head strain (XI–4–2) in 1916 on thoroughly sick soil. Owing to the warm weather favorable to the disease this season they underwent an especially severe test. Only one plant out of 45 of these Brunswick heads was seriously infected with yellows while the commercial variety planted alongside was practically destroyed by the disease. The evidence from the trials of the two seasons taken in combination justified the conclusion that this selection represented a sufficiently resistant type of Brunswick to warrant its perpetuation for distribution to the growers. Several of the most desirable heads were therefore selected for further seed growing in 1917.

TRIALS OF SECOND GENERATION BRUNSWICK SELECTIONS IN 1917

In 1916 seed representing the second generation was secured from a number of the heads selected in 1915 and these were tested in 1917 under their respective serial numbers with the following results. This 1917 trial was on the same soil which had been proved so sick in 1916 and the season was sufficiently favorable again for the Fusarium to give a good trial.

TABLE IV.—RESULTS WITH SECOND GENERATIONS OF BRUNSWICK SELECTED IN 1915 AND TESTED IN 1917.

Head Strain	Plants infected	Plants killed by yellows
	Per cent	*Per cent*
No. XI—6—12	17	4.2
No. XI—6—15	22	2.0
No. XI—6—13	25	5.8
No. XI—6—11	25	7.7
No. XI—6—10	35	7.5
Commercial Brunswick, control	80	54.0

A small quantity of the resistant Brunswick seed was also given out for trial by growers in 1917 and fortunately some of these plants were placed in a field at Union Grove, Wisconsin, where the soil was quite "sick." A visit to this field in September showed the selected strain to be standing up almost perfectly while commercial strains alongside it were badly affected by yellows. (See Fig. 4). In 1917 a small amount of this seed was placed with other state experiment stations for trial. Se

far as we know only one sample was planted on Fusarium sick soil. This was placed through the cooperation of Prof. H. S. Jackson of the Indiana Experiment Station with M. Humpfer at Hammond, Indiana. In October, Mr. Humpfer reported that with this he planted 10,800 square feet, or about one-quarter acre of badly diseased land. It gave him about 98 per cent stand, yielding 5 tons of cabbage. On one side of this he had commercial Copenhagen Market which gave only 25 per cent of

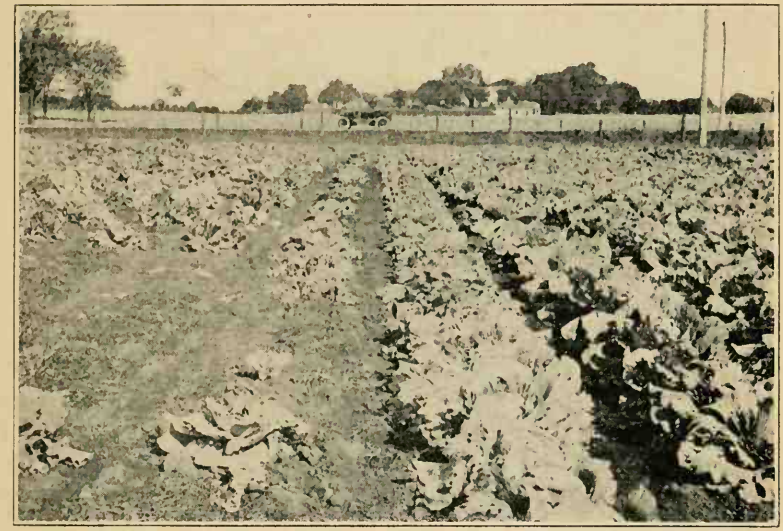

FIG. 4.—RESISTANT WISCONSIN BRUNSWICK

Cabbage trials on Fusarium sick land made by a farmer in 1917. One row of Wisconsin Brunswick (resistant) at right of center showing complete stand; balance of field on the right was Wisconsin Hollander, also resistant; remainder of field at left commercial Hollander (non-resistant) where the loss was due partly to yellows and partly to black leg.

a stand and on the other, commercial Glory of Enkhuizen which gave 50 per cent of a stand. He tried Wisconsin Hollander on the same field and found that this and the Brunswick showed about equally high resistance, the Hollander giving a 95 per cent stand whereas commercial Hollander alongside gave about a 33 per cent stand.

While the results to date have not in general shown the Brunswick strains quite equal in resistance to the best Wisconsin Hollander strains, the trials have justified the conclusion that the best selected strain deserves commercial distribution and the

seed has therefore been put out under the name Wisconsin Brunswick.

TRIALS OF WISCONSIN BRUNSWICK IN 1918 AND 1919

Trials of 1918. The trials of Wisconsin Brunswick were repeated on "sick" soil at Racine in 1918 using four of the head strains of seed grown in 1917 with the encouraging results shown in Table V.

TABLE V.—RESULTS WITH THIRD GENERATION OF BRUNSWICK SELECTED IN 1916 AND TESTED IN 1918.

Strain	Plants showing yellows
	Per cent
Wisconsin Brunswick head strain No. XI—7—1	0.0
Wisconsin Brunswick head strain No. XI—7—3	0.7
Wisconsin Brunswick head strain No. XI—7—4	0.7
Wisconsin Brunswick head strain No. XI—7—8	8.3
Commercial Brunswick, control	69.9

Trials of 1919. Owing to the severe midsummer heat the trials of 1919 were unusually severe. In 1918 seed had been secured from only one new head strain of Brunswick, XI–8–1. Therefore, two of the head strains from which seed was grown in 1917 were included, X1–7–1, which had proved the best of those tested in 1918, and XI–7–10, a strain which had been omitted through lack of room from the trials of 1918. Since no commercial Brunswick of reliable character was available for control purposes, comparison is made with the commercial Hollander planted in the trial grounds.

TABLE VI.—WISCONSIN BRUNSWICK TRIALS, 1919.

Head strain	Yellows	Lived	Headed
	Per cent	*Per cent*	*Per cent*
Wisconsin Brunswick XI—8—1	54.8	96.7	23.9
Wisconsin Brunswick XI—7—1	16.2	97.6	67.5
Wisconsin Brunswick XI—7—10	55.2	86.5	64.2
Control, commercial Hollander	98.9	9.3	7.6

Since the evidence is clear that the 1918 strain, XI–8–1, was inferior in disease resistance to both of the 1917 strains, XI–7–1

and XI–7–10, heads for further seed growing were saved only from the latter.

These trials have shown that the Wisconsin Brunswick which is now available in limited quantities for commercial use combines very well the type of the commercial Brunswick with a sufficiently high degree of disease resistance to meet practical needs. Conferences with various growers have shown, however, that while the Wisconsin Brunswick possesses many good qualities both the commercial and the selected type have certain char-

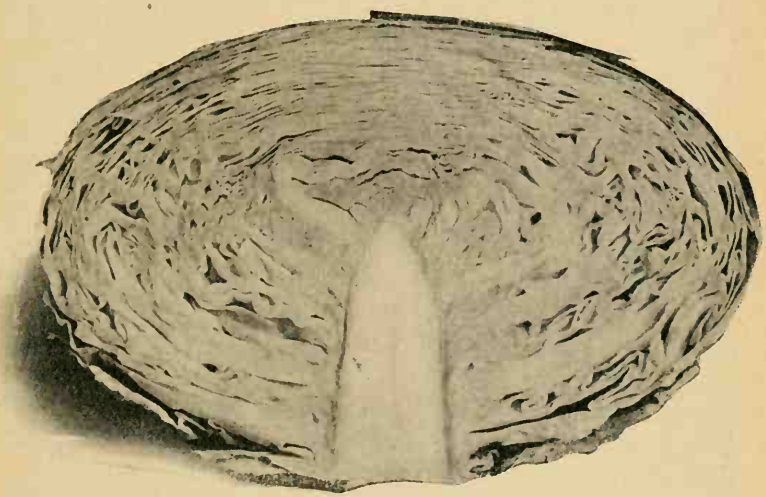

FIG. 5.—TYPICAL BRUNSWICK HEAD

Section of Wisconsin Brunswick cabbage. Note the relatively flat head and openness of spaces between the leaves of this and the other kraut type (See Fig. 6) as compared with the round, dense, hard heads of the Hollander or storage cabbage type (See Figs. 2 and 3). The characteristically very short stem or "core" of the Brunswick is also illustrated here. This commends it to the kraut manufacturers, but is not so satisfactory to the grower inasmuch as it is associated with the tendency to form a reëntrant angle at the base as explained in the text.

acteristics which stand in the way of their general acceptance for commercial krant growing. Because of the very short stem and relatively thin flat head when they grow very large, the head tends so to thicken at the sides as to form a reëntrant angle with the stem. The result is that the heads cannot be cut from the stem at harvest so easily and quickly as the All Seasons and other standard kraut types. Since it was considered possible to overcome this trouble in some degree at least, a number of heads which possessed this reëntrant stem in the minimum degree were selected from the resistant plants in the 1919 trial field. (See Fig. 5.) These have been stored for seed growing and further

trial. Since this will be a work of several years at best the resistant Wisconsin Brunswick corresponding to the commercial type will be placed in commercial distribution.

WISCONSIN ALL SEASONS

The All Seasons belongs to the same group of mid-season Flat Dutch cabbage as the Brunswick but it has a somewhat longer stem and rounder head. Because of type, quality and season

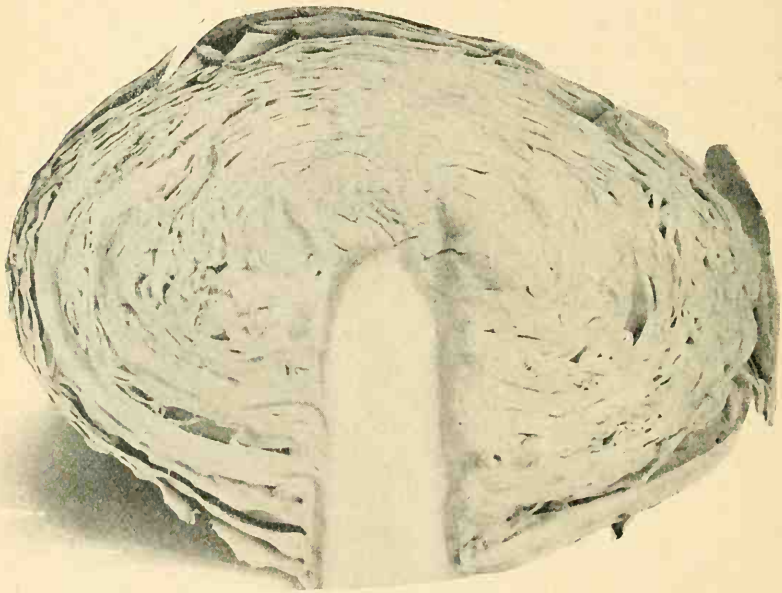

FIG. 6.—WISCONSIN ALL SEASONS

Section of typical head of Wisconsin All Seasons cabbage. This selection conforms closely in type to the standard All Seasons of the American seed trade which is a favorite variety with the majority of kraut growers. A comparison with figures 2 and 3 shows the differences between the kraut and the storage types as explained under figure 5. As compared with the Brunswick (Fig. 5) the All Seasons head is deeper with longer stem or "core," and is more rounded at the base.

it has become the standard kraut cabbage and is more widely used than any other single variety in the United States. (See Fig. 6.) Although the needs of the Wisconsin growers of the Racine district seemed fairly well met by the Wisconsin Hollander and Wisconsin Brunswick, these two varieties did not adequately meet the national situation. This fact was brought out by a survey of the kraut interests of the United States generally, undertaken jointly a few years ago by L. L. Harter and

the senior author on behalf of the Federal Bureau of Plant Industry, which showed that the second tier of states, extending from Iowa to the Atlantic seaboard, is suffering serious loss from the Fusarium disease and prefers in general the All Seasons for kraut purposes. This is preëminently the case in the Illinois, Indiana, and Ohio districts.

Since our experience has led us to believe that it is possible through selection to secure a Fusarium-resistant strain from any of the standard cabbage varieties without breaking up the horticultural type with which one is dealing, it was early decided that the next effort should be directed to securing a disease-resistant strain of All Seasons. Reference was made in our earlier bulletin[12] to the fact that Manns of the Ohio Experiment Station suggested the possibility of overcoming cabbage yellows through disease resistance. Through correspondence with Professor Selby of the Ohio Station we learned in 1914 that S. N. Green of the horticultural department of that station had already undertaken selections for this purpose. Upon request of Professor Selby, Mr. Green kindly sent us some of the seed of the most promising strain which he had selected from heads of All Seasons variety as grown in the Clyde, Ohio, district, and we sent some Wisconsin Hollander seed in return. This was tested in our 1915 series, alongside the Wisconsin Hollander and commercial varieties, and corresponding tests were made the same year near Clyde, Ohio, by J. G. Humbert of the Ohio Experiment Station, department of botany. The outcome showed that this selection of All Seasons had little if any greater disease resistance than the commercial varieties. Prof. W. J. Green of the Ohio Station recently advised the senior author that S. N. Green was no longer connected with their station and that the selections had not been continued. Although most of the plants in this strain of All Seasons in 1915 were diseased, a few appeared free from Fusarium and with the assistance of certain experienced kraut growers who inspected the field, several of the most promising of these heads were selected for seed growing. In order to save time some of these were sent to C. W. Edgerton of the Louisiana Experiment Station for winter seed growing. Seed from one of these (XXV–6–3) was returned in the spring of 1916 in time to be included in the trial grounds that year.

[12] Jones and Gilman, loc. cit.

TRIALS OF 1916 AND 1917

The disease was very severe on the trial ground in 1916 so that of the 55 selected All Seasons plants set out only six escaped infection, whereas of the selected Brunswick alongside only one plant of 45 was infected. The six heads which escaped infection were saved for seed growing and again, in order to save time, two of the most promising of these were selected for winter forcing. We were favored by the cooperation of Prof. J. B. Norton of the Bureau of Plant Industry and he secured seed from these in the greenhouse at Washington and returned to us in the spring of 1917 as follows: head strains XXV–7–2 and XXV–7–8, each grown from a single selfed plant, and strain XXV–7–2 x 8 which was the result of the crossing of these two. In addition, we had for inclusion in the 1917 trial grounds some 14 head strains of the first generation selections (XXV–6–3—XXV–6–23) grown at Madison in 1916 from heads saved in 1915. These, therefore, represented our first general selections, comparable to those tested in 1916. Furthermore, these had been grown in mixed plantation. The comparative results as shown in the following table are unusually interesting since they illustrate clearly the advantage at this stage in the work of selfing or close pollination as compared with growing in mixed plantation.

TABLE VII.—RESULTS FROM TRIALS OF SELECTED ALL SEASONS HEAD STRAINS IN 1917.*

Strain No.	Pollination	Yellows	Killed by yellows
		Per cent	*Per cent*
XXV–7–2	selfed	5	0
XXV–7–8	"	4	0
XXV–7–2x8	crossed	2	0
XXV–6–4	mixed	28	8
XXV–6–5	"	39	10
XXV–6–6	"	37	0
XXV–6–9	"	35	4
XXV–6–10	"	40	6.9
XXV–6–11	"	39	4
XXV–6–12	"	43	6.2
XXV–6–14	"	21	0
XXV–6–15	"	40	5
XXV–6–16	"	39	2
XXV–6–17	"	33	6.4
XXV–6–21	"	51.6	7.3
XXV–6–22	"	29	4
XXV–6–23	"	16	2.4
Commercial All Seasons Control		**80**	**46**

* Figure 7 shows the appearance of these plants in the field.

The showing made by all three strains of second generation seed which Professor Norton had secured was thus very encouraging indeed, both by contrast with the 1916-grown first generation strains and with the commercial. (See Fig. 7.) Thus where the commercial strain showed 80 per cent of Fusarium infection and one of the best first generation strains, XXV–6–23, showed 16 per cent, Norton's hybrid XXV–7–2 x 8 showed only 2 per cent, and the selfed strains scarcely more. This was a distinctly

FIG. 7.—ROW OF THE MOTHER HEADS OF WISCONSIN ALL SEASONS

Trial grounds of resistant All Seasons, 1917. Soil very sick, see Table VII. Commercial All Seasons in center practically destroyed by yellows. Second generation selections, XXV–7–2 and XXV–7–8, in the next two rows at the right. Of these XXV-7-2 was the best type and from it the finest heads were selected for propagation as Wisconsin All Seasons. The remaining rows at the right are the first generation selections of the same variety, which, proving less desirable, were discarded.

better showing than was made in the same parallel trials the same season with the resistant Brunswick selections and nearly as good as the best Wisconsin Hollander. It seemed clear, therefore, that we had at least three highly resistant strains of All Seasons from which to select for further increase. All were of good appearance but upon critical comparison, in which we had the advice of L. D. Coulter, cabbage expert of the D. M. Ferry Co., strain XXV–7–2 was considered to represent the best and most uniform type. Our own selections for further seed growing were restricted to this strain.

Further trials and selections have been continued with this strain of All Seasons during 1918 and 1919. The 1918 trials showed in this resistant strain (XXV–7–2) only slightly over 1 per cent of yellows and almost a full stand of heading plants (over 98 per cent) whereas the non-resistant control showed over 60 per cent of yellows and only about 15 per cent heading. In 1919, when the disease was severe owing to the high summer temperature, this strain made a very good showing, and in general proved more resistant than the best Wisconsin Hollander. Meanwhile, sufficient seed has been produced from the 1917 and 1918 selections to enable the Wisconsin cabbage growers' committee to inaugurate seed growing on a commercial scale. As will be explained later, arrangements have been made by which seed growing of the resistant All Seasons is also being carried on in a trial way under supervision in the Long Island and Puget Sound sections in addition to what is being grown in Wisconsin. The first of this seed will be ready for distribution in the autumn of 1920 under the name of Wisconsin All Seasons.

SELECTIONS OF OTHER VARIETIES

Maryland Flat Dutch. Early in our work upon the Wisconsin Hollander we learned that Close and White[13] of the Maryland Experiment Station had been noting a difference in the susceptibility of cabbage varieties to what they termed black rot; and upon our request in 1913 Professor White sent us a sample of a strain of Late Flat Dutch which they had selected and grown for such rot resistance. Owing to its origin we have termed this the Maryland Flat Dutch. This Maryland strain proved highly resistant to yellows in our 1914 trials. (See Fig. 8.) Several heads were saved from this 1914 trial and seed was grown from some of them in 1915.

Since the type did not interest the Wisconsin growers who inspected this field we did nothing more with the Maryland strain until 1919. Learning from recent conferences with kraut packers of other states that they and some other commercial cabbage interests have need for a domestic variety somwhat later in maturing than the All Seasons we decided to include some of these head strains (XXIV–5–1, XXIV–5–2, XXIV–5–3, XXIV–5–4)

[13] Close, C. P. and White, T. H. Cabbage experiments and culture. Md. Agr. Exp. Sta. Bul. 133. 1909.

in our trial plantings in 1919. They proved to be fairly resistant, being in this respect about in the class with the Wisconsin Brunswick but not equal to the better strains of Wisconsin All Seasons or Wisconsin Hollander. The type did not prove altogether satisfactory. Probably because not well suited to Wisconsin climatic conditions, especially under the high summer temperature of 1919, it did not form as large a percentage of firm heads as the other domestic varieties under trial. Further selections of the best head types were made from the more prom-

FIG. 8.—FUSARIUM-RESISTANT MARYLAND FLAT DUTCH

Trial of Maryland Flat Dutch, 1914. Three rows at right showing a practically full stand are of this variety; the next three rows at the left are commercial Houser, slightly resistant; the next three rows, with only one plant still alive, are commercial Hollander. Several of the best heads of this Flat Dutch were saved for seed growing in 1915 and gave us the head strains XXIV–5–3 and XXIV–5–4 referred to in the text.

ising strains, XXIV–5–3 and XXIV–5–4, and further selection will be made from these. Professor White advises us that he has continued to propagate this strain and that it is in successful use in Maryland.

All Head Early. The kraut packers with whom we have conferred pronounce this the favorite variety for early kraut purposes. It belongs to the early Flat Dutch group and is said to be the best cabbage of its type ever produced in Long Island where it was developed by a Mr. Strang a generation ago.[14] It

[14] Allen, C. L. Cabbage, cauliflower and allied vegetables. 1915.

has a reputation for uniformity, tenderness, and sureness of heading which make it the most promising variety of the early group from which to undertake selections for disease resistance. While for kraut purposes it is similar to the All Seasons, it is somewhat earlier in maturing, thus prolonging the packing season. A considerable acreage of this variety was grown in 1919 under contract with the John Meeter & Sons Kraut Co. in the western part of Racine and Kenosha Counties. Through the cooperation of Martin Meeter we located a field of All Head Early where the yellows was very bad (See Fig. 9) and selected for seed growing some twenty heads of good type, apparently disease-free. It is hoped that the seed secured from these may be used for further trial and selection.

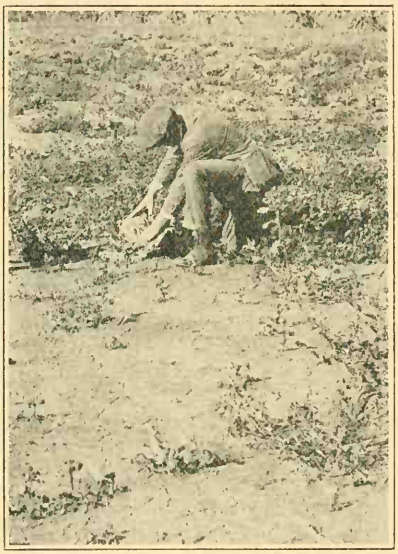

FIG. 9.—ORIGINAL SELECTIONS OF ALL HEAD EARLY AND GLORY OF ENKHUIZEN

Field of commercial All Head Early and Glory of Enkhuizen cabbage practically destroyed by yellows, at Union Grove, Wis., in 1919. Typical seed heads were selected from the few remaining resistant plants for seed production in 1920.

Glory of Enkhuizen. This is grown especially in certain sections of the east as a standard mid-season kraut variety and its use is apparently increasing in the northern Mississippi Valley. Some of this had been planted in the same "sick" field with the All Head and at least 77 per cent of the plants were killed and many of the rest showed yellows (Fig. 9). Advantage was taken of the opportunity to select heads for seed growing in 1920. It is hoped that a disease-resistant strain of this, also, may ultimately be secured.

Copenhagen Market. This is an early cabbage of excellent quality which has recently come into much favor for market garden uses and in certain localities is grown for kraut. One of the important centers of its culture is Muscatine, Iowa.

Since the yellows is serious there Drs. I. E. Melhus and J. C. Gilman of the Iowa Experiment Station have been working some two years to secure a resistant strain of this variety, and trials made in our fields in 1919 with some of the seed which they sent for this purpose, showed distinct progress toward this end with at least two strains. Inasmuch as neither of these Iowa strains conformed exactly to the standard commercial type we made additional selections from a "sick" field of Copenhagen Market near Union Grove, Wisconsin, in the autumn of 1919. It may be expected that ultimately either the Iowa or Wisconsin selection or both may furnish the desired type combined with disease resistance.

PRESENT STATUS SUMMARIZED

It is evident that individual variation in degree of susceptibility or resistance to Fusarium has been found to occur with every variety of cabbage tested on "yellows sick" soil. Experience to date justifies our confidence that this resistance is due to heritable differences and that, therefore, through the selection of such resistant heads from "sick" soil, a Fusarium-resistant strain may be secured of any of the standard cabbage varieties Our experience indicates moreover, that through careful and repeated selection this resistance may be combined with any of the other desired qualities of the standard commercial varieties, such as season of maturity, length of stem, tenderness of leaf, shape and compactness of head. In other words, resistance does not seem to be incompatible with any other of the commonly recognized variables of the cabbage. All our experience indicates that Tisdale's conclusions relative to the flax wilt[15] hold true for the cabbage, that resistance is probably determined by multiple factors. The degree of resistance is, therefore, due to the combination of these and in all cases in our experience it is partial or relative, not absolute. Moreover, this explanation is consistent with our experience that after proceeding to a certain stage with our present methods of selection little or no further progress as to disease resistance is made. This is also consistent with our general experience that the best results have in each case been secured through growing a selected head in isolation

[15] Tisdale, W. H. loc. cit.

and thus securing seed through self-pollination, but that when the benefits were once secured in this way with our best selections mass culture has been followed to advantage.

Our plan of procedure, justified alike by theory and practice, is as follows. After securing a strain showing a satisfactory degree of resistance, combined with the other desired characteristics, we release it for commercial distribution. Thereafter, our interest is primarily confined to such cooperation as is required for the maintenance of these essential standards. To this end we continue to grow each year a few hundred plants of each of these types in trial rows on soil that is "sick," i. e. thoroughly infested with the cabbage Fusarium. From these plants further selections are made with the aim of maintaining the best standards both as to type and disease resistance. Of course, there is opportunity for minor gains in this way, but our experience has not indicated that much improvement is to be expected in this direction. The surplus seed thus obtained is placed in hands of the local cabbage growers' committee for commercial increase in such manner as will best maintain general standards of excellence.

All our experience has shown that in seasons when high soil temperatures—especially during July—favor the development of yellows there will be a considerable percentage of the plants even in the most resistant of these strains which shows evidence of incipient yellows. Most of these proceed with their development to full maturity and form good heads so that the commercial loss is rarely large even in the worst cases. While the amount of the disease varies considerably with other factors such as soil and drainage, there is no evidence that the resistant character of the selected strains breaks down under any of these conditions except in the young seedling stage. The studies of W. B. Tisdale[16] have shown that seedlings of the resistant strains grown in sick soil at a temperature favorable for the development of the disease succumb almost as readily as those of susceptible strains. The plants, however, acquire a high degree of resistance after a few weeks of growth. In our experimental trials we have always aimed to grow the seedlings on healthy soil, although under Wisconsin conditions the temperature is

[16] Tisdale, W. B. Influence of soil temperature and soil moisture on the occurrence of yellows in cabbage seedlings. In manuscript.

usually too low for infection to occur while seedlings are in this susceptible period. In certain sections of the country, however, cabbage seed is sown at a time when the soil temperature is near the optimum for infection by *Fusarium conglutinans*. For best success, therefore, it is essential to make the seed bed on healthy soil. Trials have been made of one or more of these resistant strains in so many other states that we feel confident in our conclusion that the resistant qualities will be maintained without serious impairment anywhere that the cabbage will succeed.

If these conclusions are correct, it would seem, therefore, that the only serious condition yet to be met is that of the commercial production and distribution of the different varieties of resistant seed suited to local needs.

COMMERCIAL PRODUCTION AND DISTRIBUTION OF RESISTANT SEED

As soon as the merits of the Wisconsin Hollander were established the question arose as to how best to insure the production and distribution of an adequate supply of reliable seed under commercial conditions. At the outset the aim was simply to meet the needs of the Wisconsin cabbage growers, especially in the Fusarium-infested regions of the southeastern counties. This was done by the selection, at a meeting of the leading growers of this section, of a committee of five men,[17] all experienced in handling cabbage. To them was entrusted the responsibility for leadership in growing and distributing the seed. To this committee we turned over enough mother seed of the best resistant Wisconsin Hollander to inaugurate their undertaking, and we have since continued to cooperate with them by supplying them with any improved strains as these have been secured and through assistance in selecting mother heads for their seed growing. (See Fig. 10.) Through this committee somewhat over 100 pounds of Wisconsin Hollander seed was grown and distributed in 1917 and this was increased to 800 pounds in 1918. Meanwhile local growers have been encouraged to save for home seed growing the best heads from their own fields, especially where the soil is "sick" enough to insure opportunity

[17] The membership of this committee is as follows: W. J. Hansche, A. J. Piper, and S. B. Walker of Racine; Henry Broesch and W. Thompson of Kenosha; W. J. Miller of Somers. W. J. Hansche was chosen chairman and inquiries as to available seed should be addressed to him (W. J. Hansche, R. F. D. 4, Racine, Wisconsin).

for selecting especially resistant plants. In these ways the local needs have been fairly well met. The demand has, however, continued to increase from other parts of Wisconsin and from other states. It was foreseen that this would soon lead to the introduction by commercial firms of seed grown elsewhere under the regular contract method. The seed trade secures most of its cabbage seed in this way from either of three sources, Long Island, the Puget Sound region in Washington, or Europe, es

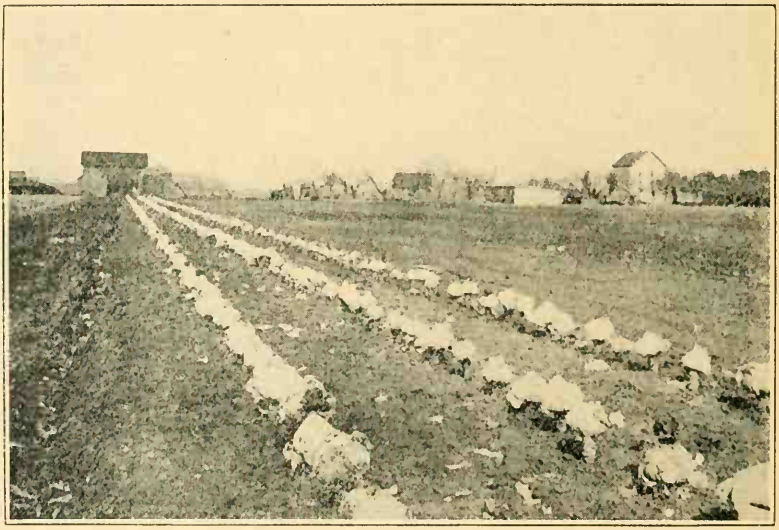

FIG. 10.—WISCONSIN HOLLANDER SEED HEADS

A farmer's plantation of Wisconsin Hollander seed heads in early spring, 1916. These were especially selected from "sick" soil in the fall of 1915, kept in a cool storage house over winter, and reset into the field for seed production in 1916. Grown by Henry Broesch, Kenosha, Wisconsin.

pecially Denmark and Holland. Since cabbage seed can be secured from these regions under contract more cheaply than it can be grown in Wisconsin it is evident that seed growing in Wisconsin on a permanent commercial scale can be encouraged only in case this method is essential for the maintenance of the disease-resistant quality in the seed. If it is demonstrated that Wisconsin grown seed is distinctly superior, it will command a sufficiently higher price to keep it on the market, otherwise the cheaper contract grown seed will ultimately replace it. Experiments were, therefore, inaugurated several years ago to determine the facts as to this matter. The most reliable

method followed by commercial seedsmen is to furnish the contract grower with the mother seed, this mother seed being secured each year from reliable sources. The essential question is, therefore, as follows: If resistant Wisconsin grown mother seed is placed for one generation in another region on non-infested soil, and a seed crop is thus secured without further selection, will such seed have lost appreciably in its disease resistant character?

The first trials for determining this were undertaken in the spring of 1915. Seed of one of the head strains of Wisconsin Hollander was sent to the Washington State Experiment Station located at Puyallup,[18] a duplicate sample of the same lot of seed being retained for later comparative trial. The seed developed from this in Washington was returned to us in the autumn of 1917 and introduced into our 1918 trial field. In addition a commercial firm secured in 1915 some Wisconsin Hollander seed which was placed under contract with a Puget Sound seed grower in 1916–17. This firm supplied us with a sample of their western grown seed for the trial. These two samples were placed on "sick" soil along with the other strains under trial in 1918 with the following results.

TABLE VIII.—SUMMARY OF 1918 TRIALS COMPARING WESTERN GROWN SEED OF WISCONSIN HOLLANDER WITH HOME GROWN.

Seed strain under trial	Amount yellows
	Per cent
Best strain Wisconsin Hollander seed grown in 1913 (VIIIa–25)	0.9
Average 5 selections made from progeny of this in 1916	0.0
Average 17 selections made from general fields W. H. in 1916	2.0
Puyallup grown seed, Wisconsin Hollander (VIIIa–15)	3.1
Sample of "mother seed" strain sent to Puyallup in 1915 for seed growing (VIIIa–15)	0.0
Commercial seedsman's Puget Sound grown seed of Wisconsin Hollander	7.0
Control: Hollander seed commercially imported	84.6

Trials of the same lot of commercial seedsman's grown Wisconsin Hollander were repeated in two plots at Racine in 1919. The results secured were as follows:

[18] This seed was grown at Puyallup under the supervision of Director W. A. Linklater and Prof. J. L. Stahl.

TABLE IX.—SUMMARY OF 1919 TRIALS COMPARING WESTERN GROWN SEED OF WISCONSIN HOLLANDER WITH HOME GROWN

Location of plot	Strain of cabbage	Per cent yellow	Per cent killed by yellows	Per cent living	Per cent headed
Drummond plot	Average 4 strains of Wisconsin grown Wisconsin Hollander	40.6	3.6	94.1	76.8
	Western grown Wisconsin Hollander	34.4	7.2	89.6	63.2
	Commercial Hollander	98.9	85.9	9.3	7.6
Broesch plot	Average 4 strains of Wisconsin grown Wisconsin Hollander	75 4	11.9	85.3	49.6
	Western grown Wisconsin Hollander	61.6	16.0	80.0	51.2
	Commercial Hollander	100.0	98.0	1.0	1.0

In 1919 the disease was severe and any sign of yellows on the plants was recorded. Thus a comparatively high percentage of disease is shown in column 1 even for the resistant strains. This condition usually occurs in a warm season like 1919, but, as previously noted, most of the resistant plants are scarcely checked by this slight attack while a large percentage of the commercial strain, when infected, dies before the end of the season. The percentage of plants killed by yellows and the percentage heading are therefore the best criteria for comparing the various strains.

From both the 1918 and 1919 figures it is evident that all of these strains of Wisconsin Hollander gave fairly good results as to Fusarium resistance. The 1918 results are the more significant and they show quite clearly that under the conditions of that trial the western grown seed was not quite so resistant as that grown in Wisconsin. Even in 1918, however, the western grown seed made a satisfactory showing and in the 1919 trial it proved practically equal to the average run of Wisconsin Hollander. It is to be remembered that in both seasons the trial was made on "sicker" soil than will commonly be used for commercial cabbage culture and therefore that the differences are more pronounced than would be evident in general field usage. Considering, therefore, the commercial advantages of growing contract seed in the intensive seed-growing districts we

are approving this method with certain reservations aiming to reduce the dangers inevitably inherent in the procedure.

The first of these dangers results from the fact that it seems inevitable that there is in all these resistant cabbage strains a tendency to progressive reversion with a consequent loss in disease resistance which can only be met by continued selection from plants grown on "sick" soil. The commercial seedsman who ignorantly or for other reasons neglects to recognize this principle may therefore fail to keep his strain up to standard. There is also always the possibility of seed admixture and of cross pollination from adjacent seed fields, both of which will require greater attention from the seedsmen and contract growers with such a strain as this than with the less specialized types.

To meet the situation with the Wisconsin Hollander we have continued to urge Wisconsin cabbage growers who have especially "sick" soil, and who have already learned how to select heads from their own fields for seed growing, to continue this practice. This will insure them at least enough seed for their own use and in certain cases they will have a surplus to sell to their neighbors or to seedsmen. Certain commercial seedsmen are already arranging to secure their "mother seed" of resistant strains from heads carefully selected from "sick" soil with regard both to disease resistance and type. We shall continue to cooperate with both local growers and seedsmen in the establishment of these practices on a sound basis.

With the kraut varieties it is more difficult for the ordinary Wisconsin grower to succeed in seed growing. One reason for this is that the earliness of maturity causes a much greater loss of heads during winter storage. The chief initial requests for this seed moreover have come not from the growers directly but from the kraut packers who, in general, purchase and distribute to the farmers the seed from which their cabbage is to be grown under contract. Most of the kraut manufacturers of the country are members of the National Kraut Packers' Association. Accordingly an arrangement has been made with this Association by which this Experiment Station and the Federal Bureau of Plant Industry have cooperated for the growing of resistant kraut seed. In this way a considerable quantity of Wisconsin All Seasons and some Wisconsin Brunswick will be available for distribution in 1921. Efforts will be made so to place this as to insure the use of as much of it as possible on "cabbage sick"

soil and so to provide as to insure the production of an adequate crop of seed annually hereafter.

It is believed that through the state and national institutions proceeding thus in cooperation with the Wisconsin cabbage growers' committee, with the National Kraut Packers' Association, and with such of the seed firms as are undertaking to handle the resistant seed, it will be possible to place the production and distribution of this seed upon a permanently reliable commercial basis. Evidence has already come to hand, however, that along with this legitimate trade development there will be some confusion through the offering of so-called disease-resistant seed of unknown origin by ignorant or unreliable dealers. Probably this is not a matter which need mislead any intelligent cabbage seed dealer or grower. In any case, it will be greatly minimized if all reliable dealers offering these Wisconsin strains of resistant seed will use the names herein given to them and will so state the source of their seed supply as to make clear the essential facts as to its origin or history.

SUMMARY AND CONCLUSIONS

1. The disease known as cabbage yellows, caused by the soil parasite *Fusarium conglutinans*, is widely distributed and seriously destructive in the United States.

2. Once introduced, it persists indefinitely in the soil and there is no known method of control except through the use of disease-resistant strains.

3. It has been found that of the commercial varieties the Volga is the most highly resistant and the Houser is somewhat resistant, but neither of these varieties meets important commercial needs.

4. The chief commercial cabbage industry in the sections where the yellows disease occurs is concerned with growing either a winter storage or shipping crop or a mid-season or autumn crop for kraut manufacture. To a lesser degree there is need for truck types.

5. Experience justifies the belief that these several needs can all be met by the selection of Fusarium-resistant strains from the standard commercial varieties now in use which are best adapted to these various purposes.

6. In undertaking such selection our first success was attained with the standard winter storage variety, Hollander or Danish

Ball Head. From this was developed the resistant strain known as Wisconsin Hollander. Since experience showed that an earlier strain of this was needed, further selection was made and a resistant strain secured which combines with earlier maturity a rounder head and shorter stem. This has been distributed under the name Early Wisconsin Hollander, and for purposes of distinction the original resistant strain is now being called Late Wisconsin Hollander.

7. In order to meet the needs of the kraut industry, resistant strains have been selected from two of the leading commercial kraut varieties, Brunswick and All Seasons, and these have been distributed under the names Wisconsin Brunswick and Wisconsin All Seasons.

8. Other Fusarium-resistant selections are receiving attention as follows: Professors White and Close of the Maryland Experiment Station have secured and distributed a resistant strain of the Late Flat Dutch; Professors Melhus and Gilman of the Iowa Experiment Station are developing a resistant Copenhagen Market. In Wisconsin the Experiment Station, in cooperation with the Bureau of Plant Industry of the U. S. Department of Agriculture, is working with resistant selections of All Head Early, Glory of Enkhuizen, and Copenhagen Market.

9. By following the proper methods any skillful cabbage grower who has Fusarium-sick soil may either undertake with reasonable confidence to develop a resistant strain of his own, or having secured one of these resistant strains he can maintain its resistance and produce his own seed.

10. It is, however, important to note that the Fusarium disease or yellows is often confused by growers with the bacterial black rot *(Bacterium campestre)*, and that these selected strains have not proved to be especially resistant to this nor to the other common cabbage diseases such as black leg (Phoma) and club root (Plasmodiophora).

11. In all cases the degree of resistance to Fusarium shown by these strains is relative, not absolute. The seedling plants are less highly resistant than they are after the transplanting stage.

12. Environmental factors, especially soil temperature, influence the development of the disease and also the disease resistance of the host. High soil temperature favors the disease and low temperature inhibits it. It does not develop even in the

non-resistant strains at a temperature below about 17°C. (62°F.) and at high soil temperatures even the most resistant strains show a considerable percentage of infection.

13. In accordance with the temperature relations noted above, the best results are obtained under Wisconsin climatic conditions by starting even the resistant strains in a non-infested seed bed to avoid possible seedling infection. These strains are then sufficiently resistant following transplantation to mature a commercially successful crop even on badly diseased soil.

14. These resistant strains have proved resistant so far as tested in other states. It seems probable that the only limitation in this respect which might occur would be in cases where they were subjected to more trying conditions as to soil temperature, especially in the seedling stage.

15. Should such conditions be met, our experience gives us confidence that through further selection resistant strains suited to any localized conditions could be secured. It is our belief, therefore, that the cabbage industry can be permanently maintained in any section of the country, in so far as the Fusarium or yellows disease is a limiting factor, through the selection of disease-resistant strains.

16. It seems probable that in case the resistant strains are propagated through successive generations without repeated selection, they will tend to lose to some extent the disease-resistant character.

17. When, therefore, it seems desirable for commercial purposes to grow the seed crop under contract in non-infested regions, it is urgently recommended that the mother seed for each such contract crop be secured from plants carefully selected for resistance and type from Fusarium infested fields. By this method it is believed that the present standards may be essentially maintained and seed successfully produced on any desired scale, by the commercial contract method.

18. Work on the disease-resistant cabbage strains will be continued by this Experiment Station in cooperation with the Bureau of Plant Industry of the U. S. Department of Agriculture and with certain other state experiment stations. While it will not be practicable for these institutions to grow or distribute seed other than for trial purposes, they will advise or cooperate with growers or seed firms in securing an adequate supply of resistant mother seed.

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The Influence of Soil Temperature on Potato Scab

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AGRICULTURAL EXPERIMENT STATION
OF THE
UNIVERSITY OF WISCONSIN
MADISON

CONTENTS

The Influence of Soil Temperature on Potato Scab

L. R. Jones, H. H. McKinney and H. Fellows

THE COMMON SCAB of the potato caused by *Actinomyces scabies* (Thaxter) Güssow is probably the most generally serious potato disease of America and with continued potato culture on the same soil the disease seems to increase steadily. Some years ago the senior author (3) had an opportunity to contrast this condition with the situation in northern Europe where potato scab is generally a minor disease, in many sections practically negligible, in spite of the highly intensive culture of this crop and the abundant use of stable manure from animals fed on cull potatoes.

This difference in the prevalence of the common scab disease in the established potato districts of Europe and America led to the conclusion that the explanation must be in the difference in environmental conditions rather than the accident of introduction of the parasite. After making full allowance for other variable factors, such as soil reaction and moisture, it seemed as though temperature variations must be an important factor in the development of the disease. Other observations have tended to strengthen this idea, some of which have been listed by the senior writers in a preliminary note (5). The causal organism belongs to a fairly high temperature group, as first shown by Shapovalov (9), who found that it thrives best in pure culture at temperatures ranging from 25-30° C.

In order to determine the influence of soil temperature on the development of scab, an effort has been made to control soil temperatures experimentally and grow potatoes in scab infested soil held at various temperatures over a reasonably wide range. Trials have been undertaken in both field and greenhouse, but thus far the best progress has been made in the greenhouse, because only here have we been able to control conditions satisfactorily. While the experimental work must be continued upon all aspects of the problem, sufficient has been learned to justify a report of progress.

EXPERIMENTAL WORK IN THE GREENHOUSE

METHODS

Five experiments have been conducted using the Wisconsin temperature tanks (4) for controlling soil temperatures. All plants have been grown in round, galvanized iron pots 6 inches in diameter and 9½ inches deep. These pots were surrounded by the water of the temperature tanks, all of which were located in one greenhouse where the air temperature was kept within as limited a range as possible during any one experiment. Soil temperatures were maintained by heating or cooling the water in the tanks by means of electric heaters or steam and cold water or ice.

SOIL

A rather fertile loam soil has been used in all of the experiments. This was always sterilized by live steam under about 1 pound pressure for four hours. The hydrogen-ion concentration of this soil after sterilization was found to have a P_H value of 7. The moisture content of the soil after inoculation and planting ranged from 18 per cent to 20 per cent (based on weight of water-free soil) in the various experiments. Both of these conditions have proved favorable for the development of the disease.

SEED

A number of varieties of potatoes were tried out in connection with the earlier series and the Irish Cobbler variety was found especially satisfactory. This variety is highly susceptible to common scab; it has a smooth white skin upon which scab lesions show distinctly and it develops a large number of tubers in a shorter time after planting than any of the other varieties tested.

Owing to the necessity for potato seed to pass through a period of dormancy before sprouting, it has not been practicable to use northern seed in experiments started in the autumn. For this reason, early grown southern seed was used in all but one experiment. From the outset of the work it was planned to use Cobbler seed from the same source (Warsaw, N. C.) in all of the work and this was done in the first three experiments. In the fourth experiment, since it was not possible to obtain this Carolina seed, use was made of some of the same variety from Madison County, Illinois. In the case of the fifth experiment, which

was not started until the spring period of the year, Wisconsin seed (Cobbler) was used.

All seed was treated at least two hours in a 1-1000 solution of mercuric chloride previous to planting in order that the work might not be complicated by Rhizoctonia and other tuber-borne diseases.

Pathogen

In all of the experiments reported in this paper, the same strain of *Actinomyces scabies* was used. This organism was isolated from a scabby potato from Door County, Wisconsin. It has been increased on various types of media, but it has always been carried in stock culture on potato glucose hard agar.

While two methods of inoculation have been used, only one has been found entirely successful. In one case the tubers were grown in sterilized soil and the inoculum, consisting of a water suspension of the spores and mycelium of *A. scabies*, was applied to the injured and uninjured surfaces of the uncovered tubers. In later experiments, however, the organism was increased on leaf mold and fine cut straw and added to the sterilized soil at the time of planting, and finally it was found that very satisfactory results could be obtained by merely adding the organism in a water suspension to the sterilized soil before planting the seed.

The latter method consisted in increasing the organism on the surface of potato glucose agar. After the organism had sporulated abundantly, the surface growth was removed and well macerated. This was put in water and the resulting suspension of the organism was sprinkled on the sterilized soil. A sufficient amount of water was used to bring the sterilized soil up to the required moisture content.

Two methods have been employed in increasing the organism on agar. The first consisted in introducing the potato glucose agar medium, containing 3 per cent agar, into Erlenmeyer flasks to a depth of ⅝ of an inch. The flasks were then plugged and sterilized for 20 minutes under 8 to 10 pounds steam pressure. Inoculations were made before the condensation water, which collects on the inside wall of the flasks during the sterilization process, was absorbed by the solid agar. The condensation water made it possible to prepare a suspension of the organsim within the flask. This suspension was then well distributed over the surface of the agar by revolving the flask at the proper angle to en-

able the suspension to pass over the whole surface of the medium, thus giving a uniform growth.

The second method consisted in pouring the glucose agar into Petri dishes. The agar was then inoculated by spraying the agar surface with a water suspension of the spores of *A. scabies*. This method can be used successfully when a culture room or other suitable place is available in order to reduce contamination.

Soil was always shoveled and screened from five to six times after the addition of the organism in order that uniform distribution might be obtained. Soil for the control plants was always handled the same as that used in the inoculated series except that the organism was not added.

PLANTING

The seed tubers used were of medium size, ranging from 2 to 3 inches in diameter. Each tuber was cut once through the stem and eye ends and one piece was planted in each pot. The seed piece was placed 6 inches below the surface of the soil with the cut surface down.

MANIPULATION OF EXPERIMENTS

Soil moistures were adjusted by weighing the pots at frequent intervals throughout the experiments and replacing the water lost. At the high temperatures this was done daily or oftener if necessary, while at the lower temperatures it was done less frequently, depending upon the conditions.

In practice it has been found that the temperature of that region of the soil where the greatest number of tubers develop remains practically the same as that of the surrounding water. In the case of the first three experiments, temperatures were adjusted three times during 24 hours. The water in the tanks which were operated above 18° C. (about room temperature) was raised one degree above the scheduled temperature at the time of adjustment, while in the case of those which were run at temperatures below 18° C. the water temperature was lowered one degree below the scheduled temperatures. This method allowed for a drop of one degree below or a rise of one degree above the scheduled temperature during the eight-hour period.

In experiments III, IV and V all tanks operated at temperatures above 15° C. were equipped with electric heaters and thermostats, which made it possible to reduce temperature variations very materially.

The average temperature variation in any of the tanks was less than one degree centigrade either up or down from the temperature at which the soil was intended to be held.

METHODS OF DETERMINING AMOUNT OF SCAB

Two methods have been used in connection with determining the amount of scab developing at the various temperatures. One consisted in determining the relative number of tubers scabbed and expressing the factor as a percentage of the total number of tubers produced in the infested soil and the other consisted in determining the relative proportion of the total tuber surface scabbed.

While the first method is the one which has heretofore been used most commonly for determining the amount of scab, there are some objections to it since it gives no indication of the severity of the disease on the tubers. For this reason it was considered advisable to record the results by both methods. The data secured in this way show that the maximum amount of disease as expressed on a basis of the number of tubers does not always coincide with the maximum as expressed upon the basis of percentage of tuber surface scabbed. This brings up the question as to which of these factors expresses more nearly the truth as to the optimum soil temperature range for the development of the disease. While this may depend to some extent upon point of view, the writers feel that it is not possible to decide this point at this stage in our knowledge of potato scab. It may be that the percentage of tubers scabbed expresses more nearly the optimum temperature for infection, while the percentage of tuber surface scabbed may express more nearly the optimum temperature for the development and progress of the disease after infection takes place.

In determining the percentage of tuber surface scabbed, it was necessary to measure all tubers and determine as nearly as possible the surface area of each tuber developing at each of the temperatures. After this process, it was necessary to measure or estimate the area of all scab lesions. From these data the per-

centages of scab surface were determined for each temperature. In cases of doubt this work was done by two people working together and independently.

In all of the greenhouse work herein reported, it is believed that all factors have been sufficiently well controlled to justify the conclusion that the differences in scab secured are due primarily to the recorded variations in soil temperature.

EXPERIMENT 1

This experiment was started November 8, 1918, and terminated January 25, 1919. While the main object of this series was to determine suitable methods of inoculation for further work, the experiment was so planned that some results might be obtained on the influence of soil temperature on the disease.

The soil used in this experiment contained 18 per cent moisture based on the weight of water-free soil. The seed was of good quality obtained from North Carolina.

The experiment was divided into two parts on a basis of the method of inoculation. One method consisted in inoculating the soil at the time of planting with 50 cc. of a spore and mycelium suspension of the scab organism. The tough leathery surface growth of the fungus was scraped from a thin layer of agar contained in six 750 cc. Erlenmeyer flasks. This material was thoroughly macerated and put in two liters of sterile water and thoroughly agitated before applying to the soil.

The second method of inoculation consisted in planting potatoes in sterilized soil and allowing plants to grow at room temperature (15-20° C.). When tubers commenced to develop (7 weeks after planting), part of the soil was removed from the upper roots and tubers of the plants. When the tubers were located, they were inoculated either on the uninjured or on a scratched surface with the sporulating growth of the scab organism. Soil was carefully replaced over the tubers and roots and the pots were placed in the temperature tanks.

Four temperatures were maintained, 12° C., 18° C., 24° C., 30° C. Three inoculated pots and one control pot for each method of inoculation were placed at or reserved for each soil temperature. The pots in the soil inoculation series were allowed to remain at 18° C. for a week after planting, then placed in the

temperature tanks. The pots in the tuber inoculation series were not placed in the tanks until after inoculation (7 weeks after planting).

On January 25 (eleven weeks after planting) all of the tubers were removed and the final data taken upon tuber development and the amount of scabbiness.

The results of this experiment showed the superiority of soil inoculation over tuber inoculation. The influence of temperature upon the development of the tubers and plants was quite marked in the series which was carried through the entire period in the temperature tanks. In the case of both series there was a marked temperature influence on the development of scab. In the direct

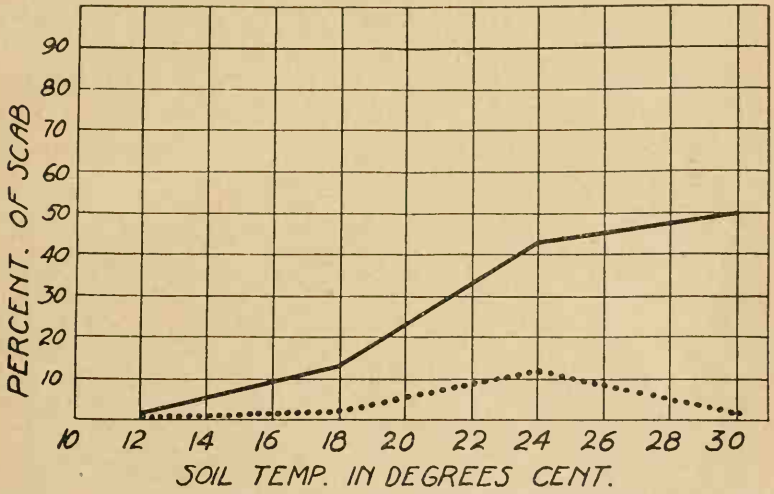


FIG. 1. THE INFLUENCE OF SOIL TEMPERATURE ON SCAB IN EXPERIMENT I.

Solid line represents the percentage of tubers scabbed.
Dotted line represents the percentage of the total tuber surface scabbed.

tuber inoculation series the greatest amount of scab developed in the tank held near 24°, but owing to the exceedingly small population of tubers these data are not included with the rest of the scab results. In the soil inoculation series the largest percentage of scabby tubers were produced in the tank held near 30°, but the greatest percentage of tuber surface scabbed was in the tank held near 24°. Reference to Table I, Fig. 1, and Plate I will give a clear idea concerning the development of the disease at the different temperatures in the soil inoculation series.

EXPERIMENT II

This experiment was started February 3, 1919, and terminated March 24, 1919. In this series seven temperatures were maintained, 12°, 15°, 18°, 21°, 24°, 27°, 27-30° C. The high temperature tank was operated on an alternate rather than a constant basis. For five days the temperature was held near 27° and for two days near 30°. This plan was followed throughout the experiment; a fair development of tubers was obtained from the experimental standpoint.

The soil moisture content in this experiment was 18 per cent based on weight of water-free soil.

The inoculum consisted of leaf mold cultures and a water suspension of the spores of *A. scabies* added to the soil previous to planting the seed. The seed was obtained from North Carolina and was a part of the supply which was used in Experiment I.

Two plants were grown at each temperature in uninoculated sterilized soil and with the exception of the 24° temperature six plants were grown in inoculated soil at each temperature. In the case of the 24° temperature two of the plants failed to grow, making only four plants in this tank.

The plants in the tanks held at temperatures above 15° C. were removed on March 24. In the case of the 12° and 15° tanks one and two plants, respectively, were removed on this date and on account of the slow development at these temperatures, the remaining plants were allowed to develop for 18 days longer when they were removed from the soil and the tubers examined.

The results of this experiment were much the same as those obtained in the previous one, as shown by Table I and Figure 2. There was a marked temperature influence on the host, which will be taken up later, and also on the development of the disease. While the disease developed at all temperatures, the most favorable one was near 24° C. In spite of the fact that most of the tubers from the 12° and the 15° tanks were not removed until 18 days after the removal of the plants growing at the high temperatures, very little scab developed in these two low temperature tanks.

In order to get some idea as to the influence of time on the development of scab at the low soil temperatures, most of the potato plants were not removed from the 12° and 15° tanks until 18 days after the removal of all the plants in the other tanks. At

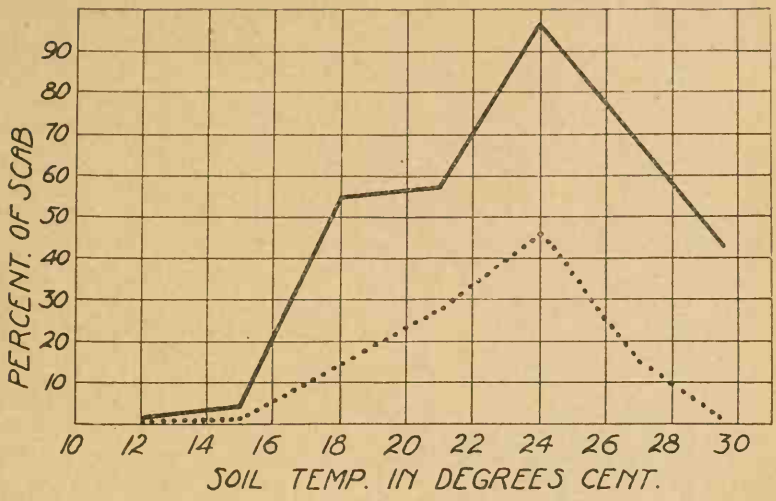


FIG. 2. THE INFLUENCE OF SOIL TEMPERATURE ON SCAB IN EXPERIMENT II.

Solid line represents the percentage of tubers scabbed.
Dotted line represents the percentage of the total tuber surface scabbed.

the end of this period, the tubers had reached sizes equal to or larger than the tubers produced by the plants removed earlier from the higher temperature tanks, but the amount of scab on these tubers was very slight as compared with the amount developed at the higher soil temperature. This observation indicates that the reduced amount of scab at the low temperatures is due to a direct temperature relation and not altogether to the delayed tuber development.

EXPERIMENT III

This experiment was started January 20, 1920, and terminated March 20 and 21, 1920. It was practically a duplication of Experiment II. The amount of inoculum used was reduced slightly on account of the extreme infection resulting in the previous experiment.

The soil contained 19 per cent moisture based on weight of water-free soil. As in Experiment II, seven temperatures were maintained, 12°, 15°, 18°, 21°, 24°, 27°, and 28.5-30° C. The highest tank temperature was held around 28.5° until March 8, when it was decided to raise the temperature to 30°. This was done on account of the fact that there was very little difference

between the development of these plants and that of the plants growing at 27° C. The greenhouse temperature ranged between 15.5° and 18.5° C., about 3 degrees lower than in the case of Experiments I and II.

The results of this experiment are shown in Table I and Fig. 3. These results are in accord with those obtained in the two previous ones in that a marked temperature influence was obtained both in the development of the host and in the development of the disease. While the disease developed at all temperatures, there

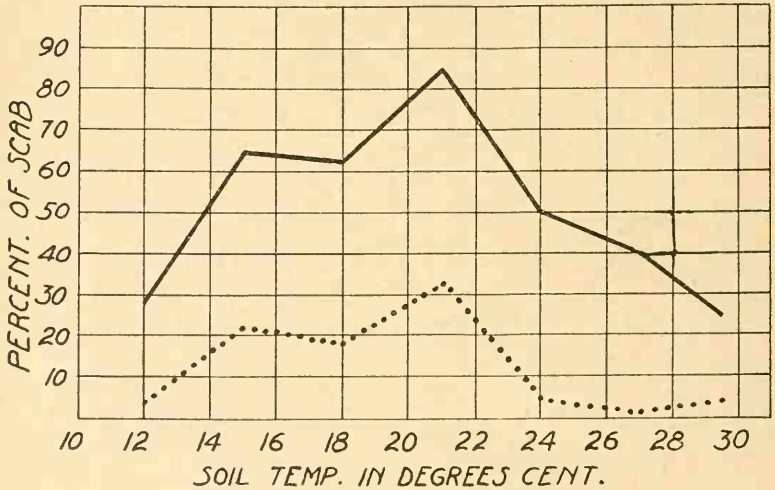


FIG. 3. THE INFLUENCE OF SOIL TEMPERATURE ON SCAB IN EXPERIMENT III.

Solid line represents the percentage of tubers scabbed.
Dotted line represents the percentage of the total tuber surface scabbed.

was very little at the extremes. In this series the greatest amount of disease developed in the tank held near 21°. This is a little lower than was the case in the previous experiments. The exact cause of this shift in optimum is not known. This point will be taken up later under the discussion on the host plant.

EXPERIMENT IV

This experiment was started December 25, 1920, and terminated March 19 and 20, 1921. As in the previous experiments, seven temperatures were maintained in this series, but there was some slight modification in the temperatures at which the individual tanks were operated. Tanks were held near the following temperatures, 11°, 14.5°, 18°, 21.5°, 25°, 28.5°, and 30.5° C.

The methods used were essentially the same as in Experiment III. The inoculum consisted of a water suspension of *A. scabies* added to the soil. A considerably larger quantity of the organism was used in this experiment than in the previous ones for the reason that it was feared the organism might have lost some of its virulence. The final results, however, show that this was not the case and a decided over-infection resulted.

While the results (Table I and Figure 4) of this experiment show that soil temperature has a marked influence on the disease, the extreme degree of infection made it difficult to interpret the relative amount of tuber surface scabbed. As in the previous experiments, the disease developed at all temperatures with the least amount developing at the extreme temperatures.

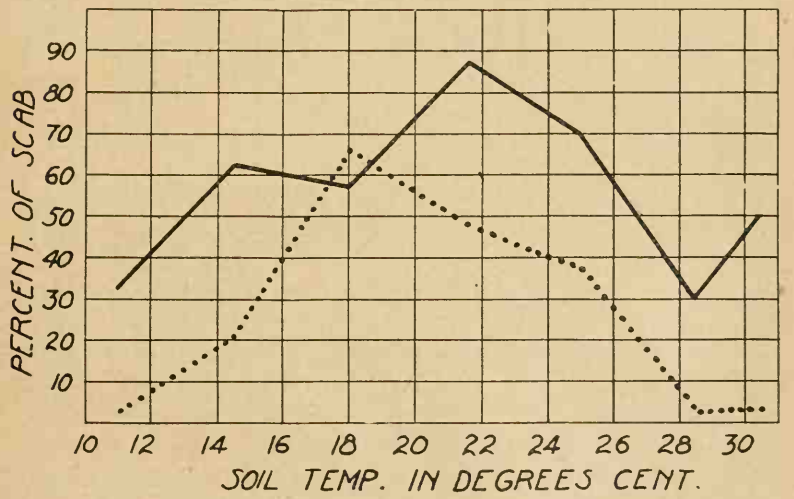


FIG. 4. THE INFLUENCE OF SOIL TEMPERATURE ON SCAB IN EXPERIMENT IV.

Solid line represents the percentage of tubers scabbed.
Dotted line represents the percentage of the total tuber surface scabbed.

Taking the number of tubers scabbed as a basis, it will be noted from Table I and Figure 4 that the maximum percentage of diseased tubers developed at 21° C., as was also the case in the previous experiment; however, it will be noted that the greatest percentage of tuber surface scabbed was at 18° C. This shifting of the optimum temperature for the development of the disease will be taken up in the discussion of the temperature tank results. The increased rate of scabbing shown at the highest temperature

is not looked upon as being very significant since only two tubers developed.

EXPERIMENT V

This experiment was practically a duplication of Experiment IV with the exception of a few minor details. The series was started March 23, 1921, and terminated May 27 and 28, 1921. As in Experiment IV, seven tanks were held at or near 11°, 14.5°, 18°, 21.5°, 25°, 28.5°, and 30.5° C.

The amount of inoculum used was reduced considerably under the amount used in Experiment IV and a considerable reduction in the severity of the disease resulted.

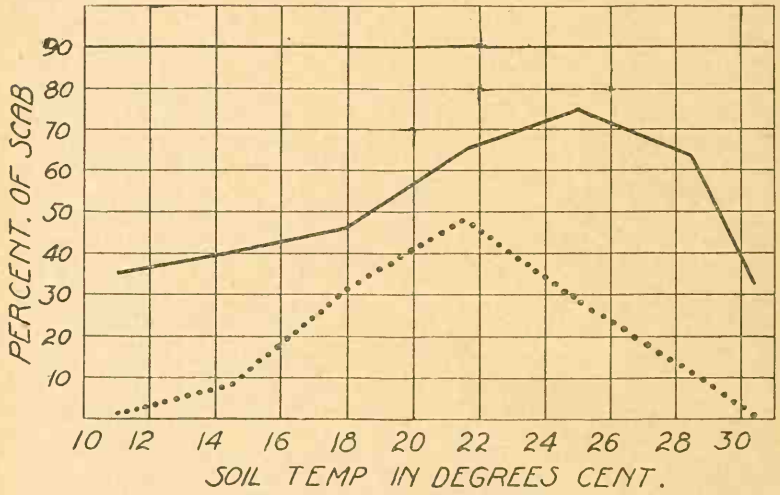


FIG. 5. THE INFLUENCE OF SOIL TEMPERATURE ON SCAB IN EXPERIMENT V.

Solid line represents the percentage of tubers scabbed.
Dotted line represents the percentage of the total tuber surface scabbed.

The results of this series are shown in Table I and Figure 5. The optimum temperature for the development of the disease as determined by the percentage of tubers infected was found to be at or near 24°, while the greatest percentage of tuber surface was scabbed at 21.5°. Plate II shows one fifth of the tuber population in the inoculated pots in Experiment V. These tubers were selected so as to show as near as possible the true variations in the development of the tubers and in the amount of scab at the various temperatures.

TABLE I—TABULATED DATA SHOWING THE INFLUENCE OF SOIL TEMPERATURE ON THE DEVELOPMENT OF POTATO SCAB IN THE TEMPERATURE TANK EXPERIMENTS.

Experiment I.

Soil temperature °C.	12	15	18	21	24	27	30
No. tubers produced	11		11		10		2
Per cent tubers scabbed	0		12.5		43.0		50.0
Per cent surface scabbed	0		.31		11.7		1.3

Experiment II.

Soil temperature °C.	12	15	18	21	24	27	27 to 30
No. tubers produced	4	40	120	146	66	180	128
Per cent tubers scabbed	0	2.5	54.1	54.8	97.0	67.7	42.1
Per cent surface scabbed	0	.0001	14.5	27.4	46.3	15.0	1.62

Experiment III.

Soil temperature °C.	12	15	18	21	24	27	28.5 to 30
No. tubers produced	82	80	49	46	55	58	69
Per cent tubers scabbed	28.0	65.0	61.6	85.1	50.0	40.9	25.3
Per cent surface scabbed	2.7	21.7	18.5	33.3	5.5	1.8	8.6

Experiment IV.

Soil temperature °C.	11	14.5	18	21.5	25	28.5	30.5
No. tubers produced	64	40	53	49	66	28	2
Per cent tubers scabbed	32.8	62.1	57.6	88.4	70.0	30.0	50.0
Per cent surface scabbed	1.83	21.0	67.3	49.2	37.5	3.1	5.5

Experiment V.

Soil temperature °C.	11	14.5	18	21.5	25	28.5	30.5
No. tubers produced	26	60	42	55	93	87	33
Per cent tubers scabbed	34.5	40.0	45.2	65.4	75.2	64.3	32.2
Per cent surface scabbed	.12	9.33	31.7	48.1	28.1	10.1	.43

RESULTS

Table I includes all scab data obtained in the preceding experiments and Figure 6 shows the average of all these data graphically. While the results have shown some fluctuation in the temperature optima for the development of scab, the average of all experiments indicates that while the disease operates over quite a wide range of temperatures, it develops in greatest abundance at soil temperatures ranging from about 20.5° to 23° C. (70-73° F.). As pointed out earlier in this bulletin, there is some question as to which factor (percentage of total number of tubers scabbed or the percentage of total tuber surface scabbed) is the most signi-

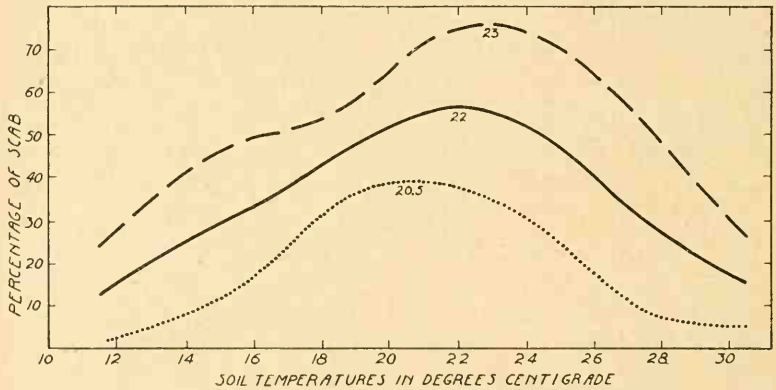


FIG. 6. THE INFLUENCE OF SOIL TEMPERATURE ON SCAB IN EXPERIMENTS I, II, III, IV AND V.

Dotted line represents the average percentage of the total tuber surface scabbed.
Dashed line represents the average percentage of tubers scabbed.
Solid line represents the average between the percentage of the number of tubers scabbed and the percentage of the total tuber surface scabbed. The latter is probably the most significant curve since it takes into account both the number of tubers scabbed and the degree of scabbiness. The authors are therefore accepting this as the basis for their final conclusions as to the relation of soil temperature to the development of potato scab.

ficant in expressing the amount of disease developed at the various temperatures. However this may be, an average between the curves for both these factors, as shown in Figure 6, gives an optimum temperature of 22° and it is this temperature which the writers are considering for the present as the optimum for the development of the disease.

It has been noted in this work that the temperature optimum for scab development has shifted from time to time. This shifting seems to indicate that certain aerial factors, not so well con-

trolled as the soil factors, may have had an influence on the development of the disease.

It is of interest to note that the fluctuation of the scab temperature optimum in the several experiments is more or less correlated with the shifting of the temperature optimum for the rate of tuber development as expressed in terms of the average weight per tuber. These correlations are shown in Tables I and V and in Figure 7.

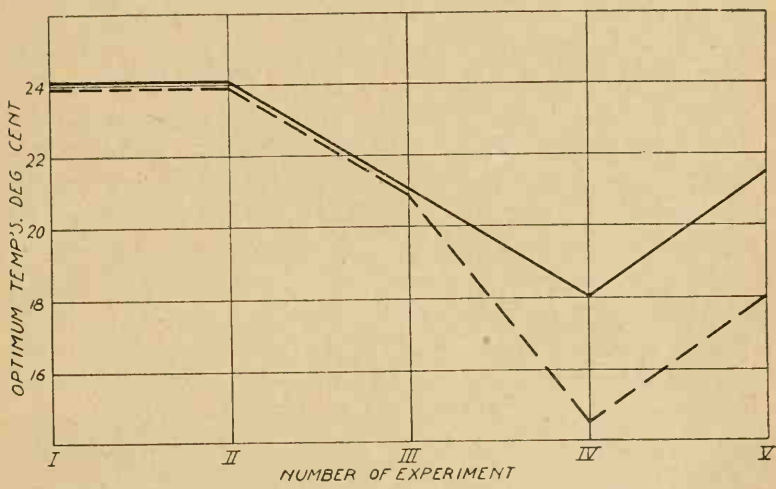


FIG. 7. THE TENDENCY TOWARD CORRELATION BETWEEN THE SOIL TEMPERATURE OPTIMA FOR SCAB DEVELOPMENT AND THE RATE OF TUBER DEVELOPMENT IN THE FIVE TEMPERATURE TANK EXPERIMENTS.

Points connected by the solid line represent the temperature optima for scab in the different experiments.

Points connected by the dotted line represent the temperature optima for rate of tuber development in the different experiments.

The rate of tuber development is calculated on a basis of the average weight per tuber produced at each soil temperature. The soil temperature giving the highest average weight per tuber is considered the optimum for the rate of tuber development.

The tendency towards correlation between scab development and the average weight per tuber suggests that scab development is dependent on tuber development and that rapidly growing tubers tend to be more scabby than those which develop slowly.

While the optimum for the development of scab did not fall as low as that for tuber development in Experiments IV and V, it will be noted that the scab optimum seemed to be influenced by the extreme drop of the tuber optimum in Experiment IV. Observations indicate that scab develops only on growing tubers. In all of the work carried on by the writers scab has never developed on any seed piece planted in inoculated soil. In the case of experiments involving the application of inoculum directly to the

surface of developing tubers it has been found that a large percentage of such tubers never develop after this disturbance. In all such cases scab has never developed on such tubers, whereas a large percentage of inoculated tubers which proceeded in their development did develop scab in a greater or less amount. These observations are in accord with the results obtained by Weiss and Orton (13) in connection with the black wart disease of potato, and there is also considerable evidence[1] which indicates that the development of scab lesions caused by *Venturia inaequalis* on the fruit and leaves of the apple is dependent more or less upon the growth of the host.

The tuber development and disease correlation in this work suggests that rapidly growing tubers are, within certain limits, more likely to become severely scabbed than tubers developing more slowly. In the event of the ultimate verification of this relation, it will not be difficult to understand how such aerial factors as light, temperature, humidity, and gas balance may within certain limits influence the development of scab through a direct influence on the above ground parts and thence upon the tuber.

In the experimental work involving the use of the scab organism it has been found that the underground bases of the stems of the potato plant and also the stolons often develop severe scab lesions (Plate III D, E) which usually originate in the lenticels. This condition seldom occurs at soil temperatures below 24° C. and it becomes more pronounced as the temperature rises. As in the case with tubers, stems are clean and white in the control pots not containing the scab organism (Plate III C). Güssow (2) has also noted that the scab organism attacks the underground stems of potato plants.

EXPERIMENTAL WORK IN THE FIELD

METHODS

Soil temperature influences have been studied in a preliminary way under field conditions. In this connection two methods have been employed in studying variations in soil temperature. One consisted in maintaining three soil temperature gradations in small plots by means of special apparatus and the other consisted in planting inoculated seed at intervals throughout the season in

[1] Unpublished observations made by G. W. Keitt, of the Department of Plant Pathology, University of Wisconsin.

order to get the benefits of the seasonal change in temperature. While the first method has given fairly good satisfaction, the second method has not been successful owing to the long period over which tuberization takes place. During these long periods too many changes in temperature and other influencing factors take place, thus making it difficult or impossible to interpret the results obtained.

One experiment was carried on with soil temperature control in field plots during the summer of 1919. In this experiment three temperature variations were maintained. At the outset it was somewhat of a question as to just how successful such a method might be, and it was recognized that the undertaking was largely an experiment in methods of temperature control.

Three plots, each 12 feet long and 9 feet wide, were used in this experiment. The soil was a dark rather heavy loam which, as far as known, had not produced a crop of potatoes during the past 9 or 10 years.

Treated Wisconsin grown Early Ohio seed free from scab and Rhizoctonia was planted in three rows the long way of the plot. The seed trenches were 3 feet apart and the seed placed 14 inches apart in the rows, making 27 hills per plot. In each plot 6 hills were left uninoculated for controls.

Inoculation consisted in dipping the cut seed in a heavy water suspension of *Actinomyces scabies* and applying the suspension to the soil used to cover the seed. This inoculum consisted of 15 gallons of water to which was added the surface growth of *A. scabies* growing on about 200 square inches of potato hard agar. The inoculated soil was well mixed and put back in the trenches. The uninoculated seed was covered with uninoculated soil and due precautions were taken to prevent contamination from the inoculated portions of the plot.

On June 3 the plants commenced to come through the soil and on June 14 the temperature apparatus was installed.

Temperature Apparatus

This experiment was planned from the standpoint of maintaining three temperature ranges. Every effort was made to maintain one plot at as low a soil temperature as was practicable, one plot at a medium temperature and one at as high a temperature as possible under the conditions.

Low temperatures were maintained by means of burying one inch water pipes just under the surface of the soil. Three of these pipes 5 inches apart were placed on each side of each row of potatoes. These pipes were connected at one end to a distributing pipe and the opposite ends were fitted with pet cocks which could be opened or closed at will. This pipe system was connected with a large coil of pipe placed inside of a well insulated box containing ice, which was buried in the soil. The whole system was connected with the local water mains and water was allowed to pass through the cooling coil, thence through the soil pipes and out through the cocks in the end of each pipe. The surface of this plot was insulated with sphagnum moss.

The soil in the medium temperature plot was merely covered with sphagnum moss.

The high temperatures were maintained by placing hot-bed sash close to the surface of the soil in one plot. Each pane of glass was lifted lightly at the over-lapping end so as to allow rain to reach the soil.

An electric thermo-couple was placed 4 inches under the surface of the soil in the center of the middle row of plants in each plot. Temperatures were determined and recorded at 7 a. m. and 5 p. m. each day throughout the experiment after the temperature apparatus was in place. Figure 8 shows the daily mean for each plot during this period. It will be noted that up to July 15 the soil temperature of the piped plot tended to run higher than the soil temperature of the plot intended to run at the medium temperature. This was due to the low capacity of the ice box and coil, allowing warm water to enter the soil pipes and actually raise the soil temperature. An increase in the capacity of the ice box and coil corrected this difficulty and the temperature curves remained well apart thereafter. Since tuberization had not taken place to any extent on July 10, this temperature irregularity doubtless had but little influence upon the final amount of scab recorded.

The tops of the plants were commencing to die on August 24 and the mature tubers were removed from the soil shortly after this date.

RESULTS

All tubers including those grown as controls showed a peculiar surface checking, the exact cause of which is not known. This

same condition occurs quite often in certain potato districts and it is considered by growers and others to be caused by some abnormal soil condition. While this checking made it more difficult to diagnose scab than would have been the case if the checking had not been present, it was possible to determine the typical scab lesions with considerable certainty. In some cases doubtful lesions were encountered. These were recorded separately from the pronounced scab lesions and it is noteworthy that their abundance was directly proportional to the amount of scab, suggesting the idea that they were common scab lesions lacking some of the typical characteristics or similar lesions caused by some other organism present in the soil[1].

Table II and Figures 8 and 9 give the results of this experiment. It is to be noted that the amount of disease increased with the rise in soil temperature[2]. The greatest amount developed in the high-temperature plot. While there was some difference in the moisture content of the three plots, it is considered doubtful if this factor altered the general trend of the curve very materially.

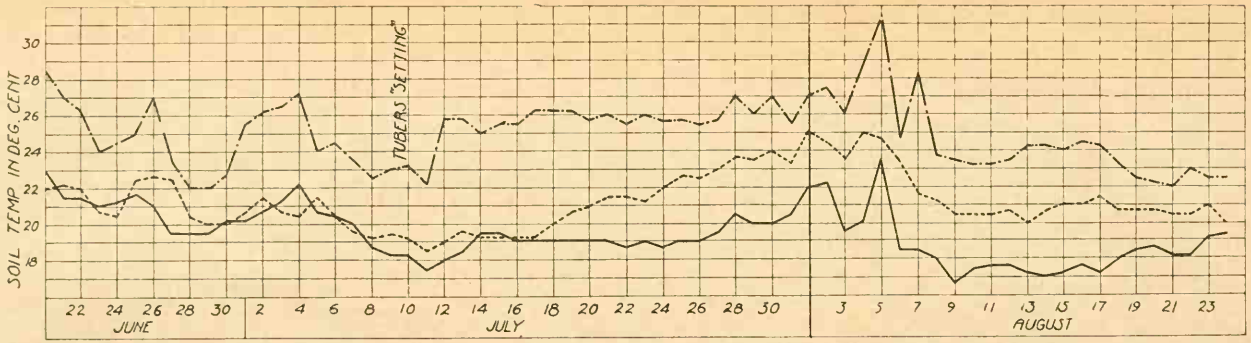
Table II—Results Obtained in the Field Temperature Plot Experiment.

Mean soil temperatures for July and August	"Low" 19°C	"Medium" 21°C	"High" 25°C
No. of tubers produced	188	232	162
Percentage of tubers scabbed	6.25	13.23	30.55
Percentage of tubers with doubtful scab	0.00	1.96	22.9
Percentage of tubers with Rhizoctonia sclerotia	41.8	20.68	19.13

Preliminary moisture experiments which have been carried on in connection with the development of scab seem to indicate that medium soil moistures ranging about 19 per cent (based on water-free soil) are more favorable for scab development than moistures held at 15 per cent and at 26 per cent respectively. In the case of the field plots under discussion, the soil in the low temperature plot contained about 24 per cent moisture, while the moisture

[1] Wollenweber (14) has shown that a number of species of Actinomyces are pathogenic on potato tubers causing lesions which differ in a number of respects from the common scab lesions produced by *A. scabies*.

[2] The soil used in this experiment contained some Rhizoctonia infestation and consequently the tubers showed some Rhizoctonia sclerotia at the time of digging. It is of interest to note from Table II that the greatest amount of Rhizoctonia developed in the low temperature plot while the least amount developed in the plot held at the highest temperatures. These results are in line with the results obtained by Richards (8) in his soil temperature studies on potato stem injury due to *Rhizoctonia solani*.



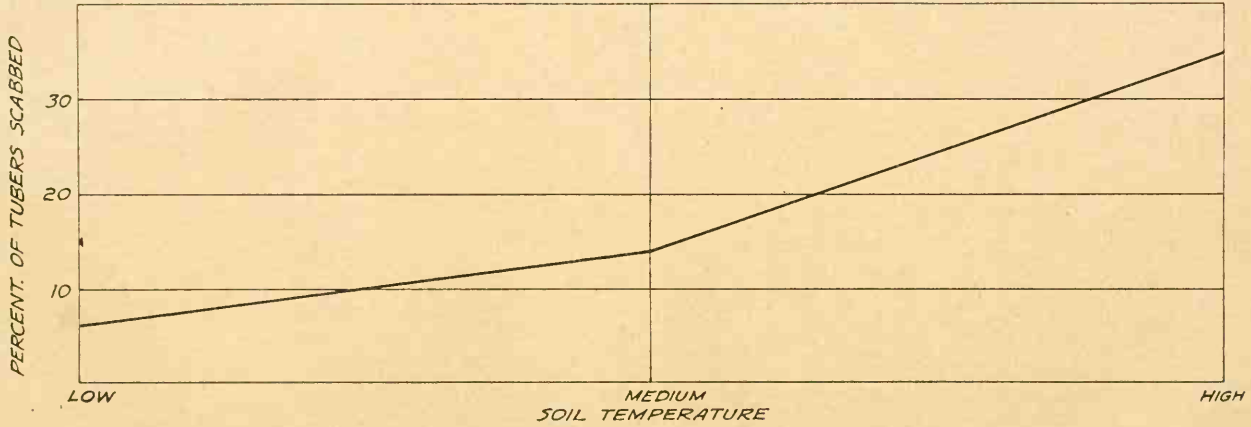


Fig. 8 (above) shows the mean of the daily soil temperatures in the field temperature plots two inches below the surface. Note that these were not satisfactorily controlled until early July, but that thereafter they were fairly well maintained relatively at a fairly high, fairly low and a median grade. Tuber formation and scab development occurred during this later period.

Fig. 9 (below) shows the percentage of tubers scabbed in the three plots as indicated by the heavy line. Note that percentage of scab rises with soil temperature in this field experiment in a way similar to that shown in the tank experiments. (For details see Table 11 and accompanying text.)

content of the soil in the medium and high temperature plots contained around 19 per cent and 16 per cent respectively. On this basis, had the soil in all the plots contained 18 or 19 per cent moisture there would have been slight increases in the amount of scab developing in both the high and the low temperature plots.

While it is recognized that in certain respects the results of this experiment do not carry as much weight as those obtained in the greenhouse on account of the difficulty encountered in controlling soil moisture, it seems justifiable to conclude that the difference in soil temperature in the three plots very largely explains the variations in the development of scab. In any case it is evident that these results which are based on mature tubers and fluctuating soil temperatures are more nearly comparable to field conditions than are the data obtained in the temperature tanks. It is, therefore, significent to note that in their main features the relations between temperature and scab are alike in the two series.

GENERAL OBSERVATIONS

As pointed out in a preliminary note by the senior authors (5), field observations made in Europe and America indicate a greater prevalence of scab in regions having warm growing seasons than seems to be the case in regions where cool summers prevail, and likewise a greater amount of scab seems to develop in a given locality during a warm season than during a cool one.

The observations made by the senior author in connection with the relative scarcity of scab in northern European potato districts led to an examination of weather records for these districts. These examinations revealed the fact that the mean temperature during the growing season in northern European potato districts is considerably lower than that for the principal potato districts in the United States. According to Orton (6) the July isotherm of 65° F. (18.3° C.) runs south to the principal potato districts of Great Britain and northern Germany, while in America it is only in Aroostock County, Maine, and parts of northern New York that we have extensive potato culture north of this isotherm. According to Smith (11) the July isotherm of 70° F. (21.1° C.) practically marks the southern boundary of successful main crop potato production in the United States.

Orton (7) reports that scab is not a serious disease in the Bermuda Islands and in correspondence with the authors, Prof. E. J. Wortley, then pathologist of the Bermuda Agricultural Station, stated that very little common scab occurs in these Islands in spite of the fact that the soils are highly calcarious. Upon consulting the temperature records of Verrill (12) for the Bermuda Islands, it is found that the mean monthly temperatures during their main potato-growing season, October to February, run from 74° F. (23.3° C.) for October down to 63° F. (17° C.) for February. After planting, the temperature goes down rapidly and at the beginning of the period of tuber formation the temperature is very close to 67° F. (19.5° C.) or below and steadily going down to 63° F. (17° C.). In the United States we have a complete reversal of this temperature and crop relation, in that the temperature steadily rises after potato planting and it is near or at its height during the period of tuber formation.

In Wisconsin, general observations show that potatoes from the Door County peninsula are freer from scab than tubers grown in other potato districts in the state despite the fact that potatoes have been grown in the county for many years and that the soil is calcarious in origin. Table III gives the monthly mean temperature for Sturgeon Bay, Door County, Wisconsin, and other Wisconsin cities located in the chief potato-growing belt of the state for the tuber developing months (June, July and August). These data show that Sturgeon Bay has a lower average normal temperature during this period than is the case of the other locations. Moreover, the chief potato district is in that portion of the peninsula lying north of Sturgeon Bay where the summer temperature will average somewhat lower.

In Wisconsin it has been noted that scab is more prevalent certain seasons than in others. John Brann, chief inspector of certified potato seed stock in Wisconsin, found that scab was unusually prevalent in the state during the seasons of 1916 and 1921, it being necessary to reject more seed on account of scab those seasons than usual. The records of the U. S. Weather Bureau show that the summers of 1916 and 1921 were among the hottest on record for this state.

TABLE III—NORMAL MONTHLY TEMPERATURES FOR TOWNS LOCATED IN THE CHIEF POTATO DISTRICTS IN WISCONSIN.

(Based on the records of the U. S. Weather Bureau.)

Cities in the Wisconsin potato district	June		July		August	
	°F	°C	°F	°C	°F	°C
Sturgeon Bay (Door Co.)	**60.0**	**15.5**	**65.6**	**18.6**	**65.5**	
Spooner	64.2	17.8	68.7	20.3	65.9	18.8
Eau Claire	66.9	19.3	70.8	21.5	68.8	20.4
Minoqua	63.6	17.5	67.2	20.6	64.1	17.8
Medford	64.9	18.2	68.5	20.2	66.6	19.2
Oconto	64.5	18.0	69.0	20.5	66.9	19.3
Waupaca	64.7	18.1	67.6	19.7	70.6	21.4
Antigo	64.2	17.8	67.6	19.7	64.8	18.2

DISCUSSION

As pointed out earlier, the writers are mindful of the fact that all factors were not under full control during the experiments reported in this paper. While this would, of course, have been highly desirable, it is felt that the various factors were sufficiently well controlled and the probable errors clearly enough evaluated to warrant the conclusions that the differences in scab development here noted are due primarily to the recorded differences in the soil temperatures.

The difficulty of rightly comparing the influence of fluctuating field temperatures with the influence of constant temperatures under experimental conditions is fully recognized. While it is possible to calculate a mean temperature for a given period, it is not definitely known if such a mean has the same relative influence on the disease as a like temperature maintained constantly over the same period. Naturally many things, such as the maximum temperatures and the duration of each in relation to the period of susceptibility of the tuber and the incubation period for the disease have to be taken into consideration, and at best such a relation becomes exceedingly complex. However, certain observations, in addition to the experimental results obtained seem to throw some light on the situation.

In going over weather records and the available observational data on the seasonal prevalence of scab, it is found, as heretofore noted, that the disease is more prevalent during the hottest growing seasons. The results obtained in the "constant" temperature

experiments, however, show that medium high soil temperatures are more favorable than high temperatures. This led to a comparison between the mean temperatures for the hottest seasons (July and August) on record in the Wisconsin potato belt, and the "constant" temperatures maintained in the tank experimental work. It was found that the mean air temperatures for these seasons ranged close to 23° C. and in the case of the month of July, 1921, the hottest month recorded in late years at Waupaca, Wisconsin, the mean did not go above 26° C. With August of the same season reaching a mean of 21° C. we have an average mean air temperature of 23.5° C.[1] during July and August, the tuber and scab developing period at Waupaca. It will be noted that this figure is strikingly close to or within the optimum temperature range (Figure 6) for the development of the disease obtained in the "constant" temperature experiments. In consideration of these observations and of the results obtained in the field temperature experiments, it is believed that the medium high mean soil temperature of a hot growing season produces essentially similar comparative results in scab production as the same temperature maintained constantly throughout an experiment. Conversely, it seems evident that the scab results obtained in the temperature tank experiments are a safe index to the relative influence of soil temperatures on the development of common scab under actual field culture conditions.

While scab developed at the low temperatures in our trials, it seems evident from Shapovalov's (9) work with the organism that these low temperatures are not as favorable for the growth and increase of *A. scabies* in the soil as are the higher temperatures. It is definitely known that under favorable conditions the organism lives for long periods in the soil and it also increases readily on such organic materials as are commonly found in "potato soils". Shapovalov (9) reports the optimum temperature for the growth of the organism in pure culture to be between 25° and 30° C. The writers have also found that the organism grows best within this range of temperature on agar, also leaf mold and

[1] Records obtained by the writers show that soil temperatures in unshaded soil at a depth of two inches average from 1 to 8° C. higher than recorded air temperatures at the same location in mid-summer. When the same soil is shaded, the temperature is approximately the same as the air temperature. In a potato field we have the latter condition and soil temperatures at a depth of four inches (about the center of a potato hill) will average from 1 to 2° C. below the air temperatures depending upon the condition. It is, therefore, reasonable to believe that the mean air temperature of 23.5° would be reduced to at least 22 or 23° C. in terms of soil temperature.

straw, hence it seems reasonable to believe that soil temperatures approaching this range will also be the most favorable for the increase of the organism in the field. This indicates that the stimulating influence of high soil temperature upon the occurrence of the scab organism will be cumulative from year to year. It seems very likely, therefore, that the slight amount of disease occurring in cool potato districts may be accounted for in part through a reduced amount of the casual organism in the soil as well as on the basis of unfavorable temperatures for the development of the disease. Hence, with such considerations in mind, we would not expect to find as great an amount of scab during a year of favorably warm temperature in a normally cool region as would be the case in another locality having this same warm temperature for the normal.

These studies show that the optimum temperature for the development of the disease does not necessarily agree with the opitmum for the growth of the parasite in pure culture. Consideration must, therefore, be given to the temperature relations of the host as well as of the parasite.

Our data indicate that the temperature optimum for scab lies between that for the rate of tuber development and that for the growth of the parasite in pure culture, being somewhat closer to the optimum for tuber development. This general relation indicates that the development of the disease may be likened to the "resultant" of two "forces". One such "force" is represented by the influence of certain variable factors upon the host, the other by the influence of the same upon the parasite. These may in some cases tend in like direction, in other cases they may be opposed as is apparently the case in the scab disease.

It seems very probable that soil temperature may in part account for the irregular results obtained in much of the seed treatment work for scab control. Very frequently farmers and experimenters report good results from certain seed treatments and in following years the same treatments may fail to control the disease on the same soil. Soils containing only a moderate infestation of the scab organism would not produce much scab during a cool year, whereas a considerable amount of the disease would be likely to occur during a hot year, even though the seed be well disinfected. Of course, it must not be forgotten that other influencing factors, such as soil moisture and soil reaction,

also play their part with soil temperature in explaining such irregular results with seed treatments.

There is some indication that excessive scabbing in certain early varieties may in part be due to the fact that tuber development in such varieties takes place during the hot part of the season. Preliminary field experiments have shown that varieties differ considerably in their habits of setting and maturing their

TABLE IV—DATA SHOWING THE TENDENCY FOR SCAB TO BE MORE PREVALENT AT THE STEM END OF TUBERS.

Data from 63 Scabby Tubers from a Commercial Bin.

Location of infection on tuber	Percentage of Tubers
Total stem ends infected	85.4
Total seed ends infected	31.7
Stem end only	46.0
Seed end only	9.5
Central portion only	22.2
General infection	6.3

Data from 1380 Scabby Tubers from Experiment II, III, IV and V.

Location of infection on tuber	Percentage of Tubers
Total stem ends infected	63.2
Total seed ends infected	28.5
Stem end only	29.1
Seed end only	4.6
General infection	18.9

tubers, thus causing variations among varieties in their exposure of growing tubers to seasonal variations in temperature. Obviously, this point must be taken into consideration in work dealing with resistance to scab in order that the purely environmental factors may be segregated from any true resistance factor.

In this connection, it is of interest to note that scab tends to be more or less localized at or near the stem end region of the tuber as shown in Table IV. This localization has been noted on the experimental tubers and on tubers from commercial lots. In the experimental work it has been observed that the localization of the disease on the tuber is less pronounced at the optimum soil temperature for the disease than at the higher and lower temperatures. The explanation for this segregation cannot be given at this time. It may be suggested that the tuber is more susceptible to infection at the stem end than at other points. It is also to be noted that the stem end of the potato has the oldest issue, and has thus been exposed to infection longer than other parts of the tuber.

THE INFLUENCE OF SOIL TEMPERATURE ON CERTAIN ORGANS OF THE POTATO PLANT

In connection with the studies on scab, it should be noted that the host plant also reacts readily to variations in soil temperature. It is not within the scope of this paper to develop various aspects of this phase, but it is pertinent to summarize some of the most evident relations which come under observation. A limited amount of host data is shown in Tables V, VI and VII.

TABLE V—GIVING DATA ON THE NUMBER OF HILLS, AVERAGE NUMBER OF TUBERS PER HILL AND THE AVERAGE WEIGHT PER TUBER IN GRAMS FROM THE TANK EXPERIMENTS.

Exp. I	Soil temperature °C.	12		18		24		30
	No. of hills	4	-------	4	-------	4	-------	2
	Av. no. tubers to hill	2.75	-------	2.75	-------	2.5	-------	1.0
	Av. wt. per tuber	3.18	-------	7.34	-------	8.00	-------	4.75
Exp. II	Soil temperature °C.	*12	*15	18	21	24	27	27 to 30
	No. of hills	1/5	2/4	6	6	4	6	6
	Av. no. tubers to hill	4/18	20/16.7	20.0	24.3	16.5	30.0	21.3
	Av. wt. per tuber	.75/1.9	1.5/3.4	1.80	1.12	1.87	.55	.22
Exp. III	Soil temperature °C.	12	15	18	21	24	27	28.5 to 30
	No. of hills	8	8	8	8	8	8	8
	Av. no. tubers to hill	13.7	13.3	8.2	7.75	9.1	9.75	11.5
	Av. wt. per tuber	2.46	4.54	6.9	7.46	6.41	5.32	2.31
Exp. IV	Soil temperature °C.	11	14.5	18	21.5	25	28.5	30.5
	No. of hills	8	8	8	8	8	5	1
	Av. no. tubers to hill	10.7	6.62	8.87	8.12	11.1	4.6	2.0
	Av. wt. per tuber	3.03	8.9	6.43	6.2	3.9	.64	1.5
Exp. V	Soil temperature °C.	11	14.5	18	21.5	25	28.5	30.5
	No. of hills	8	8	8	8	8	8	7
	Av. no. tubers to hill	4.33	10.0	7.0	9.16	15.5	14.5	5.5
	Av. wt. per tuber	2.8	7.02	12.85	9.25	4.71	1.99	.34

*One and two plants were removed from 12° and 15° C. tanks respectively at the time the data was taken on the plants growing at the other temperatures in the experiment; these data are represented by the numerators while the denominators are the data for the remaining plants grown at 12 and 15° C. which were removed eighteen days after the removal of the plants from the higher temperature tanks. The numerator figures are comparable with the figures recorded for each of the temperatures above 15°.

UNDERGROUND PARTS

Tubers

Variations in soil temperature seem to have less consistent influence on the number of tubers produced per hill than on any of the other host activities thus far observed. However, there does seem to be a tendency for the greatest number of tubers to develop at 15° C. and at 25°-28° C. There is a reduction in the number of tubers per hill at the intervening and the extreme temperatures. This curve tends to be the reverse of all other curves representing the plant activities observed in this work.

The size of tubers is influenced considerably by variations in soil temperature. During the period involved in the various tank experiments, the largest tubers were produced at soil temperatures ranging from 15° to 22° C. with the optimum at or near 18° C. However, when plants are allowed to develop beyond the period of the main experiment, as was the case with the plants held at 12° and 15° C. in Experiment II, the tubers progress rapidly and increase in size as shown in Table V. It will not be surprising to find that the optimum soil temperature for mature tuber development is somewhat lower than just indicated, when a complete series can be carried through to maturity.

Observations have not been made to determine the soil temperatures at which tubers commence to set first, but it is believed that this takes place at the temperatures which favor early sprouting and the emergence of the sprouts from the soil. The indications are that the optimum temperature for the early setting of tubers is somewhat higher than the optimum for a yield of large tubers.

The shape of tubers is influenced to a considerable extent by

TABLE VI—THE RATIO OF WIDTH TO LENGTH IN TUBERS GROWN AT DIFFERENT SOIL TEMPERATURES.

These data are based on measurements made on the whole population of Experiment V.[1]

Soil temperature °C.	11	14.5	18	21.5	25	28.5	30.5
Ratio of tuber width to length.	1:0.9	1:1.02	1:1	1:1.12	1:1.14	1:1.25	1:1.6

[1] The Irish Cobbler seed used in this work was typical of the variety which tends towards the globular shape. Most of the tubers were practically circular in the cross section intersecting the stem and bud axis at right angles, and for this reason width is designated as a single factor in Table VI.

soil temperatures as shown in Plates I, II and IV. At low soil temperatures the length of Irish Cobbler tubers is less along the stem-bud axis than along the transverse axis, while at the higher temperatures the stem-bud is much the longer and tubers tend to become egg or pear shaped, as shown in Plates I, II and IV. Actual measurements made on the population of a complete temperature series show that the ratio of the width to the length of tubers grown at 11° C. is about 1:0.9, while at temperatures near 30° C. this ratio approximates 1:1.6. The ratios developed at the intervening temperatures gradually approach the latter ratio as the temperature rises forming a rather regular curve as is shown by the data in Table VI.

Fitch (1) has noted that under certain conditions potato tubers tend to elongate and develop the pear shape. He associates this condition with drouth, the "running out" of seed stock and seasonal conditions. While factors other than soil temperature may influence the proportional dimensions of tubers, the results herein recorded show definitely that soil temperature is an important factor in determining tuber shape, a point which is of economic interest to growers who produce exhibition seed stock.

It is a matter of common observation that lenticel development on potato tubers is stimulated by certain moisture conditions. In these experiments where soil moisture was kept approximately uniform, it has been found that lenticel character has also been influenced considerably by soil temperature. These organs have not been conspicuous on tubers developed at low soil temperatures, but have become large and protruding at the high temperatures. An evident suggestion is that their relative development may be associated with respiratory metabolism.

The influence of soil temperature upon the chemical composition of the tuber has not been a matter of direct inquiry in connection with this work. It may be assumed that the composition is so influenced and a type of evidence that bears upon this deserves record. In Plate V is shown a complete temperature series of tubers grown in uninoculated soil. These tubers were photographed after storage in 70 per cent ethyl alcohol for five months after their removal from the soil. The tubers produced at the low temperatures are jet black in color, whereas those grown at the medium temperatures show practically no discoloration. At 30° C. the tubers show a slight darkening, but to

TABLE VII—DATA SHOWING THE INFLUENCE OF SOIL TEMPERATURE ON CERTAIN HOST DEVELOPMENTS.

These data are the average of the results from experiments II, III, IV and V. All data are based on determinations made at the close of each experiment. All weights are in grams and linear measurements in centimeters.

Soil temp. degrees C.*	11 12	14.5 15	18	21 21.5	24 25	27 28.5	27 30.5
Av. No. of tubers in inoc. hills	8.2	12.5	11.2	12.5	12.8	14.4	10.2
Av. Wt. of tubers per hill	22	42.7	56.0	54.2	51.0	25.0	9.5
Av. Wt. per tuber	2.55	5.44	7.0	6.0	4.2	2.1	.80
Above ground parts							
Av. Wt. of green tops per hill (Exp. IV and V)	55.1	77.0	82.2	89.0	87.0	70.0	50.0
Av. No. of stems per hill (Exp. II, III, IV and V)	2.8	3.6	4.2	4.6	4.0	3.21	2.8
Av. height of stems per hill Exp. II, III, IV and V)	22.0	23.0	22.6	23.5	25.0	30.2	14.0
Av. diam. of stems (Exp.V)	7.1	8.5	7.9	7.7	6.7	3.9	3.3
Av. No. days for plants to come through soil (Exp. III, IV and V)	23.0	17.5	14.5	12.8	12.8	16.5	24.6

a less degree than is the case with the tubers produced at the low temperatures. The black coloration suggests a melanin relation involving the reaction of various enzymes, tyrosin and other complex proteins. While this variation cannot be interpreted at this time, it is evident that the chemical composition of at least the surface tissues of these tubers was influenced by the soil temperatures. The question naturally arises as to what relation such changes may have upon susceptibility or resistance to scab infection or development. These are evidently matters deserving further investigation.

Stem bases and stolons

These parts of the potato plant seem to be greatly modified by variations in soil temperature, as noted previously by Richards (8). At the high temperatures they become relatively large in diameter and fleshy in nature. The stems are very much larger below ground than they are above the soil line, whereas at the low temperatures the reverse is true, the underground stems being more slender in proportion than the above ground stems. As the temperature advances toward the higher limits of endurance the basal portion of the main stem as well as the stolons evidently

* The soil temperatures given are those used throughout all the experiments. Since there was so little difference between the temperatures used in Experiments II and III, and those used in Experiments IV and V all data in this table were averaged in the usual manner.

assumes a storage function. This may be correlated in some degree with an inhibition of normal tuber development and consequently with a disturbance of translocation processes. As in the case of the tuber, lenticel development is very marked on stems and stolons at high temperatures and much reduced at low ones.

Above Ground Parts

Richards (8) has published upon data which are essentially in harmony with those obtained by the writers concerning the response of the above ground parts of the potato plant. The results herein reported, however, cover somewhat longer periods of development and a larger number of experiments than those which he discussed.

Stems

Soil temperature greatly influences the length of time required for germination and the emergence of the sprouts from the soil as shown in Table VII. Sprouts emerge first in soil held from 21°-24° C. and in general emergence is earlier at the high than at the low temperatures. During the early life of the plants the height of tops is directly correlated with the time of emergence. A soil temperature of about 27° C. soon becomes the optimum for these organs with a sharp decline in the curve above this temperature. Later, however, these plants at the higher soil temperatures commence to slacken their growth and come to maturity, whereas the plants at the low temperatures come on slowly and finally surpass plants grown at the high temperatures. In general the life cycle of the potato plant is short at high and long at low soil temperatures.

The green weight of "stems" is also influenced by variations in soil temperature, but this factor seems to vary more or less directly with the number of "stems" produced at the various temperatures. Apparently the optimum soil temperature for the production of green weight and that for the production of number of "stems" per hill is at or near 21° C. The two curves differ, however, in that the green weight curve tends to show a wider optimum range than the "stem" curve. Both curves decline gradually from the optimum point.

The development of "wings" on the "stems" is much reduced and the swelling of the nodes is much increased by high soil tem-

peratures and in some cases aerial tubers and auxiliary branches develop at the higher temperatures.

The leaves of the potato plant also show variations due to soil temperature. In general the ratio of width to length in leaflets follows the same trend as is the case with tubers. At the low soil temperatures leaflets are wider in proportion to their length, whereas they are longer than wide at the high temperatures (Plate III A, B). At these latter temperatures leaflets are decidedly lanceolate in form, but they tend towards the round type at the low soil temperature.

While these observations have dealt primarily with the evident characters, chiefly morphological, it is realized that the more important modifications may be those occurring in the physiological processes, especially in nutrition and including food translocation or storage. This is indicated not only in the nodal swellings and aerial tuber developments noted above, but also in the influence of soil temperature upon chlorophyll, the foliage tending in general to a deeper green at the lower soil temperatures and a lighter color at the higher.

We have not the data to go far in the discussion of the details as to the relation of even this single variable factor, soil temperature, to the normal development of the potato plant and its tuberization processes. At the same time it is evident that an adequate discussion of these must include some consideration of their interrelation with the other variables in the environment, especially air temperatures and light.

While these observations relate primarily to the host plant, their significance must not be overlooked from the standpoint of the disease. As pointed out earlier, the evidence at hand indicates that potato scab as a disease is influenced by the conditions of tuber development. These include the suggestion that rapidly growing tubers may scab more severely than slow growing ones, and that there may be such differences in the chemical composition of the tubers developing under different conditions as may influence their relative susceptibility to infection. The rate of the tuber's development, as well as its composition is, of course, correlated, with the metabolic and growth processes of the rest of the plant. It is obvious, therefore, that the ultimate understanding of the influence of soil temperature or other variable factors upon the development of a disease like potato scab is conditioned upon further studies concerning the relation of these to host as well as to parasite.

SUMMARY

1. The development of potato scab, caused by *Actinomyces scabies,* is evidently influenced by several environmental factors. An attempt has been made to secure evidence as to such possible influence of soil temperature.

2. Five series of experiments have been conducted in greenhouses using the "Wisconsin tank" method. These have included seven gradations of soil temperature ranging from about 11° to 30.5 C. In all cases under this method the aim is to maintain the other soil conditions including moisture alike and approximately constant, and in each experimental series all the plants are exposed to the same aerial conditions.

3. The results show that under these circumstances the development of the scab disease is influenced by soil temperature.

4. The disease developed at all soil temperatures, 11° to 30.5° C., but was comparatively slight at either extreme.

5. The optimum soil temperature for scab development as measured by the number of scabby tubers was found to be about 23° C., while the optimum for the percentage of the total tuber surface scabbed was a little lower, about 20.5° C. The conclusion reached is that all things considered 22° C. may be accepted as about the optimum soil temperature for scab development, where the "Wisconsin tank" method is used.

6. A field trial was also conducted in which three gradations of soil temperature were maintained during the season of tuber development. Of these the highest, approximately 25° C., proved to be most conducive to scab development.

7. Field observations seem in general to accord with the results obtained by experiments. They indicate that potato scab is comparatively more prevalent in regions having high summer temperatures than in those of lower temperature, and also that in the same district in Wisconsin the disease development is greater during hot than during cool summers. It is to be noted, however, that such observational data is relatively meager and is to be considered only as suggestive. Attention should be directed to this question by other observers.

8. As bearing more definitely upon this matter it is found that in the leading Wisconsin potato districts the mean temperatures

for July and August during the hottest midsummers approximate those found in our experiments to be the optimum for scab development.

9. Examinations of the scabby tubers from the controlled temperature experiments as well as of samples from commercial sources show that the scab lesions tend to be segregated upon the "stem end" portion of the tuber.

10. The evidence from the soil temperature tank series shows that such segregation is less evident at or near the optimum temperature for scab development and more apparent at the extreme temperatures.

11. The influence of temperature upon the development of the disease must obviously bear a relation on the one hand to effects upon the parasite and on the other hand to effects upon the host.

12. The available evidence indicates that the scab parasite as an independent organism is favored by relatively high temperatures, whereas the potato plant functions better in general at relatively low temperatures. It is, however, noteworthy that the influence of temperature upon the different potato organs is not uniform and that it varies also with the stage in their development.

13. The influence of temperature upon the prevalence of the parasite in the soil may be cumulative from season to season whereas the influence upon the host is immediate and temporary.

14. The immediate relation of temperature to the development of scab seems to be more closely correlated with its influence upon potato tuber development than with that upon the growth of the parasite.

15. It seems evident that a satisfactory interpretation of the relation of soil temperature to the development of potato scab must await on the one hand more critical study of its relation to tuber development and on the other hand a fuller knowledge of the details as to tuber infection and subsequent scab development.

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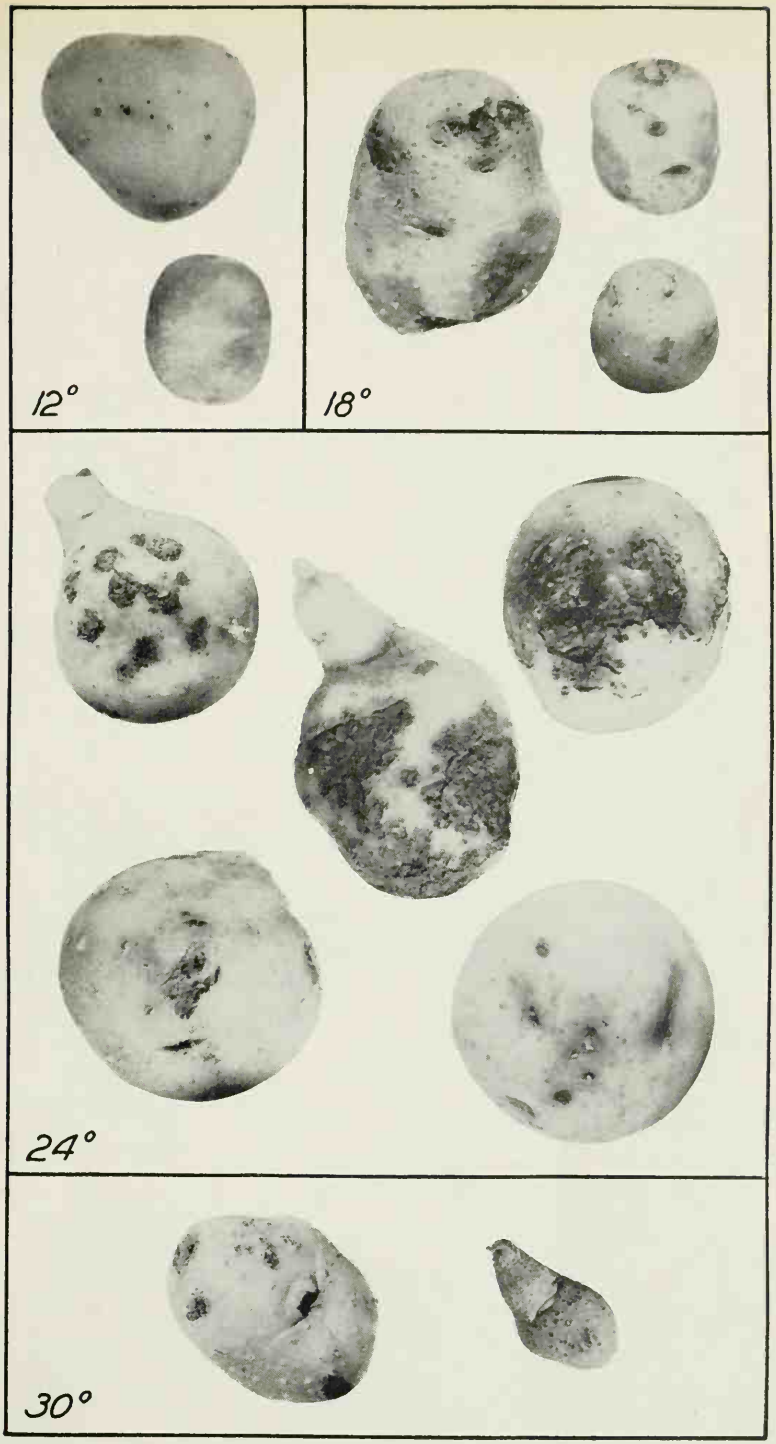

PLATE I. PART OF THE TUBERS FROM EXPERIMENT I SHOWING THE INFLUENCE OF SOIL TEMPERATURE ON THE DEVELOPMENT OF POTATO SCAB.

Note the severity of the infection on the tubers grown in the soil held near 24° C. and the tendency towards tuber elongation and the development of pear-shaped tubers at the higher temperatures.

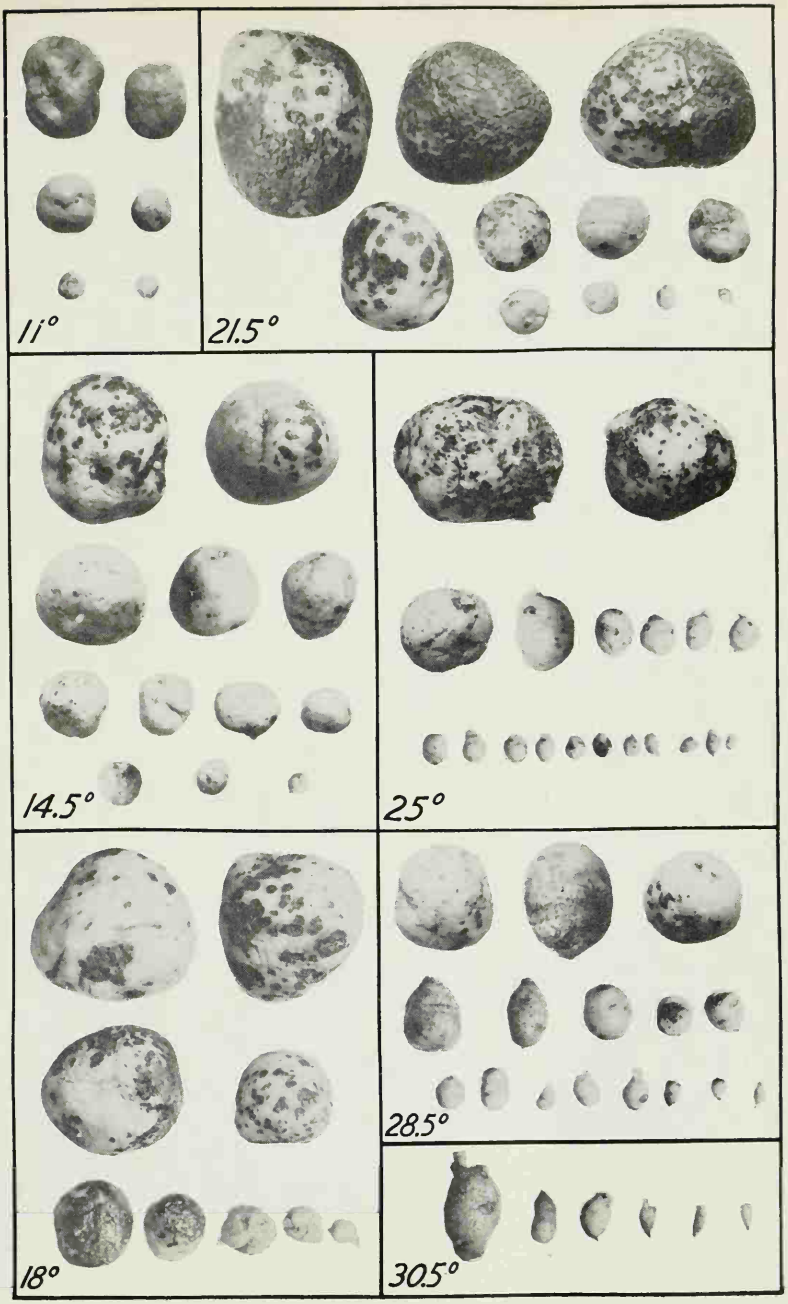

PLATE II. A REPRESENTATIVE ONE-FIFTH OF THE INOCULATED TUBER POPULATION FROM EXPERIMENT V.

The greatest amount of scab is shown on the tubers grown at 21° C. (soil temperature). Note the tendency for tubers to elongate at the high temperatures and to thicken transversely at the lowest temperatures.

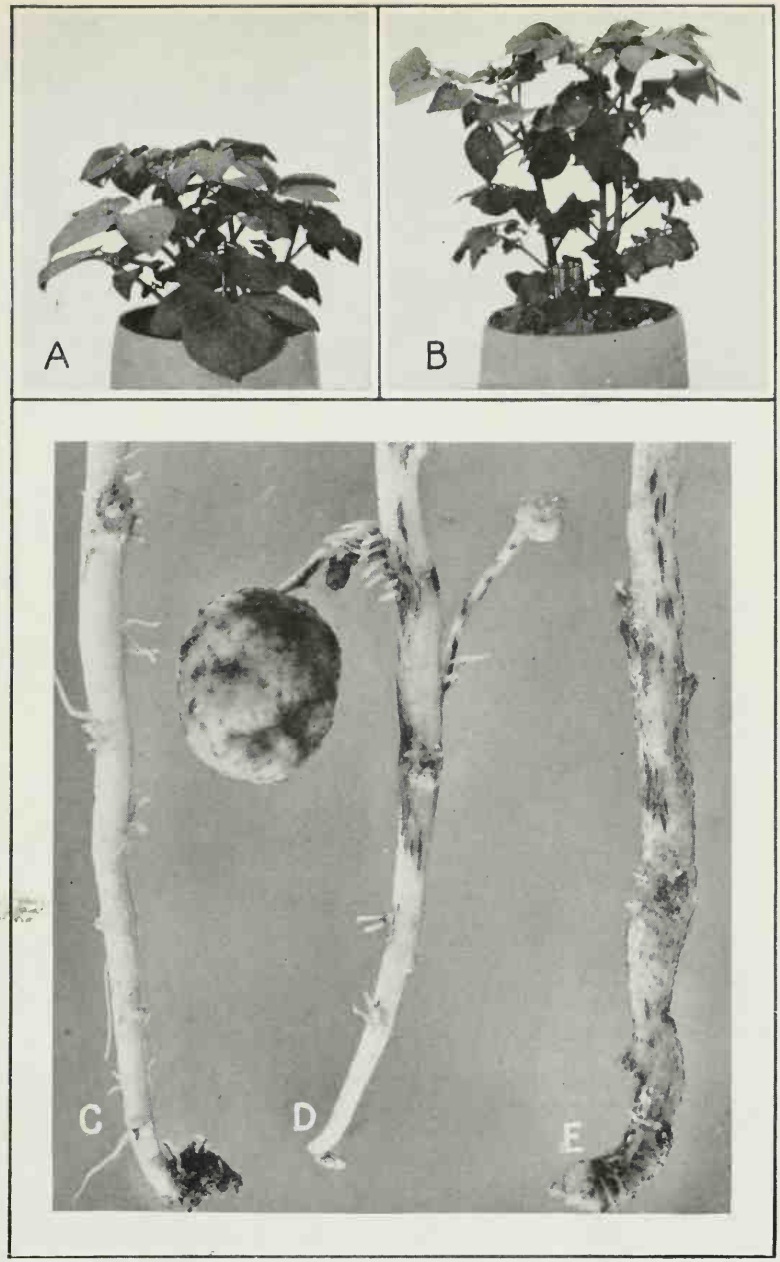

PLATE III. EFFECT OF TEMPERATURE ON PLANTS.

A. A typical plant grown at a soil temperature of 15° C. Note the low stocky development and the wide rounded leaflets.

B. A typical plant grown at a soil temperature of 27° C. Note the relative elongation of leaflets, some approaching the lanceolate form, also the elongation of stems and the more upright habit of growth.

C. An underground stem grown in uninfested soil. Note that this stem is free from any evidence of scab lesions.

D. and E. Underground stems, stolons and tubers grown in disinfected soil, which was infested with a pure culture of *A. scabies* previous to planting the seed. Note the scab lesions on the stems and stolons as well as on the tubers. Stem and stolon lesions seem to originate in the lenticels.

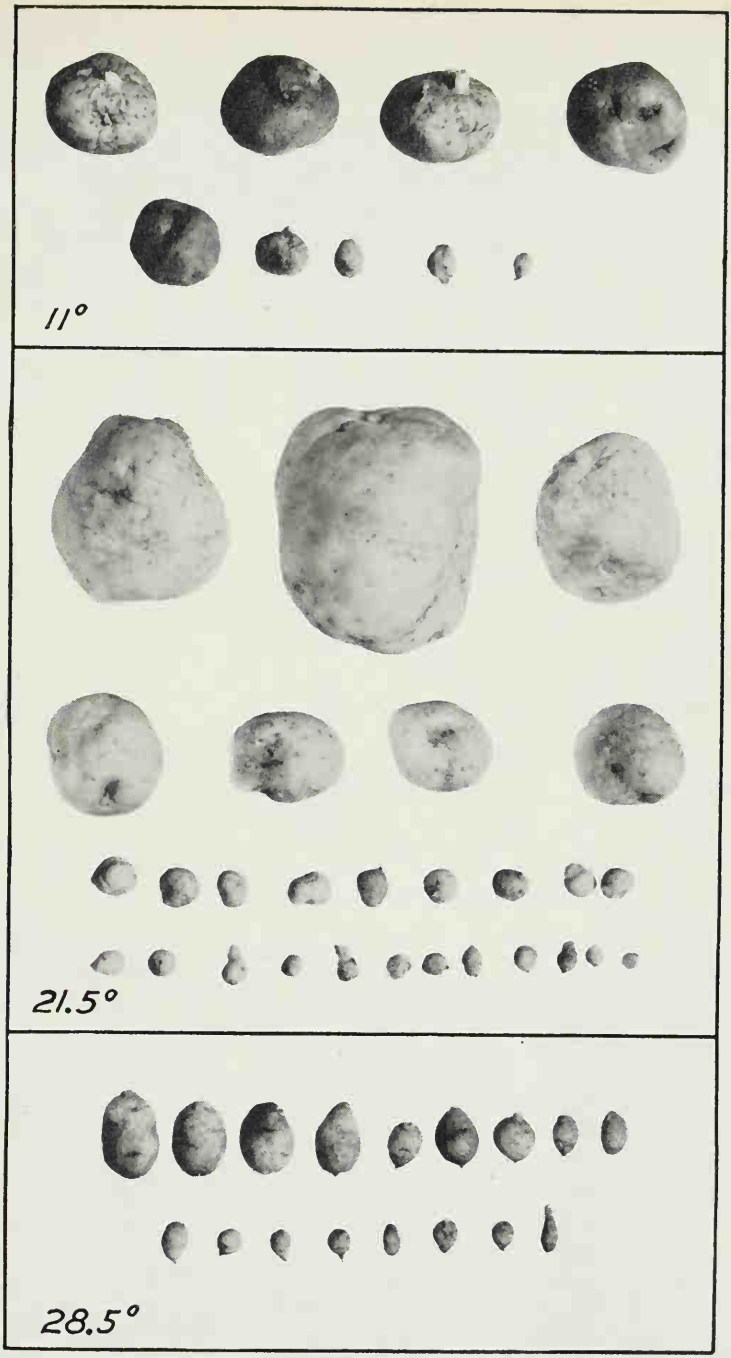

PLATE IV. A REPRESENTATIVE ONE-FIFTH OF THE TUBERS FROM THE UNINOCULATED OR CONTROL POTS FROM REPRESENTATIVE TEMPERATURES IN EXPERIMENT V.

All the control tubers were scab free. The influence of soil temperature on tuber shape is shown in this series by the elongated tubers at the high and the short tubers at the low temperatures. The dark pigment development is shown in the tubers produced at the extreme temperatures as is the case in Plate V. This darkening takes place after the tubers are taken from the soil and continues in the preserving fluid (formalin or alcohol) until the tubers produced at the lowest temperatures become jet black.

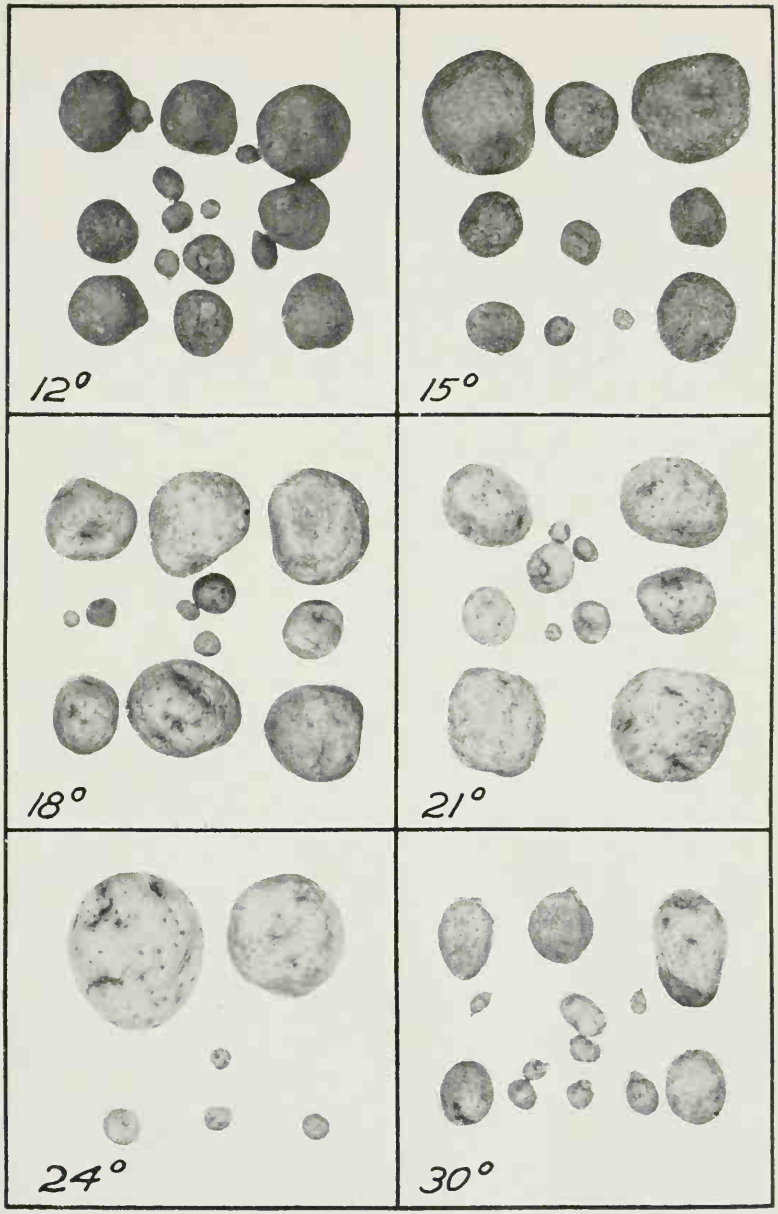

PLATE V. ALL OF THE UNINOCULATED CONTROL TUBERS FROM EXPERIMENT III SHOWING THE INFLUENCE OF SOIL TEMPERATURE ON THE DISCOLORATION OF TUBERS.

This material was photographed five months after removal from the soil, during which period it was preserved in 70 per cent grain alcohol. These results indicate a difference in the chemical nature of the tubers produced at the various soil temperatures. Such a difference may be significant in the final interpretation of the results obtained with scab at the various soil temperatures. All of these tubers were free from scab.

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Anthracnose of Cane Fruits and Its Control on Black Raspberries in Wisconsin

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Contents

Anthracnose of Cane Fruits and Its Control on Black Raspberries in Wisconsin[1]

A SURVEY of the cane fruit industry of Wisconsin by the writer (1920) in 1919 showed that anthracnose was one of the chief factors responsible for the drastic decline of the black raspberry acreage of the state in the last decade. Consequently, it was deemed advisable to make a careful study of this disease, with special reference to control measures. Field and laboratory experiments were conducted at Madison, Wisconsin, in the period of 1920-23.

The results of the writer's studies as they relate to history and geographic distribution of the disease, pathological histology, taxonomy, and morphology confirm those reported by Burkholder (1917). Burkholder's account of these subjects is so satisfactory that it appears unnecessary to treat them in this publication.

THE DISEASE

In the United States the disease caused by the fungus *Plectodiscella veneta* Burk. (*Gleosporium venetum* Speg.) appears to be widespread throughout the north and also in hilly southern regions, coinciding with the ranges of its hosts. It is generally distributed on the following hosts in Wisconsin as shown by the writer (1920): red raspberry (*Rubus idaeus* var. *aculeatissimus* (Mey.) Regel and Tiling), black raspberry (*Rubus occidentalis* L.), purple-cane raspberry (*Rubus neglectus* Peck), and blackberry (*Rubus* sp.). The relationships of the pathogens causing anthracnose on the above named hosts have not been definitely determined by cross inoculations, except as reported by Burkholder (1917) that the organism isolated from purple-cane raspberry through inoculations produced infection on black and red raspberry.

Description

The symptoms manifested on the various hosts of *P. veneta* are somewhat similar, although they vary in the color, shape and size of the lesions produced, depending on the host and the severity of the attack. Canes, leaves, petioles, peduncles, pedicels and fruits may be attacked, although the symptoms on the canes are usually the most noticeable. Descriptions here given refer to symptoms on Cumberland black raspberry unless otherwise noted.

On canes. Elliptical to circular greenish-brown lesions one-half to one millimeter in diameter usually appear on the young shoots in early spring when the latter are eight to ten inches high. These lesions are slightly sunken, with a stromatic development of the fungus in the center, which is somewhat darker and raised. Under binoculars the

[1] The writer wishes to express his indebtedness to Dr. G. W. Keitt, of the University of Wisconsin, under whose direction the work was performed.

affected tissue has a slightly water-soaked appearance. The lesions enlarge slowly and the centers become a pale buff to white, while the advancing margin is raised and reddish-brown to purple in color. Mature lesions (Plate I) are circular to oval and seldom become more than one centimeter in diameter, although they often become confluent, making large irregular patches that may encircle the cane. In cases of severe attack, the canes may crack longitudinally (Plate I, B). These cracks, usually small, may split the cane to the pith for a distance of two to three inches. The lateral branches often become seriously infected. The resultant lesions are similar to those on the canes, but smaller, and often cause the death of the young branches.

On leaves. The first spotting of leaves in the spring appears about the same time as that on the canes, although not so abundantly. The lesions first appear as yellow or straw-colored oval to irregular spots one-half to one millimeter in diameter. The center of the lesion is raised and brownish, while under binoculars the veins of the leaf at the outer edge of the lesion are slightly purple. The mature lesions are one to two millimeters in diameter with light colored centers and purple margins. These spots may drop out, giving the leaf a ragged or "shot-hole" appearance. In cases of severe infection of red raspberries, the leaves may turn yellow and drop. On the leaves the symptoms of this disease are often confused with those of common leaf spot (*Mycosphaerella rubi* Roark). The Mycosphaerella leaf spot lesions differ from those of anthracnose in being irregular in outline and somewhat larger, with minute black pycnidia usually present.

On petioles, peduncles and pedicels. Anthacnose has been found commonly on these plant parts. The lesions produced are similar to those on the canes, although smaller and often without the purple margin. On the peduncles and pedicels especially, they may coalesce into white scab-like patches (Plate II) that cause these parts to become brittle. These white patches often retard growth on one side of the attacked part, causing it to curl and crack.

On fruit. Infection occurs less frequently on the fruit than on the other parts of the host. On one occasion the writer observed fruit infection on the variety Plum Farmer in Wisconsin. One or several drupelets become brown and sunken. Frequently the whole fruit becomes brown, dry and woody, while the healthy berries are still green.

Economic Importance

Anthracnose is one of the most serious diseases of black raspberry and blackberry, although it seldom causes much injury to red raspberry. Burrill (1882) cites an instance of a plantation that had yielded a profit of $400.00 per acre, on which one attack of this disease reduced the proceeds so that expenses were not met. Scribner (1888) estimates the losses in southern Missouri due to anthracnose on black raspberries at 10 to 12 per cent. Burkholder (1917) states that in certain localities in New York state growers have been obliged to discontinue berry growing due to anthracnose, and that it is evident

that anthracnose is correlated with reduction of yields. Anderson (1920) states that "Anthracnose has entirely eliminated the growing of raspberries in some sections of Illinois, and many growers are compelled to renew their patches after two years of bearing." He also estimates that in Illinois in 1908 the loss from anthracnose was 50 per cent of the crop, and that 25 per cent of the berry crop is lost there annually because of this disease. In a survey of Wisconsin the writer (1920) found that anthracnose was one of the most important diseases of cane fruits, and was found wherever raspberries were grown, although it was of very little importance on blackberries and purple-cane raspberries. Red raspberries usually show a light spotting of the canes but the writer has not noted important anthracnose injury on red varieties in Wisconsin except in the vicinity of Eau Claire. In this district in 1919 there was considerable spotting of the leaves, which caused yellowing and dropping of the foliage. The disease is most important in the state on black raspberries and, in association with crown gall injury, it is a limiting factor in the black raspberry industry.

The disease affects the canes and leaves in the first season of growth, thereby weakening the plant and causing a decrease in fruit yield the ensuing year. The diseased canes are also more subject to winter injury than healthy ones. A very important injury in Wisconsin is caused by the lesions on peduncles and pedicels. Abundant infection on these host parts causes the fruit to be small or to dry up before maturity (Plate II).

In order to obtain data on the decrease in fruit yield due to anthracnose the writer made counts and weighed the fruit harvested from sprayed and unsprayed plots of Cumberland raspberries in 1921. The sprayed plot, consisting of 24 plants, had received two applications of lime-sulfur with gelatin as a spreader, as outlined on later pages. The disease had been very satisfactorily controlled on this plot the previous season, while the unsprayed plot, consisting of 12 plants in the same planting, had never received any spray treatment and the plants were abundantly infected with the disease. A summary of the data obtained is presented in Table I.

TABLE I.—COMPARISON OF FRUIT YIELDS OF SPRAYED AND UNSPRAYED CUMBERLAND RASPBERRY PLANTS, H. FISCHER PLANTING, MADISON, WIS., 1921

Plot No.	Treatment[a]	Average number of berries		Average weight of berries per plant
		Per plant	Per pint	
		No.	No.	Pounds
1	Unsprayed	143	227	0.42
3	L-S. +gelatin, 1, 2	217	239	0.62

[a] L—S=liquid lime-sulfur (1) delayed-dormant spray, 1-10, (2) second application of spray about one week before blooming, 1-40. One-half pound of gelatin was added to each 100 gallons of spray.

These data show that the sprayed plants on which the disease had been controlled satisfactorily for two consecutive years produced about

32 per cent more fruit by weight than abundantly infected plants that had never received any treatment for the control of the disease. The loss caused by the disease was very noticeable during the season of 1921, due to the fact that the disease was very severe and was followed by a long dry period prior to and during harvest. The average number of berries per pint was slightly higher on the sprayed plants than on the unsprayed since the smaller berries of the latter part of the season on the unsprayed plants dried up and were not harvested, while all the small ones ripened on the sprayed plants. For a comparison of the control of the disease obtained with various treatments during the season of 1921 see Table VI. Photographs taken at the time of harvest in 1921 show the condition of fruiting branches which had been sprayed as compared with that of unsprayed fruiting branches seriously injured by anthracnose (Plate VIII).

THE CAUSAL ORGANISM

Cultural Studies

Considerable difficulty has been experienced by investigators in obtaining pure cultures of the anthracnose organism. Stoneman (1898) states that the fungus "....does not adapt itself readily to artificial culture." The growth of the organism is so slow that contamination is likely to occur, but it is possible to obtain pure cultures by placing fragments of diseased tissue in poured agar plates. The exterior of the tissue from which the isolation is to be made is best sterilized by being dipped into 100 per cent alcohol and flamed. Fragments of tissue may then be removed from below the surface with a sterilized scalpel. On a 15 per cent dextrose-potato agar a straw-colored growth may be detected with a hand lens at the side of the fragment of tissues in five to seven days and may be transferred to an agar slant. The easiest method of isolation, however, is to place on the inside of the lid of a Petri dish fragments of a cane lesion bearing ascocarps. At Madison, Wisconsin, it has been possible to obtain mature ascocarps in the field from early March through June. If the cane tissue is moistened with water the spores are shot onto the agar in the lower part of the Petri dish. Germination of single ascospores may be watched and the resultant growth transferred to an agar slant in a test tube by the method described by Keitt (1915).

The growth after 14 days on a 15 per cent dextrose-potato agar is light russet-vinaceous to maroon, with a reddish discoloration of the medium. There is very little aerial mycelium produced in the young cultures. The colonies are formed by a piling up of cells that have a glistening appearance. As the cultures become older, however, fine aerial hyphae are formed over the compact growth. In cultures that are a month old these hyphae give a white, downy appearance to the maroon mass of cells underneath. Conidia are seldom produced in culture. However, a sudden increase in humidity usually stimulates their production.

The writer has been able to produce conidia by transferring culture fragments from dextrose-potato agar to the side of sterile sweet

clover stems in tubes, the stems resting on a small amount of absorbent cotton and in an abundance of water. After three days this culture may be removed to a tube of sterile distilled water, in which the conidia drop off readily. In order to obtain an abundance of conidia for inoculation work it was found advisable to pour a spore suspension on poured agar plates. After ten days large pieces of agar bearing the fungus may be transferred to a sterile glass slide in a sterile moist chamber, consisting of a Petri dish lined with moistened filter paper. After three days the cultures may be removed to sterile distilled water and the conidia shaken from the fungal growth.

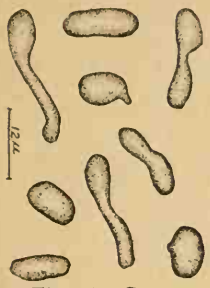

Fig. 1. Camera lucida drawing of germinating conidia of *P. veneta* after 16 hours in sterile distilled water at 24° C.

A study has been made of the relation of temperature to the growth of the organism on dextrose-potato agar. The most rapid growth was obtained at 22° to 26° C. while no growth occurred at 10° or at 32° C. Plate III, A shows the growth that was made in seven days at constant temperatures ranging from 11° to 32° C. The platings were made from a suspension of conidia in water, one loop of the suspension being removed to the center of each Petri dish, into which a 5 per cent dextrose-potato agar had been poured.

Germination Studies

Conidia germinate readily in sterile distilled water or on dextrose-potato agar or "water agar" (2 per cent agar in water). In sterile distilled water on slides in moist chambers at 24° C. the conidia become twice their original size in 12 to 24 hours and a few may become one-septate, or produce short germ tubes (Fig. 1). During the next 24 hours elongation occurs and three or four septa and possibly a small amount of branching may be observed. Conidia are budded off from any of the cells, most abundantly, however, from those at the ends (Fig. 2). Further growth takes place with profuse branching and piling up of cells, forming a stromatic mass about 50 microns in diameter after 96 hours. From this mass of cells filaments radiate for 25 to 50 microns. There is seldom any further growth in sterile water. The germination on dextrose-potato agar is somewhat similar except that true germ tubes and conidia are seldom produced and that there is a greater tendency towards the massing of cells. After 96 hours at 24° C. the colonies on this medium have an average diameter of about 200 microns.

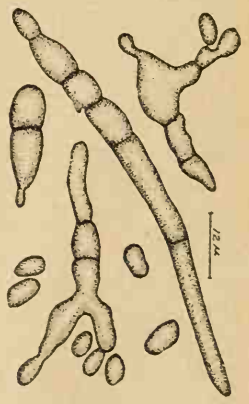

Fig. 2. Camera lucidia drawing of germinated conidia of *P. veneta* showing the growth produced after 44 hours in sterile distilled water at 24 C. and the production of secondary conidia.

Experiments on conidial germination have been conducted at controlled temperatures in sterile distilled water and on dextrose-potato agar and "water agar." Six series have been run on dextrose-potato agar, five series in sterile distilled water, and two series on "water agar," at the following temperatures: 4, 8, 11, 15, 17, 19, 22, 24, 26, 30, 32, and 34° C. No germination has been observed on any medium at temperatures below 11° C. and only slow germination with slight growth at 15° C. The optimum for germination and growth was found to lie between 22° and 26° C. Germination takes place readily at 30° while no germination has been observed at either 32° or 34° C.

The ascospores germinate readily in sterile distilled water and on dextrose-potato agar. In sterile water the spore becomes slightly swollen and from five to seven conidia are usually budded off within 16 hours. These conidia have not been observed to germinate in sterile water. On dextrose-potato agar the five to seven conidia are budded off and produce germ tubes 20 to 30 microns long within 24 hours (Fig. 3). Within 72 hours the germ tubes become branched and produce masses of cells, making a colony about 50 microns in diameter with numerous strands of branching mycelium growing for a distance of about 35 microns from the outer edge of the stromatic mass.

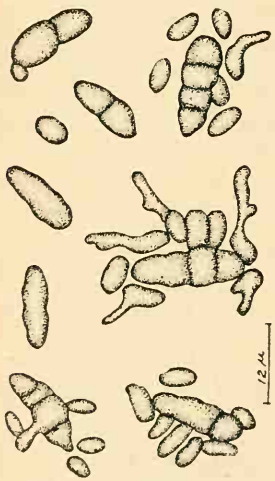

Fig. 3. Camera lucida drawing of germinating ascospores of *P. veneta* showing production and germination of secondary conidia.

Modes of germination of the spores of this organism are very variable, depending on such factors as temperature and media. The writer projects making this problem the subject of a future publication.

Pathogenicity

Lawrence (1910) inoculated fruit of blackberries with conidia from leaves and canes and reports obtaining typical lesions after an incubation period of 15 to 48 hours. Burkholder (1917) inoculated young shoots with a water suspension of conidia from lesions on canes and from pure cultures. He obtained infection in 18 out of 56 inoculation trials, with an inoculation period of three to seven days.

The writer made inoculation experiments on Cumberland raspberry plants at Sturgeon Bay, Wisconsin. The apical foot of each young cane was placed in a bag made of partially water-proofed, translucent "glassine" paper for seven days immediately preceding the inoculation, in order to preclude possibility of natural infection. At the time of inoculation the bags were removed and the young canes atomized with sterile distilled water (controls) or a water suspension of conidia from pure cultures of a single ascospore isolation from a black raspberry cane lesion. The

bags were replaced and the canes were kept moist by hanging inside of the bags Erlenmeyer flasks of water from which cheese cloth wicks were wound around the canes. The bags and wicks were removed five days after the inoculations were made, at which time no disease was apparent on the young bagged parts. Observations were made every day after the wicks were removed and the number of resultant lesions recorded. The results of these inoculations (Table II) show an incubation period of six to nine days.

TABLE II.—RESULTS OF INOCULATIONS MADE WITH *P. veneta* ON CUMBERLAND RASPBERRY CANES AT STURGEON BAY, WIS., JUNE 6, 1921

Inoculation No.	Inoculum	Number of lesions observed on stated dates				
		June 12	June 13	June 14	June 15	June 16
1A	Control	0	0	0	0	0
1B	Spore suspension	0	8	8	8	8
2	Spore suspension	0	0	8	16	16
3	Control	0	0	0	0	0
4	Spore suspension	12	17	17	17	17
5	Spore suspension	0	2	2	2	2
6	Control	0	0	0	0	0

Black and red raspberry plants have been grown in the greenhouse in early spring and attempts to inoculate them have met with little success. Eight series of such inoculations have been made with conidial suspensions from cultures obtained through single ascospore isolations. Only one of these series gave positive results. Life history studies made during the seasons of 1921 and 1922 indicate that only the young growing canes are susceptible to the disease (Tables V and VII). The plants which were grown in the greenhouse during winter and early spring did not produce a succulent type of growth, which probably accounts for the lack of positive results from inoculation experiments with these plants.

LIFE HISTORY

Seasonal Development of Disease

The disease first appears on the young growing canes and leaves in early spring, usually when the canes are eight to ten inches high. At Madison, Wisconsin, the first lesions have been observed on the following dates during the four years of observation: May 20, 1919; May 13, 1920; May 15, 1921; and May 20, 1922. The lesions on the canes, leaves, laterals and fruiting branches continue to increase in number on the young growing tissue throughout early summer. There appears to be little or no increase in disease after the middle of July as is shown in data obtained during the seasons of 1921 and 1922 (Plates V and VI).

Production of Spores

The immature ascocarps are first observed during the latter part of August. Some of the ascocarps are mature at Madison, Wisconsin, as

early as March 1, as the writer has been able to cause the discharge of mature ascospores from freshly collected cane lesions at this time of the year. The asci and ascospores, however, continue to mature through the spring and early summer. Conidia are produced during the spring on the old cane lesions and abundant production of conidia follows the development of lesions on the new growth during spring and summer. On the fruiting canes the fungus probably dies after the production of conidia in the spring, as the writer has been unable to obtain conidia or make cultures from the lesions on these canes through the summer.

Source of Inoculum in Nature

The primary sources of natural inoculum are the ascospores, which are ejected forcibly from the asci, and the conidia from the overwintered lesions on the canes. The ascospores continue to be a source of inoculum through the spring and early summer. Burkholder (1917) reports that the ascocarps are very rare and probably do not play an important part in the dissemination of the disease. The writer has, however, observed an abundance of ascocarps (Plate III, B) on black and red raspberries in Wisconsin and considers the ascigerous stage an important factor in the overwintering of the disease and its spread in the spring under Wisconsin climatic conditions. The conidia produced in the lesions (Plate III, C) on the current year's growth form the source of secondary infection through the spring and summer.

Experiments have been conducted at Packwaukee, Wisconsin, in order to obtain more definite information relative to the spread of the disease. Three rows, each 250 feet long, of Cumberland raspberry plants were set out April 15, 1920. The rows were 12 feet apart and the plants were spaced five feet apart in the rows. The planting was one-half mile from any other raspberry planting, on land where grain had been grown for 15 years. A careful survey of the surrounding country showed no wild hosts of the disease within one-half mile of the new planting. These plants were obtained from layered tips that were removed from the vicinity of the old plants one month before the appearance of the disease in the spring, and before the new shoots had appeared above the ground. Care was taken to remove all of the old cane stubs from the new plants, in order to avoid carrying any source of inoculum to the new planting. All of the soil was removed from the young plants by washing, after which they were dipped into mercuric chloride solution, 1-1000, and then rinsed before they were planted. On April 14, 1921, one year after the planting was made, these plants were entirely free from anthracnose lesions.

Observations made April 13, 1922, two years after the planting was made, showed an abundance of disease on these plants. Of the 125 plants that were living, 99 were diseased, 13 of them being severely infected. The remaining 26 plants, which were not infected with anthracnose, were scattered among the diseased ones.

The ascigerous stage of the fungus had been found abundantly on the diseased canes in the old planting, one-half mile from the ex-

perimental planting. Ascospores carried by winds that blew over the old planting toward the new one were probably the source of infection for the new planting.

Conidia are chiefly water borne, as emphasized by Burkholder (1917). The writer has endeavored to blow the conidia free from the conidiophores with air from an aspirator, but with little success. However, the conidia drop off readily when the stromatic mass is placed in water. Consequently, when the fungus mass was atomized with water an abundance of spores was washed off.

CONTROL MEASURES

Sanitation

Most writers have emphasized the importance of keeping the plantation free from badly diseased canes. Longyear (1904), Jackson (1913), Cook (1918) and Swartwout (1921) recommend cutting out all old canes and the most severely diseased new ones soon after harvest. This is a good cultural practice but it is of little value in checking the disease during the current season, since the writer's observations show that little or no infection takes place after harvest. When thinning out the canes in the spring, it is advisable to prune out the more severely diseased ones, thereby reducing the source of early inoculum.

Good cultural practices during the growing season are advisable in order to remove weeds from the rows. Weeds and compact growth of canes interfere with air drainage, and facilitate the collection of moisture, which is favorable to the spread of the disease.

Six to twelve inches of the old canes are left on black raspberry nursery stock by nurserymen to facilitate handling. The disease is often abundant on these old cane stubs, and is therefore disseminated to the new plantings. Before nursery stock is planted these old canes should be carefully removed. Young plants obtained from the vicinity of old plants in the spring should be removed to the new planting before they are four to six inches high, since infection of the young plants usually occurs soon after they have attained this much growth.

There is little possibility that the fungus lives over on fragments of plants on the ground. Observations by Burkholder (1917) and by the writer show that on the old fruiting canes the organism probably dies after the conidia have been produced in the spring. Therefore, it would appear that new plantings would not necessarily have to be made on land formerly free from the disease. However, it would be advisable to make plantings on new soil, because of the prevalence of the crown gall organism in soil previously used for cane fruit culture.

Spraying

Spraying for the control of anthracnose has been recommended by many writers, but most of the numerous attempts to control the disease in this manner have given questionable or conflicting results.

Burkholder (1917) reviews the earlier literature on spraying and reports that a dormant application of lime-sulfur, 1-8, proved to be of no benefit in the control of raspberry anthracnose. After considerable experimental work he states that: "More data relating to the effect of diseased canes on the yield of fruit are needed, and until they are obtained no conclusive proofs can be furnished that spraying to combat the anthracnose of raspberry is a profitable practice."

Dutton (1918) reports control of the disease from three applications of lime-sulfur before the blooming period, and further reports that in 1915 one dormant spray of lime-sulfur, 1-20, gave good control.

There is considerable controversy as to the possibility of spray injury from the use of lime-sulfur and Bordeaux mixture. Most writers agree that raspberry plants are very susceptible to spray injury. Without doubt, a considerable portion of the difference in the amount of injury reported as occurring in spraying experiments has been due to the fact that in their reports most workers have not differentiated between red raspberries, black raspberries, purple-cane raspberries and blackberries. There is certainly a difference in susceptibility to spray injury among these different kinds of cane fruits.

Goff (1891) experimented with ammoniacal copper carbonate, and with mixtures of ammoniacal copper carbonate and copper sulfate. These had an injurious effect on the foliage of Cuthbert, Tyler and Gregg varieties, and Bordeaux mixture, 4-6-50, caused great injury to the foliage. He concludes that the foliage of the raspberry is very delicate and can not endure applications of a corrosive nature, and that the foliage of the blackberry, though more resistant than that of the raspberry, is more susceptible to injury than that of the apple.

On black raspberries the foliage of old canes and fruiting branches is more susceptible to injury than that of young shoots, and injury is likely to occur in case either Bordeaux mixture or lime-sulfur is applied to the plants in hot, dry weather. From observations made in Wisconsin, foliage injury is to be expected if lime-sulfur or Bordeaux mixture is applied to the plants after blooming. The writer has not observed injury to black raspberry plants from summer strength of Bordeaux mixture or lime-sulfur applications before the blooming period of the plants. The dormant strength of these sprays, applied to the plants after the leaf buds on the old canes had opened in the early spring and only a few leaves had unfolded (Plate IV, A), occasioned no material injury to the plants in the experiments conducted by the writer.

In view of the conflicting evidence that has been presented regarding the effectiveness of spraying it was deemed advisable to carry on comprehensive spraying trials. Preliminary reports of the results of these investigations have been made by the writer (1922 and 1923). A summary of experimental treatments during the seasons of 1920, 1921 and 1922 appears in Table III.

TABLE III.—SUMMARY OF THE TREATMENT OF EXPERIMENTAL PLOTS OF CUMBERLAND RASPBERRY FOR THE CONTROL OF ANTHRACNOSE, H. FISCHER PLANTING, MADISON, WIS.

Plot No.	No. plants treated	Treatment of plots in stated years[a]		
		1920	1921	1922
1	12	Unsprayed	Unsprayed	Unsprayed
2	24	L–S.+glue, 1, 2,	L–S.+glue, 1, 2	L–S.+glue, 1, 2
3	24	L–S.+gelatin, 1, 2	L–S.+gelatin, 1, 2	L–S.+gelatin, 1, 2
4	24	L–S.+gelatin, 1	L–S.+gelatin, 1	L–S.+gelatin, 1
5	18	L–S.+glue, 1	L–S.+glue, 1	L–S.+glue, 1
5A	6	L–S.+glue, 1	L–S.+glue, 1	Unsprayed
6	24	L–S., 1	L–S., 1	L–S., 1
6A	24	L–S., 1	L–S., 1, 2	L–S., 1, 2
7	24	B.M.+cal–cas., 1	B.M.+cal–cas., 1	B.M.+cal–cas., 1
8	12	B.M.+cal–cas., 1, 2	B.M.+cal–cas., 1, 2	B.M.+cal–cas., 1, 2
9	12	B.M.+milk, 1	B.M.+milk, 1	B.M.+milk, 1
9A	12	B.M.+milk, 1	B.M.+milk, 1, 2	B.M.+milk, 1, 2
10	12	B.M.+gelatin, 1	B.M.+gelatin, 1	B.M.+gelatin, 1
11	12	B.M.+gelatin, 1, 2	B.M.+gelatin, 1, 2	B.M.+gelatin, 1, 2
12	24	B.M.+glue, 1	B.M.+glue, 1	B.M.+glue, 1
13	24	B.M.+glue, 1, 2	B.M.+glue, 1, 2	B.M.+glue, 1, 2
14	12	B.M., 1	B.M., 1	B.M., 1
14A	12	B.M., 1	B.M., 1, 2	B.M., 1, 2
15	20	----------	L–S., 1, 2	L–S., 1, 2
15A	20	----------	L–S., 1	L–S., 1
16	24	----------	L–S.+glue, 1, 2	L–S.+glue, 1, 2
16A	24	----------	L–S.+glue, 1	L–S.+glue, 1
17	18	----------	L–S.+gelatin, 1, 2	L–S.+gelatin, 1, 2
17A	24	----------	L–S.+gelatin, 1	L–S.+gelatin, 1
17B	6	----------	L–S.+gelatin, 1, 2	Unsprayed
18	24	----------	L–S.+saponin, 1, 2	L–S.+saponin, 1, 2
18A	12	----------	L–S.+saponin, 1	L–S.+saponin, 1
18B	12	----------	L–S.+saponin, 2	L–S.+saponin, 2
19	24	----------	L–S.+gelatin, 2	L–S.+gelatin, 2
20	24	----------	B.M.+gelatin, 2	B.M.+gelatin, 2
21	12	----------	B.M., 1, 2	B.M., 1, 2
21A	12	----------	B.M., 1	B.M., 1
22	12	----------	B.M.+glue, 1, 2	B.M.+glue, 1, 2
22A	12	----------	B.M.+glue, 1	B.M.+glue, 1
23	12	----------	B.M.+gelatin, 1, 2	B.M.+gelatin, 1, 2
23A	12	----------	B.M.+gelatin, 1	B.M.+gelatin, 1
24	12	----------	B.M.+cal–cas., 1, 2	B.M.+cal–cas., 1, 2
24A	12	----------	B.M.+cal–cas., 1	B.M.+cal–cas., 1
25	8	----------	Scalecide, 1; B.M.+gelatin, 2	L–S.+gelatin, 1, 2, 3
25A	8	----------	Scalecide, 1	L—S.+gelatin, 1, 2, 3[b]
26	8	----------	Scalecide, 1; B.M.+gelatin, 2	B.M.+cal–cas., 1, 2, 3
27	8	----------	Scalecide, 1; L–S.+gelatin, 2	B.M.+cal–cas., 1, 2, 3[c]
28	48	----------	Unsprayed	Unsprayed
30	20	----------	----------	L–S., 1
31	20	----------	----------	L–S., 1, 2
32	24	----------	----------	L–S.+glue, 1
33	24	----------	----------	L–S.+glue, 1, 2
34	24	----------	----------	L–S.+gelatin, 1
35	24	----------	----------	L–S.+gelatin, 1, 2
36	24	----------	----------	L–S.+saponin, 1
37	24	----------	----------	L–S.+saponin, 1, 2
38	24	----------	----------	L–S.+cal–cas., 1
39	24	----------	----------	L–S.+cal–cas., 1, 2

[a] L–S. = liquid lime-sulfur. B.M. = Bordeaux mixture. Cal–cas. = calcium caseinate spreader.

Spray applications designated as: 1 = delayed-dormant, using lime-sulfur, 1-10, or Bordeaux mixture, 6-6-50; 2 = application about one week before blooming period, using lime-sulfur, 1-40, or Bordeaux mixture, 3-3-50; 3 = application one week after blooming, using lime-sulfur, 1-40, or Bordeaux mixture, 3-3-50, except as noted in footnotes following.

For discussion of spreaders see page 12.

[b] Lime-sulfur, 1-80, plus gelatin was used in application 3.

[c] Bordeaux mixture, 1 ½-1 ½-50, plus calcium caseinate was used in application 3.

Preparation of Sprays

Lime-sulfur. Commercial liquid lime-sulfur testing 33° Baumé was used in the experiments. The required amount of liquid was added to the water to make the strengths outlined in the summary of treatment (Table III).

Bordeaux mixture. Pound to gallon "stock solutions" of lime and copper sulfate were prepared. To make the Bordeaux mixture of the 6-6-50 formula, six gallons of the lime "solution" was diluted to 25 gallons, and six gallons of the copper sulfate solution was diluted to 25 gallons after which the two were mixed with agitation. Bordeaux mixtures of other formulae were made in a corresponding manner.

Spreaders with Lime-Sulfur

Gelatin. One-half pound of white gelatin was used to each 100 gallons of spray. The gelatin was placed in solution with a small amount of water aided by slight heating. This solution was added to the diluted spray mixture and agitated.

Glue. One pound of finely ground high grade glue was added to each 100 gallons of spray. The glue was placed in solution and added to the diluted spray mixture in the same manner as the gelatin.

Saponin. One ounce of soap tree bark was placed in one quart of water and boiled for 15 minutes. The liquid was strained and used at the rate of eight ounces to ten gallons of spray mixture.

Calcium caseinate. A proprietary preparation of casein and lime was used at the rate of one pound to each 100 gallons of diluted spray. The powdered material was added to the diluted spray mixture slowly, with agitation.

Spreaders with Bordeaux Mixture

Gelatin. Added as outlined above.

Glue. Added as outlined above.

Calcium caseinate. During the season of 1922 the proprietary preparation was used as outlined above. During the seasons of 1920 and 1921 this material was made as follows: 200 grams of powdered casein was mixed thoroughly with 480 grams of hydrated lime. The dry mixture was added to the spray, slowly and with agitation, at the rate of 150 grams to 25 gallons of the diluted spray mixture.

Milk. Milk was added to the diluted spray mixture at the rate of two gallons to 100 gallons of the spray, as recommended by Lecomte (1913).

Condition of Plots

The experimental plots were located in the H. Fischer planting near Madison, Wisconsin. In 1920 four rows of 78 plants each were selected in a four-year-old Cumberland raspberry planting. The plants were four feet apart in the rows and the rows five feet apart. Plots 1 to 14, with the number of plants shown in Table III, were arranged consecutively in

the four rows. During the seasons of 1921 and 1922 an adjacent planting of Cumberland raspberries was selected for additional plots, the planting being four years old in 1921 (Plate IV, B). The plots were square or rectangular and contained the number of plants shown in Table III. Previous to 1920 no spraying had been done for the control of the disease in the H. Fischer planting, which was heavily infected with anthracnose (Plate I, A).

EXPERIMENTS IN 1920

Treatment

A summary of treatment appears in Table III, and supplementary data follow.

The delayed-dormant spray was applied on April 26 to plots 2, 3, 4 and 6, but a heavy rain washed off most of the spray before it could dry and made it necessary to discontinue the work On April 29, a partly cloudy day, all plots were sprayed, including the ones that had been sprayed on April 26. A "Perfection" hand sprayer was used. Since there was no foliage on the canes at this time it was easy to cover them thoroughly with a low pressure. An average of one-half pint of spray per plant was used. The buds on the old canes were showing from one-quarter to one-half inch of green tissue with no leaves unfolded.

The second application of spray was made on May 26, a bright, clear day. A double-action pressure pump with a barrel attachment was used, and a pressure of 150 to 200 pounds was maintained on a single disc nozzle. An average of three-quarters of a pint of spray per plant was used. The plants were grown in the hill system and tied to stakes. The foliage was so dense that it was hard to cover the old canes thoroughly. Buds were forming on the fruiting branches and the new shoots were 12 to 15 inches high.

Results

Observations made May 4 showed no apparent injury from the dormant strength spray. Observations made May 26 showed very little infection on the plots. Primary infection occurred during the rain of May 10, appearing as lesions on May 13, although the infection was very light at this time.

In order to obtain comparative data on the effectiveness of the different spray applications, a count of the number of lesions on each of 20 canes and 20 fruiting branches per plot, chosen at random, was made on June 17 and again on July 13. A summary of this count work appears in Table IV. As the plants were heavily pruned in the latter part of July, it was impossible to obtain further data. However, very little infection occurred after the last counts had been made.

The results of the counts made on the canes and fruiting branches are discussed in accordance with the objects of the experiments.

TABLE IV.—RESULTS OF SPRAYING EXPERIMENTS FOR THE CONTROL OF ANTHRACNOSE ON CUMBERLAND RASPBERRIES, H. FISCHER PLANTING, MADISON, WISCONSIN, 1920[a]

Plot No.	Treatment[b]	Average number of lesions on stated dates											
		June 17th						July 13th					
		On canes by feet					On fruiting branches	On canes by feet					On fruiting branches
		1st[c] foot	2nd foot	3rd foot	4th foot	Total		1st[c] foot	2nd foot	3rd foot	4th foot	Total	
1	Unsprayed	44.6	31.2	11.6	0.4	87.8	26.8	32.5	38.7	59.7	27.3	159.2	25.6
2	L-S.+glue, 1, 2	0.4	0.0	0.0	0.0	0.4	0.0	0.7	1.0	3.2	1.3	6.2	0.9
3	L-S.+gelatin, 1, 2	0.4	0.0	0.0	0.0	0.4	0.0	0.3	0.5	3.4	0.9	5.1	0.5
4	L-S.+gelatin, 1	0.4	0.3	0.0	0.0	0.7	0.0	0.2	0.2	3.2	6.0	9.6	0.9
5	L-S.+glue, 1	2.3	3.5	0.0	0.0	5.8	0.2	3.0	5.1	30.5	19.2	57.8	3.5
6	L-S., 1	1.0	4.1	0.3	0.0	5.4	1.9	1.6	2.8	8.7	7.4	20.5	7.5
7	B.M.+cal-cas., 1	1.8	1.6	0.0	0.0	3.4	0.1	2.8	3.7	13.1	38.2	57.8	5.9
8	B.M.+cal-cas., 1, 2	3.6	0.8	0.0	0.0	4.4	0.0	2.6	1.9	8.6	15.2	28.3	2.2
9	B.M.+milk, 1	1.6	1.9	0.0	0.0	3.5	0.5	1.3	2.0	9.2	21.4	33.9	5.4
10	B.M.+gelatin, 1	2.6	2.6	0.0	0.0	5.2	0.7	1.2	2.7	11.7	15.9	31.5	2.5
11	B.M.+gelatin, 1, 2	2.8	0.6	0.0	0.0	3.4	0.4	1.2	1.1	4.6	11.6	18.5	1.2
12	B.M.+glue, 1	6.6	3.4	0.2	0.0	11.2	1.0	1.4	3.4	7.1	16.7	28.6	2.5
13	B.M.+ glue, 1, 2	2.9	0.3	0.0	0.0	3.2	0.3	2.4	1.8	2.5	9.8	16.5	1.6
14	B.M., 1	8.8	6.2	0.1	0.0	15.2	1.1	3.4	5.7	15.2	21.5	45.8	6.1

[a] A summary of counts made on twenty canes and twenty fruiting branches per plot, chosen at random.
[b] See Table III for details of treatment. Spraying dates: 1 (delayed-dormant), April 29; 2, May 26.
[c] Basal foot of cane.

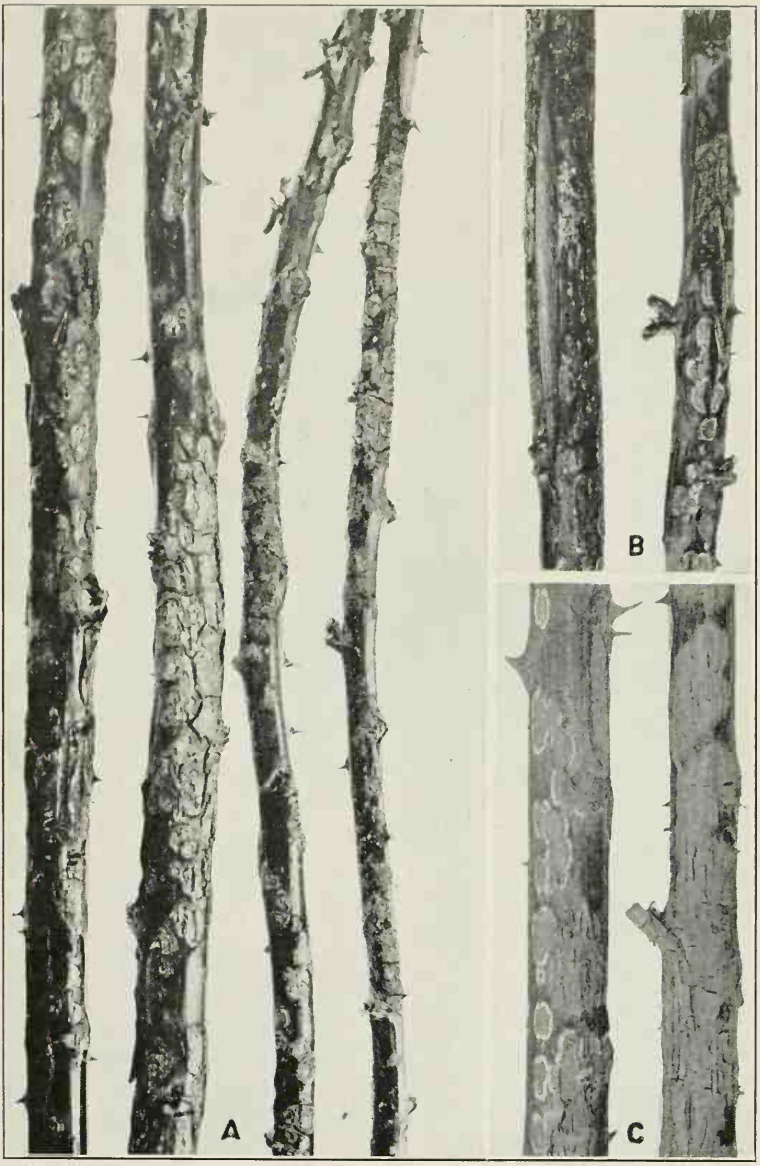

Plate I

A.—Anthracnose lesions on two-year-old canes of Cumberland raspberry from the H. Fischer planting, Madison, Wisconsin, showing the severity of the disease when the control experiments were begun in 1920.

B.—Longitudinal cracking of Cumberland canes following severe anthracnose infection.

C.—Anthracnose lesions on a Plum Farmer raspberry cane.

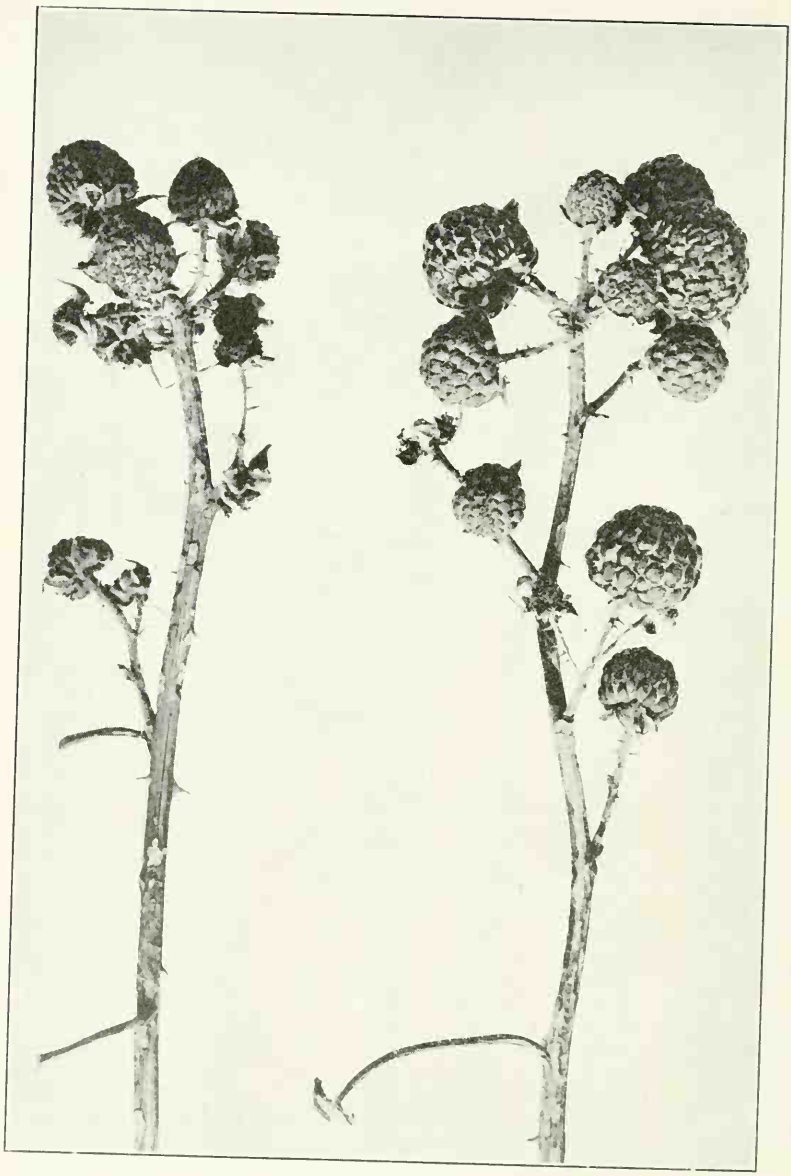

Plate II

Cumberland raspberry fruiting branches from unsprayed plants, July 7, 1921. The abundance of anthracnose lesions on peduncles and pedicels caused a reduced yield of fruit.

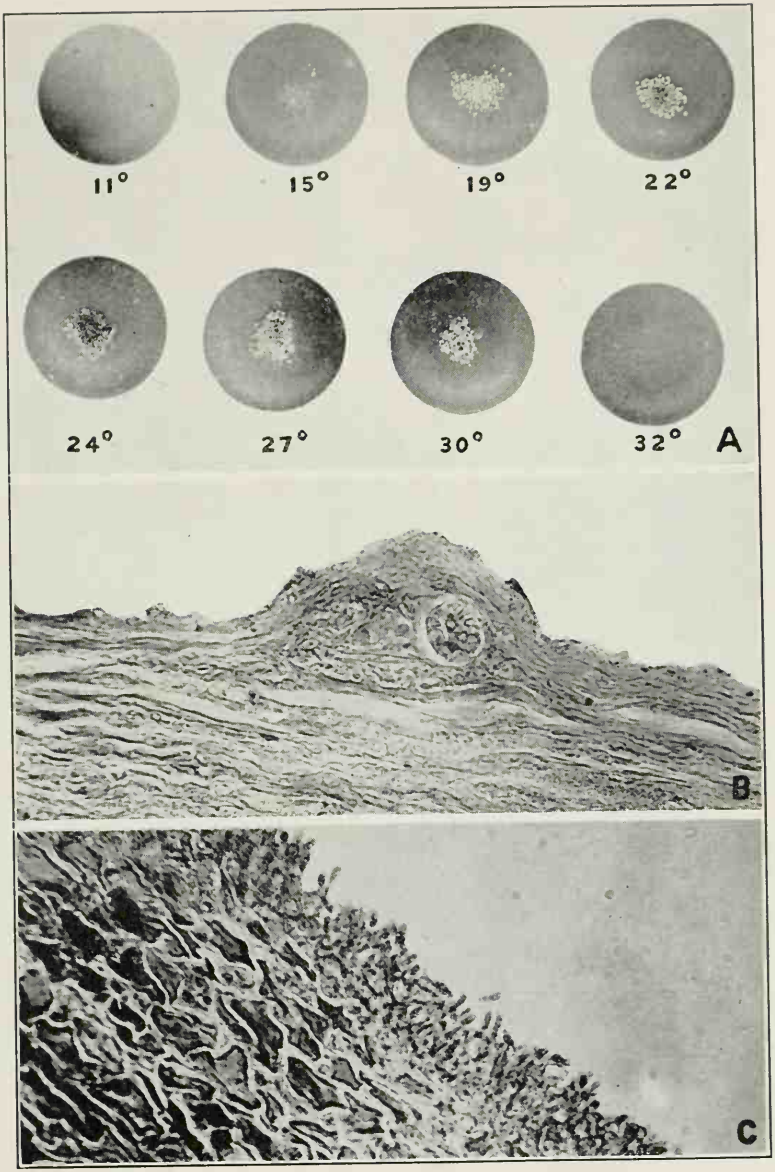

Plate III

A.—Seven-day-old growth of *P. veneta* on dextrose-potato agar (x .65). Cultures made from a conidial suspension and held at various constant temperatures (Centigrade).

B.—Photomicrograph of a cross section of an ascocarp of *P. veneta* from a cane lesion, showing one globular ascus with ascospores.

C.—Photomicrograph of a cross section of a lesion on a young cane, showing collapsed host tissue and the production of conidia.

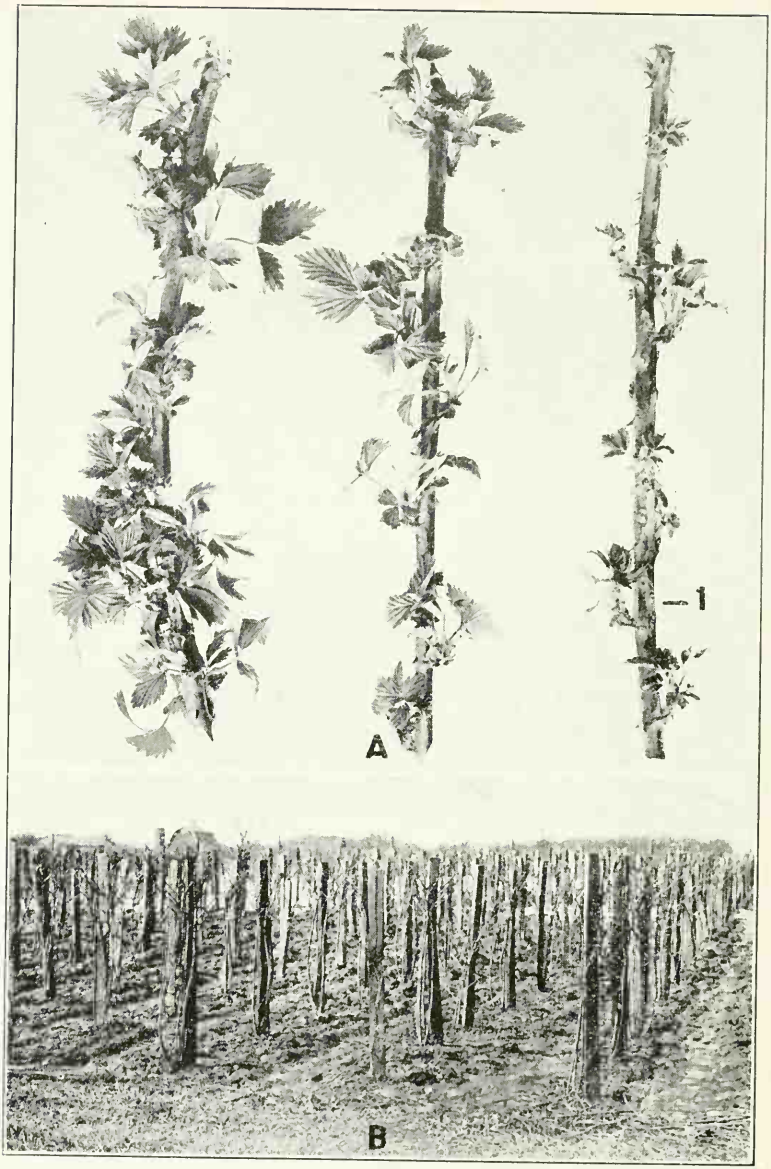

Plate IV

A.—Development of the foliage on raspberry canes in the H. Fischer planting on May 2, 1922. It is recommended that the delayed-dormant spray be applied after a few leaves have unfolded from the buds on the old canes, as shown by the cane marked 1.

B.—General view of the H. Fischer Cumberland raspberry planting where the control experiments were carried on, April 19, 1921.

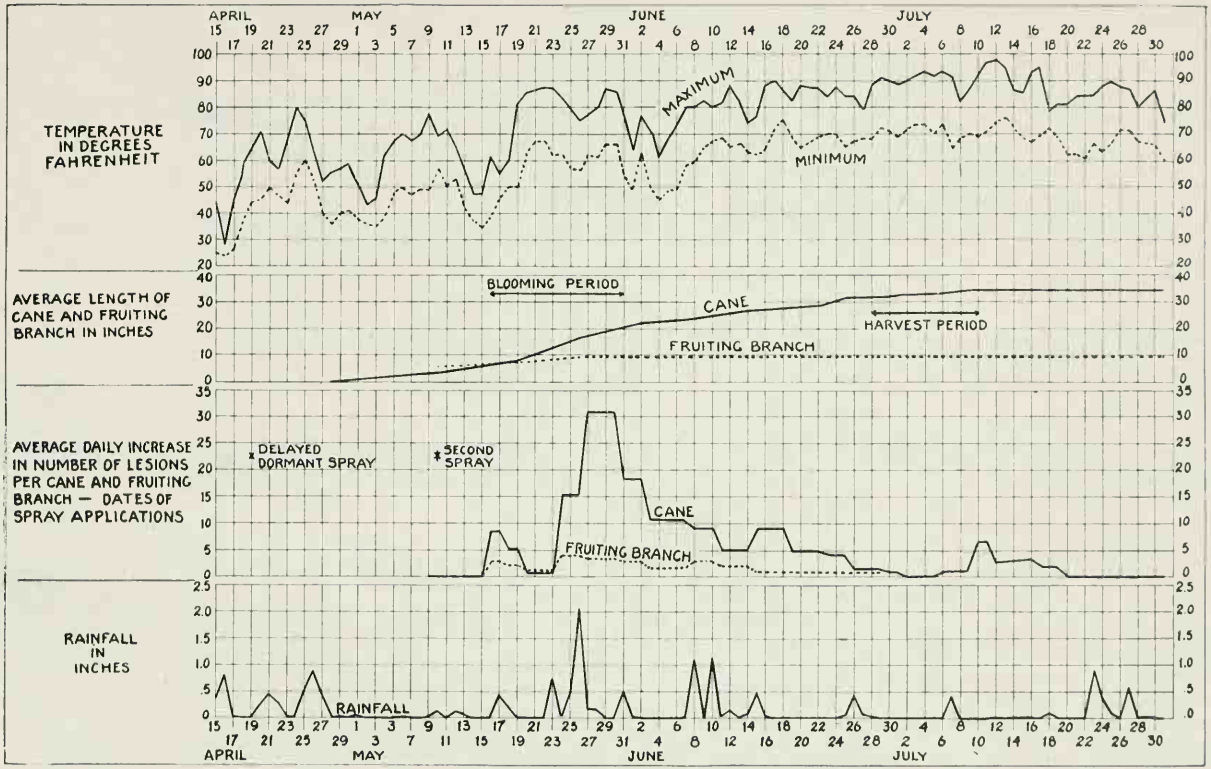

Plate V

A correlation of disease and host development with meteorological records and dates of spray applications, raspberry anthracnose experiments, H. Fischer planting, Madison, Wis., 1921 (see page15).

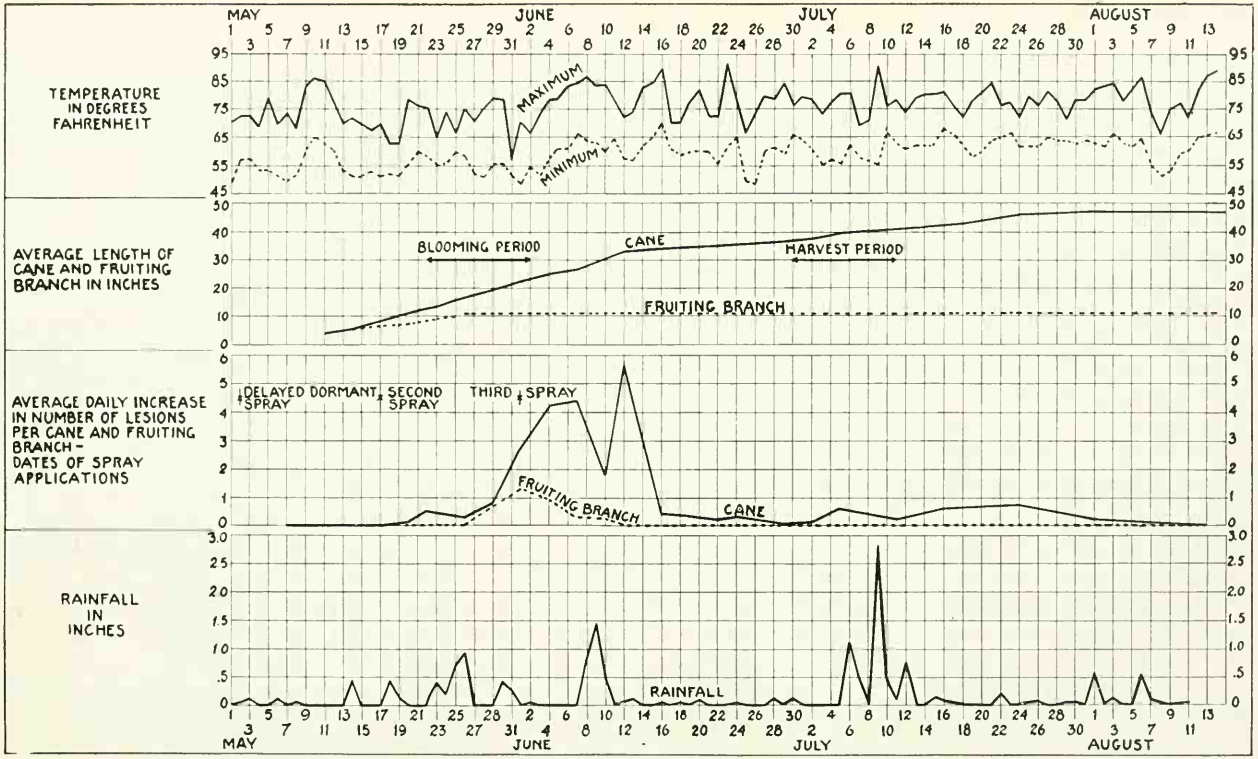

Plate VI

A correlation of disease and host development with meteorological records and dates of spray applications, raspberry anthracnose experiments, H. Fischer planting, Madison, Wis., 1922 (see page 19).

Plate VII

A.—A young Cumberland raspberry cane which received two thorough and timely applications of lime-sulfur spray with gelatin as a spreader, H. Fischer planting (plot 3). Photographed July 7, 1921.

B.—A young unsprayed cane from the same planting (plot 1), showing the extent of the disease on the canes of unsprayed plants at harvest time. Photographed July 7, 1921.

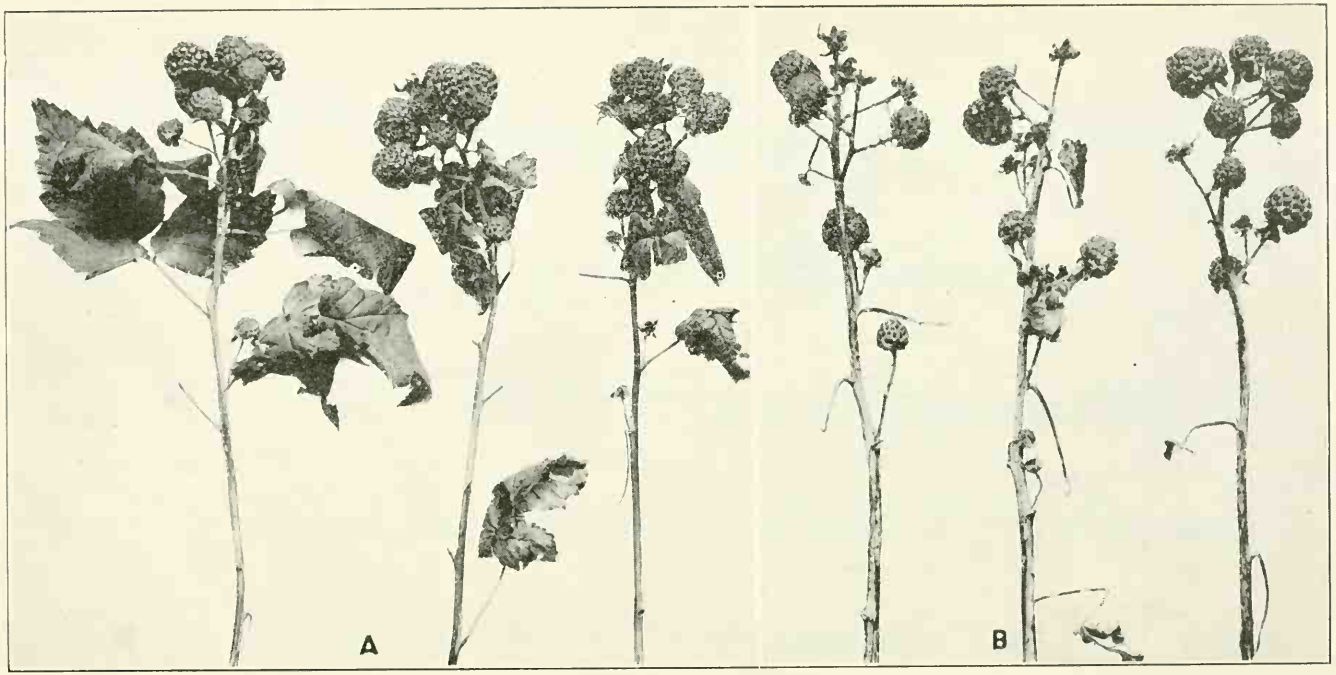

Plate VIII

A.—Cumberland raspberry fruiting branches which received two thorough and timely applications of lime-sulfur spray with gelatin as a spreader, H. Fischer planting (plot 3). Photographed July 7, 1921.

B.—Unsprayed fruiting branches from the same planting (plot 1), showing the dried condition of the fruit caused by anthracnose on branches and pedicels. Photographed July 7, 1921.

The effectiveness of lime-sulfur as compared with Bordeaux mixture. In general, lime-sulfur was more effective in controlling the disease than was Bordeaux mixture.

The effectiveness of a delayed-dormant spray only. Lime-sulfur with gelatin as a spreader gave excellent control of the disease, and lime-sulfur without a spreader controlled the disease commercially. Lime-sulfur in combination with glue as a spreader failed to check the disease in the latter part of the season.

Bordeaux mixture with glue gave commercial control of the disease, while poor control was obtained from the use of Bordeaux mixture alone or in combination with gelatin, calcium caseinate or milk.

The effectiveness of a delayed-dormant spray followed by a second application about one week before blooming. Lime-sulfur with gelatin or glue as a spreader gave very good control of the disease. Bordeaux mixture with gelatin, glue or calcium caseinate as a spreader may be classed as having given commercial control.

The effectiveness of adding spreaders to the above sprays. The addition of gelatin to lime-sulfur distinctly increased the effectiveness of the spray. Glue in combination with lime-sulfur, when two applications of spray were made, increased the effectiveness of the spray nearly as much as did the gelatin. The addition of glue gelatin, milk or calcium caseinate to Bordeaux mixture increased its effectiveness in controlling the disease, glue and gelatin being the more efficient.

EXPERIMENTS IN 1921

Seasonal Development of Host

The first exposure of green tissue in the leaf buds was observed April 8, and the warm period from April 11 to 13 caused the buds to develop until an average of one small leaf was unfolded and one-half inch of green tissue was showing. The snow and cold weather of April 15 and 16 checked the growth. The first new shoots appeared above the ground about April 28.

In order that the seasonal development of the host might be followed, 20 canes and 20 fruiting branches on the plants in the unsprayed plot were tagged on May 15. The length of these canes and fruiting branches was recorded at intervals of two to five days until no further increase was noted. These data are recorded graphically in Plate V in relation to the development of other factors important in the control of the disease.[1]

From a study of Plate V it will be seen that most rapid growth of the host occurred between May 17 and June 4, while the fruiting branches had practically ceased growing by June 2. The young canes continued to elongate until about July 9, when they averaged about 35 inches in length.

[1] The climatological data are from the records of the Madison station of the United States Weather Bureau (Climatological data. U. S. Dept. Agr. Weather Bur. Wis. Section 26: 15-32. 1921). The H. Fischer raspberry planting is located five miles east of the Weather Bureau station, and undoubtedly the climatological conditions vary somewhat from those recorded at the Weather Bureau station.

Seasonal Development of Disease

The seasonal development of the disease was followed on the above noted 20 canes and 20 fruiting branches, the increase in number of lesions being recorded at intervals of two to five days throughout the period of increase in infection. Additional records were made on August 17 and September 23 to ascertain whether any infection had taken place late in the season. The data were averaged and are recorded graphically in Plate V. Supplementary data as to the development of the disease are to be found in Table V.

From a study of Plate V it will be seen that the first lesions appeared on May 16, infection having taken place during the rains of May 10 to 13. The greatest development of the disease occurred during the last half of May, when the raspberry plants were making their most rapid growth. There was a continued increase in the amount of disease until July 20, and the more important infection periods may be traced to preceding rains, allowing from two to seven days for incubation. The disease de-

TABLE V.—AVERAGE INCREASE IN NUMBER OF ANTHRACNOSE LESIONS ON CANES AND FRUITING BRANCHES OF UNSPRAYED CUMBERLAND RASPBERRY PLANTS, H. FISCHER PLANTING, MADISON, WIS., 1921.

Dates observed		On canes by feet [a]					On fruiting branches [b]
		1st [c] foot	2nd foot	3rd foot	4th foot	Total	
		No.	No.	No.	No.	No.	No.
May	15	0.0	0.0	0.0	0.0	0.0	0.0
	17	15.8	0.0	0.0	1.1	16.9	6.6
	19	9.7	0.0	0.0	1.0	10.7	4.3
	23	2.3	0.0	0.0	0.3	2.6	5.1
	26	33.0	12.4	0.0	0.4	45.8	11.8
	30	11.3	110.0	1.0	0.1	122.4	13.5
June	2	8.4	42.2	3.7	0.1	54.4	9.0
	7	5.8	31.5	15.7	0.0	53.3	8.7
	10	3.1	17.2	6.4	0.0	26.7	8.1
	14	0.6	9.7	9.7	0.0	20.0	8.4
	18	0.2	5.1	29.4	0.0	34.7	4.1
	22	0.0	2.4	16.0	0.0	18.4	2.6
	25	0.0	1.0	10.0	0.0	11.0	1.2
	29	0.0	0.3	4.4	0.0	4.7	1.1
July	1	0.0	0.0	1.5	0.0	1.5	0.0
	5	0.0	0.0	0.1	0.0	0.1	0.0
	9	0.0	0.0	0.5	0.0	0.5	0.0
	11	0.0	0.0	0.6	12.2	12.8	0.0
	14	0.0	0.0	0.0	7.9	7.9	0.0
	16	0.0	0.0	0.0	5.4	5.4	0.0
	19	0.0	0.0	0.0	4.3	4.3	0.0
	21	0.0	0.0	0.0	0.0	0.0	0.0
Aug.	17	0.0	0.0	0.0	0.0	0.0	0.0
Sept.	23	0.0	0.0	0.0	0.0	0.0	0.0

[a] Average increase in number of lesions on twenty canes tagged on May 15.

[b] Average increase in number of lesions on twenty fruiting branches tagged on May 15.

[c] Basal foot of cane.

veloped very abundantly during the season. The lack of disease development after July 20 seems to have been due, primarily, to the cessation of host development. This is further shown in Table V where increases in the number of lesions on the young growing portions of the

canes are recorded, in contrast with the cessation of disease development on the older and hardened portions. High temperatures during the summer (Plate V) may have been an important factor in checking disease development, since the writer's investigations have shown that the maximum temperature for growth of the organism on dextrose-potato agar is about 90° F.

Treatment

A summary of treatment appears in Table III, and supplementary data follow.

The delayed-dormant spray was applied on April 19, a bright day with a ten-mile easterly wind. A barrel pump was used, and a pressure of 100 to 150 pounds was maintained on a single disc nozzle. An average of three-fourths pint of spray per plant was used. The leaf buds on the upper part of the canes had opened, averaging one leaf unfolded with two to four leaves folded but separated from the bud. The lower buds showed one-half inch of green tissue with an average of one leaf folded but separated from the bud.

The second spray application was made May 10, a cloudy, cool day. A barrel pump outfit was used, and a pressure of 100 to 150 pounds was maintained on a single disc nozzle. An average of 1¼ pints of spray per plant was used. The young shoots were three to four inches high, and the fruiting branches five to six inches long with the blossom buds well formed.

Results

Counts were made of the number of lesions on canes and fruiting branches on the various plots as in 1920, and the results appear in Table VI. Plates VII and VIII further illustrate the effectiveness of spraying for the control of this disease in 1921.

The results of the counts are discussed in accordance with the objects of the experiments.

Unsprayed. The disease was extremely abundant on the unsprayed plants and more so in the planting which had received no previous treatment for the control of the disease.

The effectiveness of lime-sulfur as compared with Bordeaux mixture. There was little or no difference in the effectiveness of these two spray materials.

The effectiveness of a delayed-dormant spray only. On the plants which had been sprayed the previous season lime-sulfur in combination with glue or gelatin as a spreader, and Bordeaux mixture with gelatin gave fair control of the disease. Lime-sulfur alone, and Bordeaux mixture alone or in combination with glue, milk or calcium caseinate were not very effective in controlling the disease. On plants which had received no previous treatment for the control of the disease all spray materials failed to control the disease commercially when only the delayed-dormant application was made. Lime-sulfur with gelatin, and Bordeaux mixture with calcium caseinate gave better control than any other spray combination in this test.

TABLE VI.—RESULTS OF SPRAYING EXPERIMENTS FOR THE CONTROL OF ANTHRACNOSE ON CUMBERLAND RASPBERRIES, H. FISCHER PLANTING, MADISON, WISCONSIN, 1921[a]

Treatment[b]	Plot No.	Average number lesions per cane	Average number lesions per fruiting branch	Plot No.	Average number lesions per cane	Average number lesions per fruiting branch
Unsprayed	1[c]	189.0	55.8	28[c]	420.3	84.9
L-S.+glue, 1, 2	2	29.5	1.0	16	30.3	2.3
L-S.+glue, 1	5	39.4	3.9	16A	106.2	7.6
L-S.+gelatin, 1, 2	3	12.4	0.9	17	40.2	3.7
L-S.+gelatin, 1	4	33.0	2.8	17A	81.5	8.0
L-S.+gelatin, 2				19	113.7	11.5
L-S.+saponin, 1, 2				18	57.8	3.9
L-S.+saponin, 1				18A	129.2	8.6
L-S.+saponin, 2				18B	121.8	8.4
L-S., 1, 2	6A	21.6	1.8	15	59.7	4.8
L-S., 1	6	60.1	3.8	15A	143.0	16.5
B.M.+glue, 1, 2	13	34.4	7.4	22	60.4	7.2
B.M.+glue, 1	12	83.4	14.4	22A	80.9	21.6
B.M.+gelatin, 1, 2	11	18.8	1.0	23	55.4	6.4
B.M.+gelatin, 1	10	36.3	8.5	23A	61.9	6.8
B.M.+gelatin, 2				20	109.5	10.6
B.M.+cal-cas., 1, 2	8	23.5	1.5	24	33.6	2.7
B.M.+cal-cas., 1	7	67.5	9.0	24A	52.5	3.4
B.M.+milk, 1, 2	9A	28.7	4.8			
B.M.+milk, 1	9	58.1	10.3			
B.M., 1, 2	14A	37.7	3.0	21	68.4	24.2
B.M., 1	14	63.0	11.0	21A	93.9	17.5
Scalecide, 1; B.M.+gelatin, 2				26	78.7	16.6
Scalecide, 1; L-S.+gelatin, 2				27	78.7	17.6
Scalecide, 1				25	99.3	11.0

[a] A summary of counts made on twenty canes and twenty fruiting branches per plot, chosen at random, August 15, 1921.

[b] See Table III for details of treatment.
Spraying dates: 1 (delayed-dormant), April 19; 2, May 10.

[c] Plots 1-14 had received the same treatment in 1920, while plots 15-28 had received no previous treatment for the control of the disease.

The effectiveness of a delayed-dormant spray followed by a second application about one week before blooming. On plants which had been treated the previous season satisfactory control was obtained from the use of lime-sulfur alone or with gelatin or glue as a spreader, and from the use of Bordeaux mixture with gelatin or calcium caseinate as a spreader. Although no satisfactory control was obtained on the plants that had not been treated the previous season, lime-sulfur with glue, and Bordeaux mixture with calcium caseinate were more effective than the other spray combinations.

The effectiveness of a single spray application about one week before blooming. Lime-sulfur with saponin or gelatin as a spreader, and Bordeaux mixture with gelatin as a spreader showed little effectiveness in controlling the disease when only the one application of spray was made, about one week before the plants were in blossom.

The effectiveness of adding spreaders to the above sprays. Added effectiveness was obtained by using spreaders with the sprays during this season, which was one of extremely abundant infection. Greater benefit was obtained from the use of gelatin or glue with lime-sulfur, and from calcium caseinate with Bordeaux mixture than from any other spreader used with either of these sprays.

EXPERIMENTS IN 1922

Seasonal Development of Host

The seasonal development of the host was followed as in 1921, and the results are shown graphically in Plate VI in relation to the development of other factors important in the control of the disease.[1]

On April 18 the buds on the old canes were showing about three-quarters of an inch of green tissue, but no leaves had unfolded. The first leaves were unfolded on April 22 and the new shoots began to appear above the ground May 1. The development of foliage on the old canes on May 2 is shown in Plate IV, A. From a study of Plate VI it will be seen that the canes continued to increase in length until August 1, and that the most rapid growth occurred between May 15 and June 12. The fruiting branches had obtained their maximum length about May 27.

Seasonal Development of Disease

The seasonal development of the disease was followed on 20 canes and 20 fruiting branches as in 1921. The data are recorded graphically in Plate VI, and supplementary data are to be found in Table VII. From a study of Plate VI it will be seen that the disease first developed in the field on May 20 and that no increase in number of lesions was observed after August 1. The greatest development of disease occurred during the early part of June when the plants were making their most rapid growth.

[1]The climatological data are from the records of the Madison station of the United States Weather Bureau as in 1921 (Climatological data. U. S. Dept. Agr. Weather Bur. Wis. Section 27: 17-32. 1922).

The disease continued to develop through a longer period in 1922 than in 1921, which may be correlated with the fact that the growth of the host plants continued for a longer period in the season of 1922. The fact that the temperature seldom reached 90° Fahrenheit during June and July of 1922 may have had some effect in favoring the longer period of infection. As in the previous season the greatest amount of disease developed when the host plants were growing most rapidly. As in 1921 the older portions of the canes developed resistance to the disease while the younger portions were being infected (Table VII), which further indicates that the rapidly growing portions of the raspberry plant are the most susceptible to the disease and that resistance to the disease is developed as the growth ceases and the plant tissues harden.

TABLE VII.—AVERAGE INCREASE IN NUMBER OF ANTHRACNOSE LESIONS ON CANES AND FRUITING BRANCHES OF UNSPRAYED CUMBERLAND RASPBERRY PLANTS, H. FISCHER PLANTING, MADISON, WIS., 1922.

Dates observed		On canes by feet[a]					On fruiting branches[b]
		1st[c] foot	2nd foot	3rd foot	4th foot	Total	
		No.	No.	No.	No.	No.	No.
May	17	0.0	0.0	0.0	0.0	0.0	0.0
	20	0.3	0.0	0.0	0.0	0.3	0.1
	22	1.0	0.0	0.0	0.0	1.0	0.2
	26	1.0	0.0	0.0	0.0	1.0	0.1
	29	2.5	0.0	0.0	0.0	2.5	0.8
June	1	5.7	1.6	0.0	0.0	7.3	2.3
	4	2.0	10.3	0.3	0.0	12.6	1.0
	7	0.5	11.3	1.5	0.0	13.3	0.3
	10	0.1	3.9	1.4	0.0	5.4	0.3
	12	0.0	10.9	0.7	0.0	11.6	0.0
	16	0.0	0.4	0.8	0.3	1.5	0.0
	19	0.0	0.9	0.0	0.0	0.9	0.0
	22	0.0	0.3	0.3	0.0	0.6	0.0
	24	0.0	0.0	0.0	0.5	0.5	0.0
	29	0.0	0.0	0.2	0.0	0.2	0.0
July	2	0.0	0.0	0.0	0.3	0.3	0.0
	5	0.0	0.0	1.4	0.4	1.8	0.0
	11	0.0	0.0	1.0	0.0	1.0	0.0
	18	0.0	0.0	2.0	1.8	3.8	0.0
	24	0.0	0.0	1.0	3.0	4.0	0.0
Aug.	1	0.0	0.0	0.0	1.3	1.3	0.0
	11	0.0	0.0	0.0	0.0	0.0	0.0
Oct.	7	0.0	0.0	0.0	0.0	0.0	0.0

[a]Average increase in number of lesions on twenty canes tagged on May 17.

[b]Average increase in number of lesions on twenty fruiting branches tagged on May 17.

[c]Basal foot of cane.

Treatment

A summary of treatment appears in Table III, and additional data follow.

The delayed-dormant spray was applied on May 2, a cloudy day with a light easterly wind. A wheelbarrow spray outfit was used, and a pressure of 100 to 150 pounds was maintained on a single disc nozzle. An average of one-half pint of spray per plant was used. The stage of development of the foliage on the old canes at the time when this spray was applied is shown in Plate IV, A. The new shoots were beginning to appear above ground.

The second application of spray was made on May 17, a cloudy, cool day with a light breeze from the southeast. The wheelbarrow spray outfit was used, and a pressure of 75 to 100 pounds was maintained on a single disc nozzle. An average of 1¼ pints of spray per plant was used. The young canes were eight to nine inches high, and the fruiting branches seven to eight inches long with the blossom buds well formed.

The third application of spray was made on June 1, at the end of the blooming period of the plants. The wheelbarrow spray outfit was used, and a pressure of 75 to 100 pounds was maintained on a single disc nozzle. An average of 1½ pints of spray per plant was used. The new canes were 22 to 23 inches high.

Results

Counts were made of the number of lesions on canes and fruiting branches on the various plots, as in 1920 and 1921, a summary of which appears in Table VIII.

The results of the counts are discussed in accordance with the objects of the experiments.

Unsprayed. The disease was fairly abundant on the unsprayed plants, although not so abundant as in the previous season. Plants which had been sprayed in 1920 and 1921 but left unsprayed in 1922 showed considerable decrease in the amount of infection on them as compared with the amount of infection on plants which had been left unsprayed the three seasons (plots 5A and 1). This cumulative benefit from spraying is not evident, however, in a comparison of results from plots 17B (sprayed in 1921, unsprayed in 1922) and 28 (unsprayed the two seasons).

The effectiveness of lime-sulfur as compared with Bordeaux mixture. In general, lime-sulfur gave slightly better control of the disease than did Bordeaux mixture.

The effectiveness of a delayed-dormant spray only. Commercial control of the disease was obtained from the use of lime-sulfur alone or in combination with glue, gelatin or calcium caseinate as a spreader, and from the use of Bordeaux mixture with calcium caseinate as a spreader. Lime-sulfur with saponin as a spreader, and Bordeaux mixture alone or in combination with glue, gelatin or milk failed to control the disease commercially.

The effectiveness of a delayed-dormant spray followed by a second application about one week before blooming. Very satisfactory control of the disease was obtained from the use of each of the spray combinations, with little difference in their effectiveness.

The effectiveness of a delayed-dormant spray followed by two applications; (A) one week before the blooming period, and (B) at the end of the blooming period. Excellent control of the disease was obtained from the use of lime-sulfur with gelatin, and Bordeaux mixture with calcium caseinate, but extreme injury to the foliage resulted from application of the sprays after the blooming period. Little or no reduction

TABLE VIII.—RESULTS OF SPRAYING EXPERIMENTS FOR THE CONTROL OF ANTHRACNOSE ON CUMBERLAND RASPBERRIES, H. FISCHER PLANTING, MADISON, WISCONSIN, 1922[a]

Treatment[b]	Plot No.	Average number lesions per cane	Average number lesions per fruiting branch	Plot No.	Average number lesions per cane	Average number lesions per fruiting branch	Plot No.	Average number lesions per cane	Average number lesions per fruiting branch
Unsprayed	1[c]	78.7	10.6	28[c]	96.8	17.1	28[c]	96.8	17.1
Unsprayed (sprayed in 1921)	5A	54.7	14.2	17B	94.5	16.2			
L-S.+glue, 1, 2	2	12.0	0.8	16	12.3	1.5	33	6.5	1.4
L-S.+glue, 1	5	26.2	6.3	16A	31.2	8.8	32	10.2	1.9
L-S.+gelatin, 1, 2	3	4.7	4.7	17	8.0	0.9	35	9.8	0.7
L-S.+gelatin, 1	4	39.5	2.6	17A	29.1	5.5	34	15.5	3.3
L-S.+gelatin, 2				19	39.9	2.4			
L-S.+gelatin, 1, 2, 3				25	6.9	1.3			
L-S.+gelatin, 1, 2, 3[d]				25A	4.9	0.9			
L-S.+saponin, 1, 2				18	18.9	3.7	37	8.4	1.1
L-S.+saponin, 1				18A	59.3	14.9	36	19.6	7.6
L-S.+saponin, 2				18B	43.9	5.8			
L-S.+cal-cas., 1, 2							39	11.0	1.8
L-S.+cal-cas., 1							38	27.5	6.3
L-S., 1, 2	6A	10.6	1.0	15	20.3	2.3	31	6.1	1.2
L-S., 1	6	26.2	4.4	15A	23.8	3.7	30	19.0	4.7
B.M.+glue, 1, 2	13	12.3	2.1	22	12.8	1.8			
B.M.+glue, 1	12	21.8	1.6	22A	35.3	10.0			
B.M.+gelatin, 1, 2	11	13.4	1.2	23	7.9	0.5			
B.M.+gelatin, 1	10	31.5	7.5	23A	41.1	7.9			
B.M.+gelatin, 2				20	74.6	16.8			
B.M.+cal-cas., 1, 2	8	7.6	1.6	24	13.6	1.3			
B.M.+cal-cas., 1	7	29.2	5.9	24A	25.4	8.9			
B.M.+cal-cas., 1, 2, 3				26	6.8	1.2			
B.M.+cal-cas., 1, 2, 3[e]				27	9.2	1.9			
B.M.+milk, 1, 2	9A	22.8	3.3						
B.M.+milk, 1	9	35.8	5.1						
B.M., 1, 2	14A	14.5	1.3	21	16.4	3.6			
B.M., 1	14	16.0	6.2	21A	44.5	13.0			

[a] A summary of counts made on twenty canes and twenty fruiting branches per plot, chosen at random, August 11, 1922.

[b] See Table III for details of treatment. Spraying dates: 1 (delayed-dormant), May 2; 2, May 17; 3, June 1.

[c] Plots 1-14 had received the same treatment in 1920 and in 1921, plots 15-28 were treated in 1921, while plots 30-39 had received no previous treatment for the control of the disease.

[d] Lime-sulfur, 1-80, plus gelatin was used in application 3.

[e] Bordeaux mixture, 1½-1½-50, plus calcium caseinate was used in application 3.

in the amount of foliage injury was brought about by reducing the strength of the summer sprays by one-half in this third application.

The effectiveness of a single spray application about one week before blooming. Lime-sulfur with gelatin or saponin as a spreader, and Bordeaux mixture with gelatin failed to control the disease commercially when only the one application of spray was made, about one week before the blooming period of the plants.

The effectiveness of adding spreaders to the above sprays. Very little benefit was obtained from the use of spreaders with the sprays during this season, which was one of only moderately abundant infection.

SUMMARY

Anthracnose, caused by the fungus *Plectodiscella veneta* Burk., manifests itself in purplish to white spotting of the canes, leaves, petioles, peduncles, and pedicels, and in drying up of the fruit.

The disease appears to be widespread with its hosts in the United States, and has been reported as common to blackberries and raspberries in Canada. The black raspberry has been observed to be more susceptible to the disease in Wisconsin than any other host. No difference in susceptibility of the different varieties of black raspberry has been observed.

Anthracnose is one of the most serious diseases of black raspberries and blackberries. It is reported as entirely eliminating the growing of raspberries in some sections of the United States, and estimates of the annual loss in fruit yield due to this disease in various sections of the United States range from 12 to 63 per cent of the crop. The writer obtained data in 1921 showing a 33.2 per cent decrease in fruit yield caused by this disease on black raspberries.

The minimal temperature for growth of the fungus on dextrose-potato agar is about 11° C., the optimal, between 20° and 26°, and the maximal, about 31° C.

Conidia are not produced readily in culture, but are obtained abundantly upon the transfer of suitable fragments of cultures from a dry to a very moist atmosphere.

Conidia germinate readily in sterile distilled water and on nutrient media, and secondary conidia are often budded off.

Ascospores on cultural media germinate usually by the production of five to seven conidia, which in turn produce germ tubes.

The period of incubation on the canes has been shown by inoculations and observations to be from three to nine days.

The disease first appears on the young growing canes and leaves in the early spring, usually when the canes are eight to ten inches high, which has been between May 13 and May 20 during the last four seasons at Madison, Wisconsin.

The lesions continue to increase in number on the young growing tissue throughout early summer, and as the plants cease growth during July resistance to the disease is developed.

Ascospores and conidia form the source of natural inoculum in the spring and early summer.

Ascospores, which are forcibly ejected from the asci, may be carried by the wind for a distance of at least one-half mile from old plantings and cause infection in new plantings.

Good cultural practices during the growing season are advisable. Weeds should be kept in check, as they increase the humidity around the canes.

In making new plantings care should be taken to remove the old canes from the young plant roots, thereby eliminating a possible source of inoculum.

During the seasons of 1920, 1921 and 1922 anthracnose on black raspberries was satisfactorily controlled by spraying, lime-sulfur giving somewhat better results than Bordeaux mixture.

Only one application of spray, about one week before blooming, failed to control the disease in any case.

The use of a spray after blooming increased the effectiveness of lime-sulfur and Bordeaux mixture in controlling the disease. Injury to the foliage from this spray application was sufficient, however, to preclude its use.

The results indicate that fair control may be obtained by applying only the delayed-dormant spray each year, using lime-sulfur, alone or with glue or gelatin as a spreader, or Bordeaux mixture with calcium caseinate or gelatin as a spreader.

The use of spreaders increased the effectiveness of the sprays, especially in seasons of abundant infection. Glue and gelatin gave the best results with lime-sulfur; gelatin and calcium caseinate, with Bordeaux mixture. It is doubtful, however, whether the use of these spreaders is warranted when careful spraying is done.

To control anthracnose on black raspberries under Wisconsin climatic conditions it is recommended that two applications of spray be made each season as follows: (1) after a few leaves have unfolded in the spring (Plate IV, A 1), using lime-sulfur, 1-10; and (2) about one week before the blooming period of the plants, using lime-sulfur, 1-40.

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Memoranda

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Experiments on the Control of Wildfire of Tobacco

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CONTENTS

Experiments on the Control of Wildfire of Tobacco[1]

THE CONTROL of the wildfire disease of tobacco caused by *Bacterium tabacum* (Wolf and Foster) has been the subject of considerable investigation since the outbreak of the disease in North Carolina in 1917 (14). The outstanding observation, bearing on control, has been the fact that the disease originates in the seed bed and that practically all cases of field infections are traceable to this source. The prevention of seed bed infection is, therefore, the most logical aim of all methods of control. This naturally involves: first, the determination of how or on what materials the causal organism lives over winter or from one crop to the next; and second, methods of preventing such infected material from being introduced into the seed beds.

Once seed bed infection occurs and is discovered, the grower must choose between discarding the infected plant beds entirely or taking a risk in using some or all of the plants, relying on unfavorable weather conditions to prevent further serious spread of the disease. This latter method is economically hazardous, as it is likely that the disease may prove disastrous to a crop if proper weather conditions for the dissemination and the development of the disease occur. Precautions to prevent dissemination in the field are of doubtful value as a means of control; their effectiveness is at least very limited, and probably more often they are effective only under relatively unfavorable weather conditions for the development of disease.

The investigations reported in this bulletin are consequently mainly concerned with a study of the factors which may account for seed bed infection, together with methods of preventing such infection. The practical conclusions arrived at are also to a considerable extent influenced by several years of observational studies made during field surveys.

Summary of Earlier Work

The control of tobacco wildfire has received some experimental attention in most of the tobacco districts in which it has occurred. While some difference of opinion exists as to the relative importance of the methods of overwintering of the causal organism, practically all investigators agree that the causal organisms may survive from one crop to the next on infected and cured tobacco leaf, except that in flue-cured tobacco sufficient heat may be used to kill the organism. The subsequent dissemination of this infective material to the seed beds

[1]Cooperative experiments with Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture.

may naturally occur in several ways, the most unusual of which has been announced by Valleau and Hubbard (13) who claim that the wildfire organism is commonly transmitted through the spitting of tobacco juice into the seed beds.

Wolf (15) and Fromme and Wingard (5) were first to point out the possibility of overwintering on seed and introduced the formalin and corrosive sublimate seed treatments respectively as control measures for tobacco wildfire. The importance of overwintering on seed in the Connecticut Valley has been questioned by Anderson and Chapman (1) and Clinton and McCormick (3).

Similarly, overwintering in soil has been suggested by the earlier workers, but this again has been questioned by more recent observations and experiments.

Information concerning the possibility of overwintering of the wildfire organism on seed bed covers (cloth and sash) and frames is especially meager. The possibility has been recognized, however, and reported in some cases as occurring (15).

Tobacco stems (leaf-midribs) both in commercial fertilizers and as untreated fertilizer material have been held responsible, by observation, for some cases of overwintering. This seems least likely in the case of the manufactured fertilizers containing stems where heat treatment is used (15). Untreated stems and stalks, since they usually carry leaf fragments which may naturally be infected, are probable overwintering carriers as pointed out by Anderson and Chapman (1).

Experimental evidence on the actual dissemination of the wildfire organism is small and fragmentary. Observational evidence is abundant but rarely convincing. Since almost any material which has been exposed so as to carry the causal organism physically may conceivably carry it from place to place, this subject is not a very fruitful one for satisfactory speculation or experimentation.

It has been suggested by various workers that long distance dissemination may occur most often through transportation of infected seed, plants, or commercial tobaccos and by dry winds. With respect to transmission of the disease from plant to plant, in seed beds and in the fields, all investigators agree on the effectiveness of rain, especially when accompanied by strong wind. Heavy storms and hail which injure the leaf surface are especially favorable to subsequent heavy infections as well as for dissemination .

The control of wildfire in the seed beds by dusting or spraying frequently with copper-lime dusts or Bordeaux mixture has been recommended by workers in the Connecticut Valley. When properly applied it is claimed to be an effective control measure. This method has not been generally adopted outside of New England and some question as to its value has already been raised in our work (8). Dusting and spraying in the field has received some attention by other workers (12) with negative results.

Since the work reported in this paper had been practically completed, Anderson (2) has published his results on overwintering of

tobacco wildfire in New England. His results indicate that the bacteria winter most successfully in situations where they are not subjected to keen competition from the growth of other organisms—principally in fairly dry situations—and that they winter least successfully under conditions moist enough for competing organisms to grow. He concludes the wildfire organism may overwinter on cured leaves in the barn, plants standing in the field, on boards, sash, and dry fragments of seed pods, but that overwintering in leaves exposed to decay or in the soil is least likely.

Overwintering Studies

The overwintering experiments were designed to determine how long and under what conditions the wildfire organism is most likely to survive the period during which its host plants normally can not be the source of its propagation. The main tests have been made with artificially infested materials which are most likely to be concerned with overwintering and seed bed infection. These have been stored under different conditions in most instances, and tested from time to time as to their ability to yield infection when placed in contact with young tobacco plants. It has been assumed that the application of infected material to a unit area, in many cases hundreds of times greater in quantity than that which would occur under natural conditions, reduces the errors which might result from working with only a relatively small amount of material. Conditions for infection have been made as ideal as possible both by wounding the plants and by maintaining favorable environmental conditions. Considerable variation in this condition is evident, however, from the results. Tests were made soon after the materials concerned were infested and before storing away in all cases to make certain that the causal organism was pathogenic at the start of the test. The results are, therefore, believed to be reliable from the experimental standpoint. From a practical standpoint we have also tested out materials supposedly infected naturally, and made a considerable number of field observations, and these factors are also taken into consideration in drawing final conclusions.

In the 1922 experiments artificial applications to seed, boards, cloth, soil, etc. were made with both pure cultures of the organism and with the juice extracted from badly infected green leaves. Two different sets of applications were made known as Series I and Series II. These materials were divided up into separate portions each suitable for one test. It was planned to store one-half of this material out-of-doors in the winter months, but this was not done in some instances because the organisms were apparently dead on those materials most commonly out-of-doors in winter, before the winter months arrived. The cured leaf material was cured under normal conditions in the shed, and the buried leaves were, of course, outside all winter.

The 1923 materials were inoculated artificially with dried crushed leaves, for the reason that this would seem from our 1922 experiments

to offer the best opportunity for the persistence of the causal organism over winter. Part of this material was stored at room temperature and part in a weather-instrument chamber out of doors where the material was protected from rainfall.

Inoculations with these materials have been tried in several ways. Frequently they were made by scrubbing or washing of the materials in a small amount of water and making fifty wound inoculations on individual plants in pots with the washings. In other cases platings on agar were made from the materials and wildfire-like colonies used for inoculation. More reliable results are obtained by placing the materials directly upon young vigorously growing seedlings in "flats" after wounding them. The flats were then well watered and kept covered with paper for one or two days, keeping the plants and paper moist in the meantime. The infested materials were removed from the flats three or four days after the inoculation was made. Our experiments have led us to question any conclusions based on negative results from inoculating individual plants with material in which the causal organism is not abundant and is in a latent state, even if such plants are vigorous, wounded and placed under good environmental conditions. Seedling inoculations in the greenhouse in which at least 100 plants are involved seems the most reliable test. Inoculations in out-of-door sections of seed beds are not apparently as reliable on account of the danger of dissemination of the organism from section to section, and less certainty in the control of the environmental conditions. Practically all of our results are based on greenhouse tests.

The first series of experiments were started in the midsummer of 1922, for the purpose of comparing the survival of the wildfire organism on or in seed, soil, cloth, boards, and dried, naturally infected leaves, cured naturally infected leaves, and green leaves buried about 4 inches in the soil, without direct contact with the soil and with mixtures of soil in proportions of 1 to 5 and 1 to 10. In addition the watery extract from green leaves and the pure culture suspension used for infesting the seed, soil, etc. was saved for comparative tests, as was the dried green leaf pulp from which the green leaf extract was made.

An attempt has been made to present the data from inoculations made with these infested materials in condensed form. The percentages of infection given are not comparative throughout for the reason that different methods of inoculation were used in some cases and because of the variable conditions for infection which cannot be avoided. It is also to be expected that the dilution of the suspension of organisms recovered from the infested materials naturally varies greatly. Within certain limits, however, the percentages are believed significant and to these attention will be called. The principal value in the results, however, lies in the outcome as to whether infection was or was not obtained after repeated trials. In this respect the results are believed to be significant to a high degree. Attention has already been called to the fact that the number of organisms involved in these tests are probably infinitely greater than would be likely to occur under normal con-

ditions, so that the small amount of material used as units (10 grams seed, 1 square foot of cloth, 16 square inches of boards, etc.) are comparable to a much larger quantity of these materials under practical conditions.

From Table I it seems evident that the wildfire organism can survive but a comparatively short period in liquids exposed to general contamination, and that its limit of survival on such material as moist soil and dried green-leaf-plup, cloth, and boards is only about one to two months, under the conditions of this experiment. On the other hand, on tobacco seed and on dried and cured leaves the organism was still alive after nine months.

It is interesting to note the comparative survival on seed and cloth. There is some indication that seed tends to exercise some protective influence on the wildfire organism and that the cloth possessed some deleterious action. This suggestion is based partly on the reversal of behavior in the two cases between the results from the pure culture and the green-leaf inoculum. In subsequent attempts to prove up these relationships, however, we failed to obtain conclusive results.

A second series of tests with infested materials similar to that in Series I was started in the early fall of 1922, and carried on simultaneously throughout the fall and winter. The methods used were in all respects similar. The data secured in this series are presented in Table II. The results agree in general with those presented in Table I regarding overwintering. Moist soil, cloth and boards inoculated with pure-cultures or extract from infected green leaves rapidly lost their infective powers, whereas seed retained it to a striking extent. The liquid extract from infected green leaves again became non-infectious after only a few days and the dried pulp from these same leaves retained infection less than one month. Tobacco stalks were infested and included in this test. We are unable to account for the fact that we were not able to get definite infection from these in three trials made before the seventh-month test, which gave good infection. Under questionable infections, however, we have been in the habit of including lesions which were so faint that they could not be readily identified as wildfire, and we are, therefore, inclined to attribute this result to conditions not being sufficiently favorable to bring out good halos with a weakened organism.

It seemed logical to conclude from the first year's tests that the presence of moisture and the protective action of host tissue were important factors in the overwintering of the wildfire organism, and that the most likely place of the survival of the parasite under practical conditions would be in dried or cured leaf material not permitted to decay. Since such dried leaf material might readily attach itself to the materials used in seed beds or in their construction, or be accidentally or otherwise transferred from buildings where it had remained dry, to seed beds, it was deemed advisable to repeat the whole set of experiments, using as a source of inoculum dried infected

leaves crushed or powdered. These were applied in the fall of 1923 by dipping the various materials into a water suspension of the infected tissue after which they were rapidly dried and stored under the desired conditions.

As the previous season's experience indicated that the most reliable results could be obtained by direct inoculation to flats (about 22"x14") containing several hundred young and vigorously growing plants, practically all results for the winter of 1923-24 are based upon this method. At the close of the experiment, 100 plants were pulled at random from the flat and the total number of lesions on these counted. Flats showing no infection in the counts were carefully searched for any single lesion which might occur. In most cases the results given are averages of duplicate inoculations.

A comparison of results with these materials, as shown in Table III, indicates that the wildfire organism survived most successfully on dried leaves, or dried leaves in dry soil, or on dry stalks; apparently not so well on boards and on seed, quite poorly on cloth and in cured leaves, and was rapidly destroyed in moist soil and rotting leaves. The variation in amount of infection is no doubt in part due to differences in amount of inoculum applied in each case, although effort was made to insure that the inoculations would have some quantitative significance. The results justify the conclusion that the causal organism survives better on some materials than on others, under similar environmental conditions.

This experiment and others have indicated that the organism survives better in infected leaves rapidly dried at air temperatures than in those going through the ordinary curing process. Considerable variation may be expected in this direction according to the condition of curing.

Table III is of practical significance, however, in showing that dried or cured leaf tissue may harbor the wildfire organism over winter either on seed, cloth, boards, stalks, or in dry soil under conditions such as occur in buildings or wherever materials may remain dry. In moist soil or in rotting leaves the organism dies out in a comparatively short time.

In order to determine the influence of different conditions of storage as to temperature and humidity upon the pathogenicity of the wildfire organism, the infected materials were stored outside all winter in an open chamber protected only from direct rainfall. The inside storage was in a relatively warm (about 75° F.) and dry room. Table IV shows a comparison of this material after several months. It may be seen from this table that practically no difference in pathogenicity occurred as a result of these differences in temperature and humidity in relatively dry materials. The influence of alternate freezing and thawing on organisms in a moist condition in cultures is discussed on page 19.

The possibility of the overwintering of wildfire in soil has been repeatedly raised by growers. While most workers are agreed that this probably rarely happens and is of little significance, sufficient obser-

vational and experimental evidence is offered to keep the matter in doubt.

The experiments here have repeatedly indicated that the wildfire bacteria cannot survive more than a month in ordinary moist loam soil. Some difference may exist in different soils in this respect, but overwintering of any organisms in soil is very doubtful except as dry infected leaf tissue is lodged in dry soil, and does not become intimately associated with it at any time for even short periods. This condition may occur in tobacco sheds or other protected places where the soil remains dry. Table V shows how well the bacteria survive in air-dry soil or in sterile soil whether wet, moist or dry, as compared with soil kept moist, or remaining moist for only a sufficient time after infestation to permit drying. The readiness with which the wildfire organism overwinters in sterile soil or dry soil as compared with unsterilized moist soil seems to be a good basis for the assumption that overwintering is largely dependent upon competition with other organisms as already has been suggested by Anderson (2). To be sure, this explanation seems to account for the comparatively rapid deterioration of the wildfire organism in contaminated liquids, rotting leaves and in ordinary moist soil as compared with otherwise sterile or dry soil. On the other hand, when comparing the persistence of the organism on seed and dried or cured leaves with its persistence on cloth and boards under similar moisture conditions, it does not seem to satisfy the requirements wholly. Although this whole matter requires further verification, we are inclined to include in the overwintering requirements the protective action of certain materials, generally host tissue, and perhaps the absence of injurious substances not commonly considered as such. On the other hand, as will be shown later, the wildfire organism may deteriorate more rapidly in pure culture than in the dormant condition on seed, in which case competing organisms do not explain their death or loss of pathogenicity.

The effectiveness of decay in destroying the wildfire organism is more clearly shown in Table VI, but, on the other hand, conditions favorable for continued decay do not seem to be required for ultimate destruction of the parasite.

Various miscellaneous experiments have been conducted with overwintering which will not be presented in detail except to say that thus far infection has been obtained from air-dried leaves after eighteen months, from cured leaves after fifteen months and from artificially infected seed after twenty months, although in some cases the period of longevity of the organism on these materials has apparently been considerably less.

It seemed likely to us that if other plants were subject to infection by the wildfire organism these might also prove to be an overwintering agent. To test out this probability a considerable number of other plants (common garden and field crops and common weeds) were artificially inoculated. Most plants tried were found to be subject to the disease when succulent young plants were inoculated under favorable

environmental conditions. The results of this phase of the work have already been published (10). No evidence, however, has been secured that sufficient infection occurs in nature on other hosts than tobacco to warrant the belief that they ever play a part in overwintering, nevertheless it seems worth while for investigators of this disease to be on the look-out for evidence of such cases.

Dissemination Studies

The question of dissemination of this disease involves a considerable number of problems, of which some are now open only to speculation while others are apparently more likely to be solved by observational than by experimental data.

The problems involved are in some respects distinct, since they involve long distance dispersal, dispersal to adjoining districts, dispersal from farm to farm, spread from plant to plant in the field, and in the seed bed, as well as the original source of infection of the seed bed. Wildfire apparently spread from North Carolina to thirteen widely separated tobacco growing states east of the Mississippi River in five years. At present there can be speculation only as to the agency of dispersal, since many might conceivably be involved. Although preliminary experiments indicate that active fermentation may destroy the causal organism, all portions of the tobacco leaf do not ferment actively. Some experiments indicate, for instance, that dry infected leaves could withstand a temperature of 100°F. for five days, although moist cured leaves could carry the organism only one to two days at this temperature. It seems likely, therefore, that commercial tobaccos of certain kinds may be a common long distance dispersal agent, since the organism may quite likely survive two years in tobacco leaf tissue. Dry wind storms may readily carry infested material for long distances and infected seed and plants may be involved in special cases. All of these, excepting wind dispersal, seem to have been excluded in certain cases of epiphytotics which have been observed.

The spread from farm to farm within a given area is still a subject of speculation. The more or less localization of the disease in districts, as in Wisconsin in 1922, seems to indicate local spread which cannot be attributed to infection from commercial tobacco, or even the use of home grown tobacco by the workers as suggested by Valleau and Hubbard (13), a practice which is quite uncommon in the north. A careful survey definitely excluded dissemination by seed or plants as a possibility in that year. Dissemination of infested material by wind, especially dry wind, within the district seemed the most logical explantation, although in isolated cases other means accounted for the spread from farm to farm.

Spread of Wildfire in the Field.

The importance of rainstorms with wind as a dispersal agent in the field and in uncovered or cloth covered seed beds is recognized by all workers on wildfire. The actual distance and amount of dissemination

following rainstorms can only be assumed, however, from the area and number of new infections occurring, which are brought out by conditions favoring infection. The causal agent may have been spread in many cases before the storm. Unless the wind is especially severe it is not generally believed that rain storms carry the disease over wide areas. In the spring of 1924, some seed bed experiments were laid out to test this subject by placing flats of plants with bare ground between them at varying distances, up to twenty feet, from a central source of infection, but, unfortunately, the results were not convincing owing to the small amount of infection obtained.

To test the possibility of man carrying the disease about in any one field or distributing it to other fields, two experiments were conducted. An artificially infected pad of cloth was used with which leaves in the field were brushed sufficiently to break the plant-hairs in one case and touched lightly in another case. Infection occurred in both cases, but was more marked in the former. One experiment was conducted during a moist period of weather and the other during a dry period. The relative results were apparently the same in both cases.

Where wet infected cloth was applied to wet leaves the best infection was secured, although good infection was also obtained with wet cloths applied on dry plants. Some infection was also secured from the dry infected cloth on wet plants, when the contact was sufficient to break the plant-hairs. When dry cloth was used on dry plants no infection resulted. These tests seem to indicate that the disease may be readily spread in an infected field by man brushing against the plants when the leaves or the clothes or both are wet, but not when these are both dry.

An important question relative to dissemination relates to the influence of the amount of infection that can be permitted to enter the field on the seedlings when transplanted, and the extent to which infection can be kept down by the removal of diseased plants or diseased leaves.

On June 28, 1923, an isolated piece of ground was selected and divided into eight plots, each 30 feet by 36 feet. One hundred and twenty plants, three feet apart each way, were set in each plot. These plants were selected according to the amount of disease present on them, the "badly diseased" ones showing lesions on all the leaves and the "slightly diseased" ones showing no actual lesions at all, although they came from a section of a seed bed which had been inoculated about two weeks earlier, but upon which no infection had occurred, owing apparently to unfavorable conditions for infection. The "considerably diseased" plants showed a few lesions on the lower leaves. The different lots were pulled and transplanted by different individuals to prevent contamination in handling. The season was unfavorable for wildfire, and at times no signs of the disease were visible in the field. Following light rains, a slight upward spread on the infected plants was noted, but no general spread occurred until following a short rain storm with strong wind about the middle of September, when the plants were full grown. Following this a heavy infection developed. On September 25, the

number of leaves infected on each plant was estimated by two different individuals. The average infection per plant is shown in Table VII. The data show mainly that the "slightly diseased" plants eventually gave almost as much disease as the "heavily diseased" plants. The spread of the disease into healthy plots was evident, more to the eastward than to the westward, and consequently the infection in Plot 1 is believed to be due largely to the organisms originally present. Similar plots conducted in 1924 corroborated the conclusions from the previous season.

The experience with the careful removal of diseased leaves at short intervals from a small center of infection in plots in 1923 and 1924 was of such a nature as to indicate little or no value resulting from this practice if favorable conditions for the disease develop later in the season. While this work has been done on a large scale in Wisconsin, in the control work in 1922 in cooperation with the State Department of Agriculture the subsequent unfavorable conditions for the development of the disease did not give a true measure of its value.

Under favorable weather conditions for infection and consequently reproduction of the parasite and for its dissemination, a very small percentage of disease in the field may rapidly develop into a large one, which may subsequently be very injurious to the crop.

Transplanting of even a very small percentage of infected plants or of only slightly diseased plants is, therefore, not believed to be warranted in view of the damage which may result.

It should be stated, however, that observational evidence on a large number of farms under apparently similar weather conditions indicates that there is no close correlation between the amount of infection in the seed bed, or the original infection in the field, and that subsequently occurring. The actual condition of the plants themselves, as a result of local field conditions, seems to play a large role.

Seed Bed Infestation.

All matters considered, the transfer of infested material into the seed bed is the most important problem to be taken into consideration in connection with the control of wildfire. It has been shown that it practically can be taken for granted that overwintering will not occur in soil lying out-of-doors.

It has been shown at this station that the wildfire organism can overwinter on artificially infested seed, and that artificially infested seed sown in seed beds may result in infection of seedings (Plate 2, bottom) A number of trials have been made however, in which infection has not been secured as a result of sowing infested seed, although conditions apparently ideal for infection to occur have been maintained. Although it is not generally believed that seed under field conditions is infested, experience here indicates that it is a factor which must be reckoned with. It is a wise precaution, therefore, not to save seed from infected fields, but if seed must be taken from such fields, it should be thoroughly disinfected before sowing. The subject of seed disinfection is discussed on page 14.

Overwintering experiments here as well as those of others, have shown that the wildfire organism readily survives the winter in dry or cured infected tobacco leaves. In the tobacco shed and stripping rooms a very considerable amount of refuse containing the living causal organism must exist following work on an infected crop. Here, apparently, lies the most important factor for seed bed infection. The dissemination of this material to the seed beds may occur in a number of different ways, unless precautions are taken to prevent it. The loose refuse should be burned or buried, followed by the precaution of placing the seed beds at a considerable distance from the tobacco shed. The use of lumber, cloth, or any other material on the beds which has been stored in the shed should also be guarded against, since this involves overwintering not necessarily on these materials themselves but on pieces of infected leaves which may be attached to these materials and carried to the seed bed.

The wildfire survey (Plate VII) in Wisconsin in 1923 brought out the following interesting observation bearing on dissemination from sheds. Out of about ninety cases in 1922, 60 growers placed their seed beds near their sheds and 27 developed wildfire in 1923. Twenty-three growers placed their seedbeds a considerable distance from the sheds, and only one developed wildfire in 1923. Out of nine new cases of wildfire in 1923 seven developed in beds placed near the sheds. This evidence seems to point towards the general importance of dissemination of infected material from the curing sheds to the seed beds. The survey in 1924 did not indicate such close correlation between location of beds and infection, but the season was unsual in many respects and other complicating factors may have played a part in infection. On farms where wildfire has previously occurred, it is an excellent precaution to keep the seed beds a considerable distance from the building which may harbor the parasite in order that wind, animals, or man may not readily transfer even small bits of infected material to the seed beds. Furthermore, seed bed boards or frames, cloth or sash, should not be stored in sheds. If they are so stored or have been on an infected bed the previous season, they should be cleaned and disinfected if again used for seed beds. According to our results, infested lumber piled out of doors in such a way that it all becomes wet will not harbor the organism from season to season.

Cloth covers are not likely to carry infected material unless stored under infected tobacco. These can be readily sterilized by boiling or steaming when desirable.

Dusting and Spraying Experiments

In the Connecticut Valley, efforts have been made to control wildfire in the seed beds by dusting with copper-lime dust and spraying with Bordeaux mixture (1, 3). Their experimental results in the green house have shown very marked reduction in the amount of infection on seedlings following these treatments, and a high degree of benefit was likewise obtained under out-of-door seed bed conditions. Neither in the

green-house nor in the field is absolute control claimed, however, by these investigators.

Experiments along a similar line were started in connection with our work in the fall of 1922 (8). Most of the work has been carried out in the green-house with seedlings in flats (about 14 inches x 22 inches) which usually contained 300 or more seedlings. One set of experiments was also conducted in 3 foot x 3 foot seed bed areas out of doors.

The first experiment was planned to show the difference in control obtained in wounded as compared with unwounded plants, inoculating artificially before and after dusting or spraying, together with a relative comparison of the effectiveness of spraying and dusting and their frequencies of applications. The flats were inoculated three times (in a few cases two times) with a water suspension of the wildfire organism from cultures. The data secured are shown in Table VIII. The percentage of infection obtained is relatively high, and it may be objected that this experiment was not a fair comparison as to the value of dusting and spraying on account of the number of inoculations and the amount of inoculum used. In the absence of any method for duplicating natural dissemination only the inoculated controls can be relied upon for comparison. While these indicate infection approximately twice as great as that of the sprayed and dusted flats they were not as badly diseased as may be frequently noted in plant beds under conditions of natural infection.

Table VIII indicates that only about 20 per cent more of the plants were infected, and only about two to four more infections per plant occurred in the wounded as compared with the unwounded seedlings. (It is estimated that each plant received on the average ten or more wounds.) In both the wounded and unwounded series, plants dusted or sprayed after the inoculation showed considerably more infection than plants dusted or sprayed before inoculation. No important consistent difference between spraying with Bordeaux and dusting could be noted in this experiment, as there was more variation between the "brand" of dust or spray used than between the methods of application. The Bordeaux paste spray used from appearance was apparently of inferior grade. "Fungi-Bordo" gave better results than "Nu-Rexo." Increasing the number of applications of "Nu-Rexo" reduced the percentage of plants infected and the number of infections per plant.

A second experiment showed that corrosive sublimate sprayed on the plants one-half hour before inoculation reduced the percentage of plants infected from 96 to 49, and the average number of infections from 5.79 to 1.44. Leaf injury was produced by the corrosive sublimate which could be reduced, however, by adding lime without materially influencing its effectiveness. Following this trial lime alone was tried in comparison with copper-lime dust. This test seemed to indicate that lime alone was as effective as the commercial copper-lime dust. An experiment was then conducted in out-of-door seed beds in the spring of 1923, running duplicates in 3 foot x 3 foot seed bed areas. Air-

slaked lime, "Limate", "Niagara D-6," "Nu-Rexo", "Corona Bordeaux," "Sanders Dust," "Fungi-Bordo," "Corona Sulphur," Bordeaux (4-4-50) spray and calicum caseinate ("Kayso") were compared. Six applications of the chemicals were made, two being applied before one light artificial inoculation of the wildfire organism made on June 14. On July 11 an examination of the beds seemed to indicate that the "Limate" and "Kayso" plots were as free from wildfire as the uninoculated controls. Slight infection was found in the others and considerable infection in the inoculated controls. "Niagara D-6" and "Sanders Dust" gave some leaf injury but not enough to seriously affect the plants.

On October 13, 1923, young seedlings in flats were dusted with "Kayso" and Limate" in comparison with "Sanders Dust", "Fungi-Bordo", and dry soil. The percentage of plants showing infection are shown in Table IX. "Kayso" alone apparently gave the best results, due probably in part to its adhesiveness. "Limate" was approximately as good as the copper-lime dusts. Soil dust for some reason increased infection above that of the inoculated controls.

These results with spraying and dusting are believed to have some bearing upon the theory and practice of this method of control for wildfire, although corroboration of the results and conclusions may be necessary to bear them out. Copper, the toxic constituent in Bordeaux spray and copper-lime dusts, has never been regarded as a good germicide and its use as a spray to prevent bacterial infection is quite unusual in the history of plant disease control, although its value in preventing fungus invasion is universally recognized. The experiments indicate further that copper is not the effective agent in the case of wildfire control. It seems more likely that the effectiveness of spraying and dusting is due in part at least to its physical rather than to its chemical action. While "Limate" or "Kayso" is not recommended for the practical control of wildfire, yet the latter could probably be used to advantage on account of its adhesiveness. Spraying and dusting, with any material used in our tests however, do apparently not prevent the occurrence of more or less wildfire in the seed beds when conditions favorable to the dissemination and development of the causal organism occur. The experiments on dissemination have shown that a very slight amount of seed bed infection, in fact an infection so small as to be undeterminable at the time of planting, may result in heavy field infection, providing conditions favorable for the dissemination and development of the disease occur in the field.

If, therefore, spraying and dusting do not wholly control the disease, the question may be raised as to the actual value of this practice. If wildfire becomes annually a common and serious seed bed trouble in any given district, spraying and dusting, or some better method of control, may need to be resorted to. Under conditions where only a low percentage of the seed beds are infested in a district, it will probably be safer in the long run for the grower to discard infested seed beds entirely in preference to taking the risk of placing even a slight amount of infection in the field, such as may occur in infested

sprayed or dusted beds. The actual value of this practice however, must finally be determined largely by the results which the growers obtain from its use rather than from experimental trials of the kinds described.

Seed Disinfection

It was pointed out earlier that while seed was not apparently a common source of wildfire infection, it is regarded as an unsafe practice to sow seed grown one or two years previously in an infected field without thorough disinfection. Formaldehyde solution (1-16) was first used for seed disinfection against wildfire. Earlier experience here with this disinfectant indicated that it was injurious to germination in some cases and this observation has also been reported from other stations, particularly from Virginia. Corrosive sublimate (1-1000) treatment was recommended as a substitute by Fromme and Wingard (6), their experiments having shown that no injury to germination of the seed occurred under their conditions. This treatment was recommended and used soon after in the northern sections where wildfire was on the increase. Experience here with the corrosive sublimate treatment like that of others (I, II) proved disastrous, for the reason that while the treated seed germinated in subsequent seed germination tests on filter paper, (Plate 3, bottom) it almost universally failed to germinate for the farmers. The injurious action of the corrosive sublimate treatment (Plate 3, top) was found to occur only when the seed was sprouted before sowing (either as mixed with rotten wood or as pure seed) as is a common practice in northern tobacco-growing districts. When sown directly in soil, the treated seed sprouts normally; and this method of sowing is the common practice in Virginia and other southern districts. Corrosive sublimate treated seed also practically fails to germinate on potato agar. The failure of corrosive sublimate treated seed to germinate is believed to be due to the toxic action of the corrosive sublimate absorbed and retained by the seed, which in contact with filter paper or soil passes from the seed, but in contact with other seeds, in decayed wood or on agar, is not absorbed from the seed. We cannot agree with Anderson and Chapman's (1) explanation of hardening of the seed coat in this respect nor that treatment with water alone may result in a similar injury, although there have been cases where seed treated with water alone and lying in cloth bags in contact with other bags, treated with corrosive sublimate, fail to germinate, apparently due to the diffusion of the toxic property from one bulk to the other.

Experiments were accordingly started with the purpose of finding some satisfactory method of disinfecting tobacco seed for tobacco districts where seed is normally sprouted before sowing. A large number of tests on modifications of the corrosive sublimate and formalin treatments were first tried. Later calcium hypochlorite, "Bacilli-Kil" (B. K.), cupra-ammonium carbonate, electrically generated ozone (with possibly nitrous oxide), heat in vacno, Seed-o-San, Semesan, Uspulun,

Bayer's Compound and other commercial compounds, and silver nitrate were tried. Following these treatments the rate and percentage of germination of seeds on filter paper and in bulk were determined, as well as the disinfection secured by sowing the treated seed on potato-dextrose agar plates. The data on this subject are too voluminous to present in detail here so the principal results only are given.

Seed stored moist for as long as forty-eight hours, and then dried, showed no injurious effect on germination either on filter paper or in "bulk". Seed treated with corrosive sublimate, kept moist for eight hours or longer after treatment, retarded germination on filter paper markedly, and no germination occurred in bulk. Corrosive sublimate treatment at various temperatures from 0° C. to 30°C. did not appreciably influence the usual result (i. e. germination on filter paper but no germination in bulk). Two to eight washings after treatment with the sublimate did not measurably alter its normal behavior. Poor drying after treatment retarded germination only slightly as compared with moderate to good drying. Soaking seed in water up to two hours before treatment with the sublimate had no influence on the result. A twenty minute treatment with corrosive sublimate, 1 to 1000, retarded germination appreciably on filter paper, as compared with shorter treatments. Five, ten and fifteen minute treatments with corrosive sublimate, 1-1000 ,gave good but not perfect disinfection of seed so far as wildfire was concerned, but did not permit germination in bulk. Corrosive sublimate (1-500) for fifteen minutes retarded germination somewhat more on filter paper than did the standard treatment. Corrosive sublimate (1-2000) was not effective as a disinfecting agent.

Soaking seed in water after treatment with corrosive sublimate up to thirty hours did not favor its germination on filter paper or in bulk but rather added to the injurious action secured.

These, or other modifications of the corrosive sublimate treatment which have been tried, including those recommended by Anderson and Chapman (1), do not permit the germination of the seed in bulk, or in decaying wood with anything like sufficient certainty to warrant its recommendation in districts where sprouting before sowing is practiced.

The formaldehyde treatments did not prove particularly injurious to the particular lots of seed used in these experiments, either on filter paper or in bulk up to about 2 per cent formaldehyde with 15 minute treatments. The objections to the formaldehyde treatment lie primarily in the fact that its disinfecting properties are not so reliable as corrosive sublimate up to strengths which are not likely to be injurious to the germination of some lots of seeds. Our experience and that of others also has been that formaldehyde (5) is much more injurious to some lots of seed than to others for reasons not fully understood, and it is, therefore, not regarded as a promising tobacco seed disinfectant.

Calcium hypochlorite (about 2 per cent Cl. water) retarded germination about 10 per cent only in treatments from two to twenty-four hours, but the seed was markedly bleached.

'Bacilli-Kil" (B. K.), about 3.38 per cent NaClo up to four hours, followed by washing, did not injure seed appreciably, but seed was bleached and it was not effective as a seed disinfectant in treatments of less than four hours duration.

Cupra-ammonium carbonate (spray formula) did not injure seed germination up to 1 hour treatment, but it did not give sufficient disinfecting action to warrant further trials. Five hours treatment killed seed but did not satisfactorily disinfect it.

Ozone (with perhaps nitrous oxide) generated electrically did not injure dry seed up to eight hours, but wet seed was killed in about four hours. Neither treatment was sufficiently effective as a disinfecting agent in our tests.

Heat treatments, even under reduced pressure, did not give satisfactory disinfection up to temperatures that killed the seed in these limited trials.

A number of commercial seed disinfectants, mostly of the organic mercury compounds, have been tested both as dust and liquid treatments. These have included principally Seed-o-San, Dupont Semesan, Dupont Dust Disinfectant No. 12, Bayer's Dust, Bayer's Compound and Uspulun. None of these met all the requirements for disinfection of tobacco seed. The dust treatments as a rule do not permit germination in bulk, and the liquid treatments retard the germination in bulk to such an extent as to render them unsafe to recommend in practice. The disinfecting value of these compounds against the wildfire organism on tobacco seed proved in all cases to be so low at any of the strengths recommended (and in some cases with increased concentrations and long treatments) that they cannot be recommended for this purpose.

In the first experiments with silver nitrate as a disinfecting agent for tobacco seed a $\frac{n}{50}$ solution (about .33 per cent) was used in treatments varying from two to thirty minutes. Germination was not appreciably injured either when tested on filter paper or in bulk, and good disinfection was secured in all cases. In a second preliminary test silver nitrate was used in strengths varying from 0.1 per cent up to 0.8 per cent for fifteen minutes and again germination was not injured appreciably even at the higher strength, and excellent disinfection was secured at all concentrations. A number of trials subsequently made with silver nitrate indicated that it is the least harmful of any disinfecting agent tried on tobacco seed, and that its disinfecting properties are as good if not better than that of corrosive sublimate (Plate IV). Accordingly, it was suggested that silver nitrate 1-1000 treating for 15 minutes be substituted for corrosive sublimate treatment of tobacco seed, especially in districts where seed is commonly sprouted before sowing.

During the spring and summer of 1924, a decided outbreak of wildfire occurred in Wisconsin, owing to very favorable weather conditions for its occurrence. In a few cases seed was suspected of being the agency of introduction into the seed bed, although the seed had been treated with silver nitrate. This led to further investigations on the

subject of seed sterilization, comparing particularly silver nitrate and corrosive sublimate treatments. For this purpose seed heavily inoculated by artificial means was used, which following treatment was plated out on potato agar, often using as many as forty dishes with around two hundred seeds included in each dish as a test for each treatment. As a result of these tests it appeared that occasionally a wildfire organism escaped the recommended treatments, sometimes one in five or ten thousand seeds. The results again indicated that silver nitrate was somewhat more effective than corrosive sublimate as a disinfecting agent. It was also especially noticeable that the former was much more effective against fungus saprohytes than the latter.

In this connection, it must be remembered however, that the seeds used in these experiments were infested with at least a hundred and probably with a thousand times more of the wildfire bacteria than commonly occur on seed under natural conditions, so that it is doubtful if more than one seed in several hundred thousand escapes disinfection in practice. Since there are, however, three to four hundred thousand seeds in an ounce, the possibility remains that occasionally wildfire may escape the present methods of seed disinfection.

In order to reduce this possibility to a minimum or eliminate it entirely, a double seed treatment with silver nitrate was resorted to, permitting the seed to dry one or more days between treatments. At the same time the length of the treatment has been reduced since early experiments showed that even a two minute treatment with silver nitrate was very effective. With the double treatment, each treatment lasting ten or even five minutes, it has been possible to disinfect the seed so completely that no wildfire organism has been recovered from seed so treated after extensive trial. Therefore, it seems that the double treatment should be used in preference to the single treatment in cases where the most reliable disinfection is required.

The germination of the seed is apparently more retarded by two treatments than where only a single treatment is given, but this has not been found to be of more than one or two days duration and, consequently, is not to be regarded as a serious objection. The advantage of two five-minute treatments lies largely in the fact that germination is retarded somewhat less than with two ten-minute treatments. It has also been noted here that it is not advisable to sprout the seed in the same cloth in which it was treated.

Loss of Virulence

The experience of most workers with the wildfire organism has been that it may relatively rapidly lose much or all of its virulence in culture. This phenomenon is common with many bacterial parasites and is said to occur in nature also.

A simple though fairly extensive experiment was conducted with *B. tabacum* for the purpose of determining in the first place the best

cultural method for this organism in order to retain its virulence, and secondly to form a possible basis of reasoning in regard to overwintering of the organism.

Three virulent strains (isolated from different sources) were selected and transferred to three different media, potato-dextrose agar, beef-peptone agar and bouillon. These media were made up in sufficient quantity to last throughout the experiment and sealed in tubes with paraffin. In all, about 650 cultures were involved. Several original transfers were kept, and transfers were then made serially weekly and monthly, fifty-two weekly and thirteen monthly transfers being made in the experiment. The cultures were kept in the refrigerator at about 8-10° C. throughout the experiment. At intervals of about one month the non-transfers and the last weekly and monthly cultures were tested for their virulence by making 50 wound inoculations with a water suspension from each culture on the leaves of young tobacco plants in pots supplying suitable conditions for infection. The results are recorded as percentages of infection. Considerable difference occurred in the rate of infection and the size and appearance of the lesions, but these cannot be gone into detail here. The results on the percentage basis were on the whole quite variable when compared from month to month, due undoubtedly to variation in environmental conditions affecting infection. Studied in detail, the results also show occasional contradiction, i. e., a culture would at one time give a higher, and at another time a lower percentage of infection when compared to another. Taken as a whole, however, the following conclusions seem warranted from the data.

The degree of virulence showed a general downward tendency on all media with increasing age, most marked on beef-peptone agar and least marked on potato dextrose agar (Plate V). In the case of potato agar the greatest loss of virulence occurred when no transfers were made (Table 10) and the least when weekly transfers were made. In the case of beef-peptone agar the greatest loss of virulence occurred in the weekly transferred series and the least in the no-transfer series. At the end of 15 months the loss of virulence was complete on beef-peptone agar in all three strains used.

In bouillon not much effect of the transplanting itself was noted, the evidence being somewhat in favor of monthly or weekly transplants above no transplanting as regards retention of virulence.

Some difference existed in the three strains used in regards to their ability to retain their virulence under any one condition, strain 3, for instance, was over twice as virulent as strain 2 on untransferred beef-peptone agar. When transferred back into potato-agar, the cultures in all cases seemed to be approximately equal in vigor of growth, but virulence was not materially altered.

None of the cultures, as a rule, gave as high percentage or as good infection as freshly isolated cultures from new lesions. Aside from this the best culture medium for *B. tabacum* seems to be potato-dextrose agar, with transfers at intervals of somewhat less than one

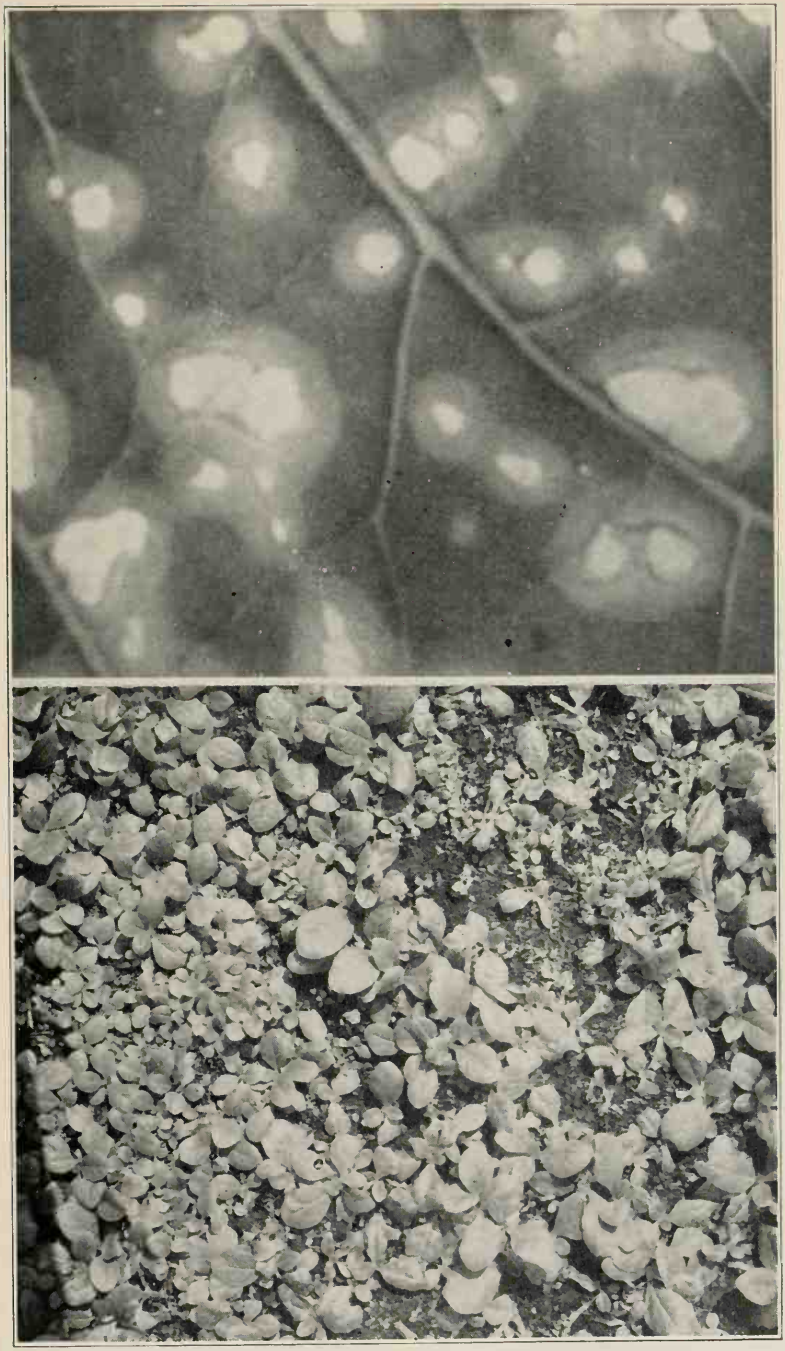
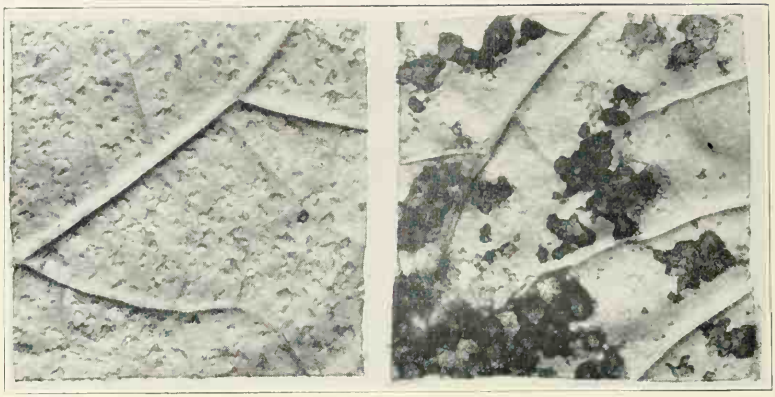


Plate I

Top—Typical symptoms of wildfire on portion of tobacco leaf. The chlorotic area or "halo" surrounding a whitish or brownish central necrotic area is characteristic of this disease.

Bottom—Wildfire infection in a seed bed often kills young plants.



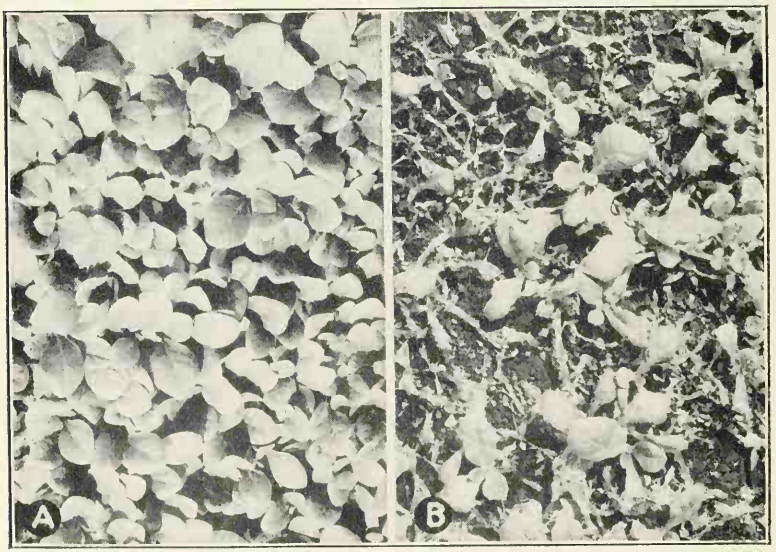


Plate II

Top—Early and Late symptoms of blackfire of tobacco. This disease differs quite strikingly from wildfire and is due to a different bacterium. The control methods, however, are much the same so far as known so that it is believed the recommendations presented in this bulletin for wildfire control apply to blackfire also.

Bottom—Wildfire may overwinter on the seed. (A) Control plot sown with uninfested seed. (B) Wildfire resulting from sowing artificially infested seed.

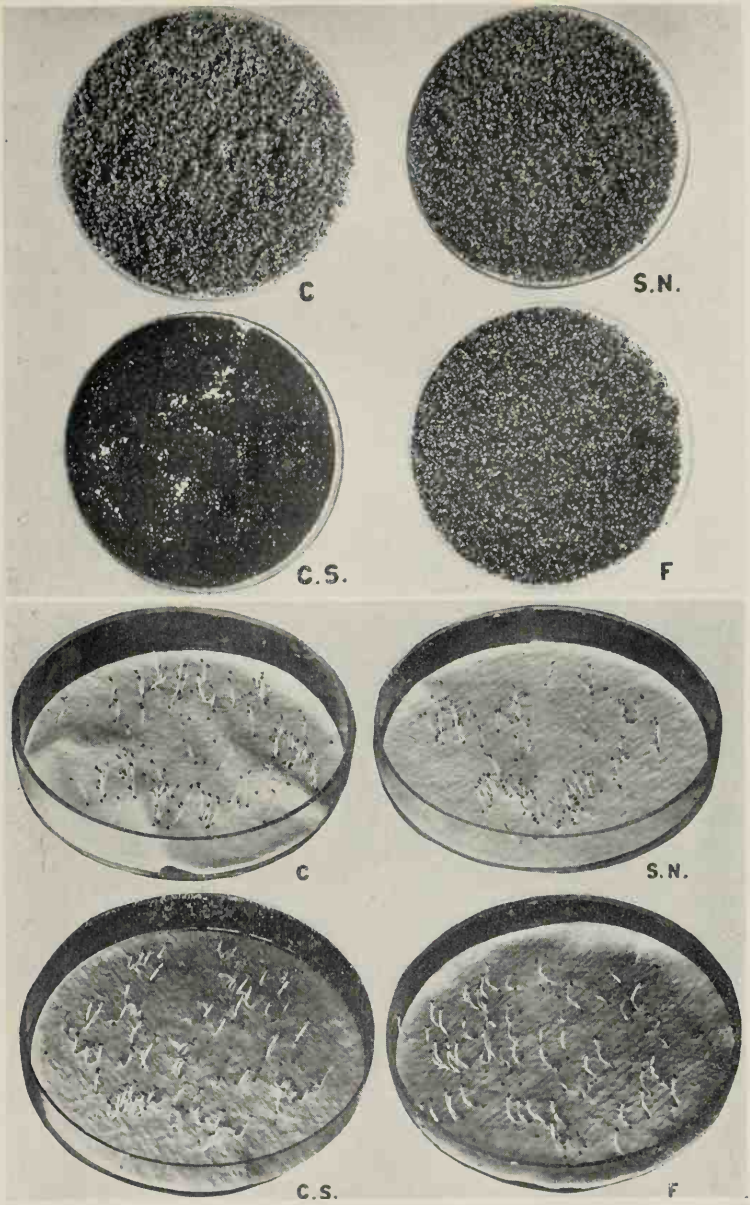


Plate III

Top—Corrosive sublimate prevents sprouting of seed in bulk. Silver nitrate treatment does not prevent sprouting by this method.

Bottom—The germination of tobacco seed after different treatments using the ordinary method for testing the seed.

(Note that corrosive sublimate does not prevent germination by this method, nor does it prevent germination when seed is sown dry in the soil.

C.—Control no treatment.

S.N.—Disinfected with silver nitrate.

C.S.—Disinfected with corrosive sublimate.

E.—Disinfected with formalin.

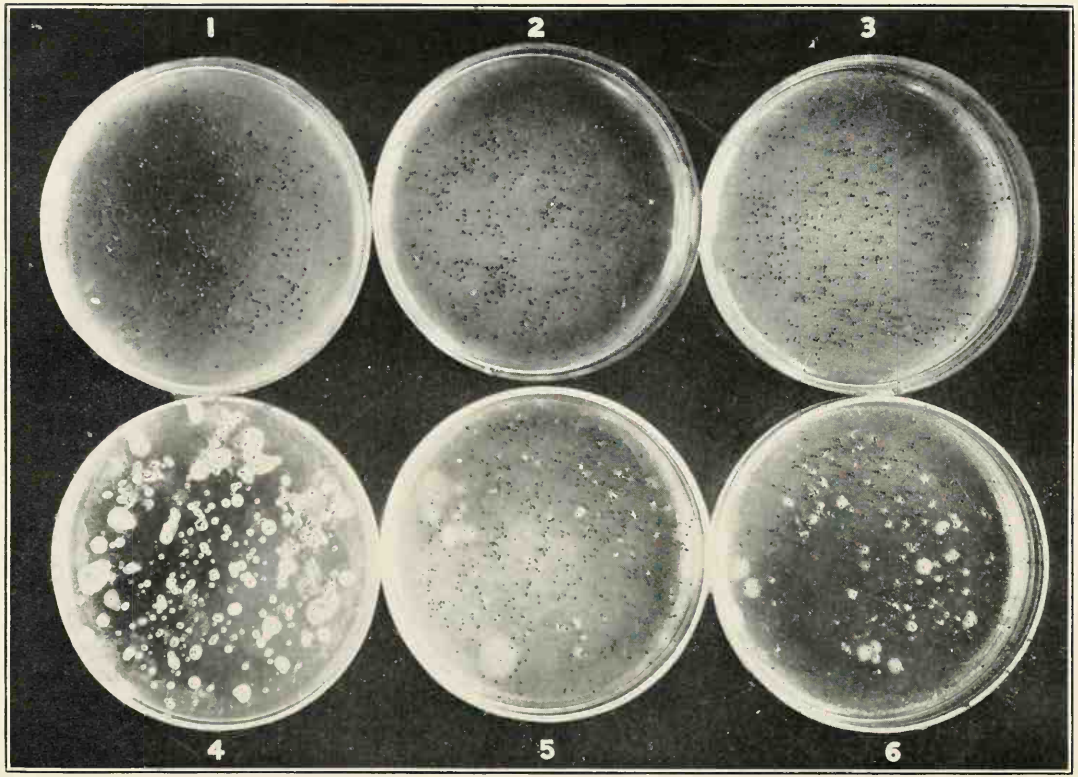


Plate IV

Method of testing the efficiency of seed disinfecting agents.

1.—Silver nitrate 5 minute treatment.
2.—Silver nitrate 10-minute treatment.
3.—Silver nitrate 15-minute treatment.
4.—Control no seed treatment.
5.—Corrosive sublimate 5-minute treatment.
6.—Corrosive sublimate 10-minute treatment.

The growth from the seed in the corrosive sublimate treatments are sapro-phytic fungi in practically all cases.

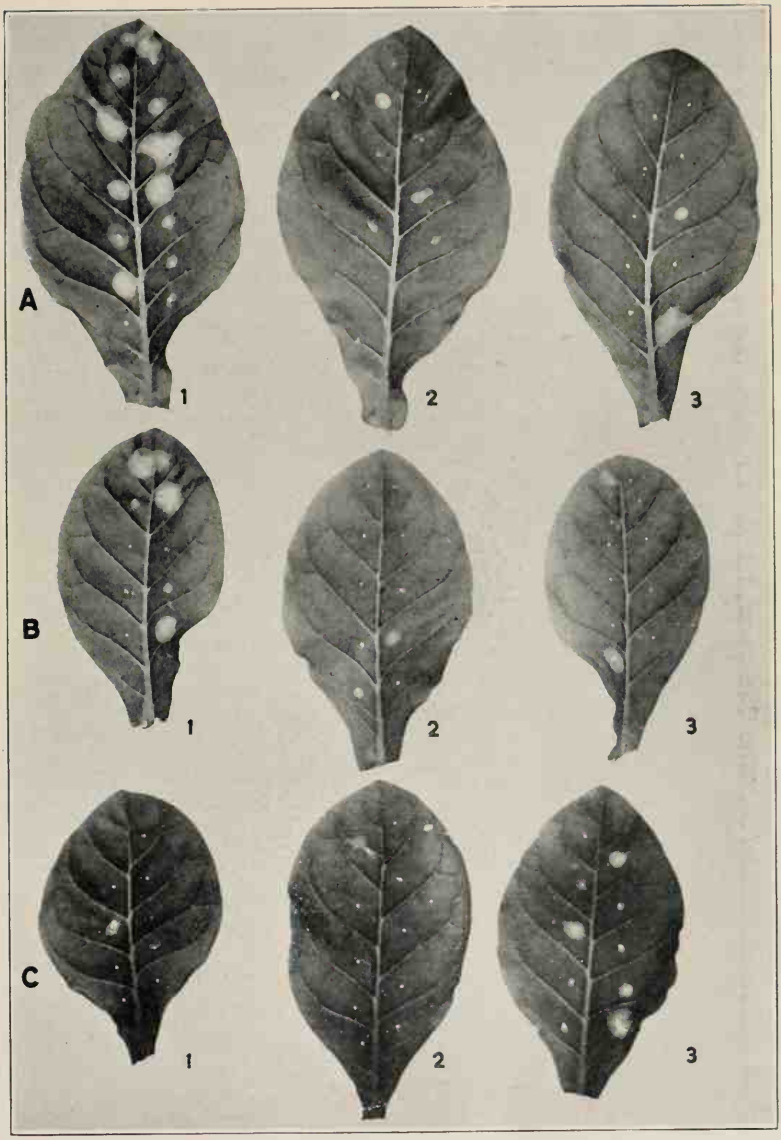


Plate V

Illustrating the comparative loss of virulence of the wildfire organism grown in pure cultures for one year on different media and with different frequencies of transfers.

A—Potato-dextrose agar.
B—Bouillon
C—Beef peptone agar.

1—Transferred weekly.
2—Transferred monthly.
3—No transfer.

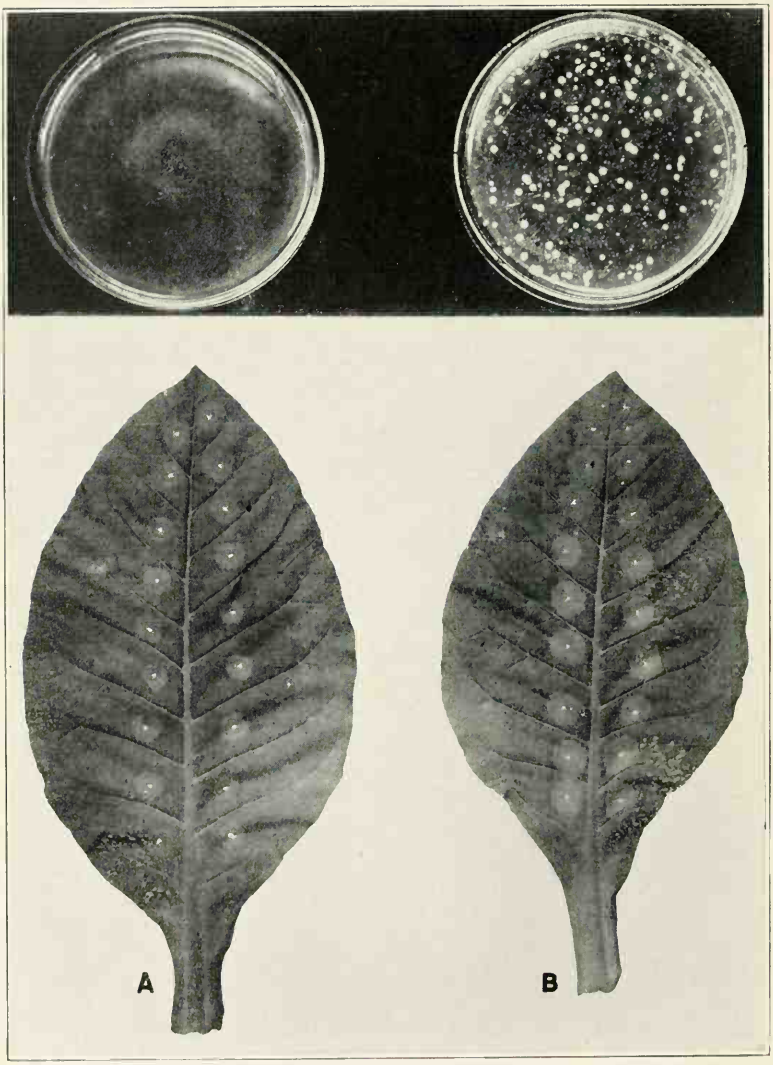


Plate VI

Wildfire symptoms produced by toxin only.

A.—Leaf inoculated with a sterile filtrate from wildfire cultures. Dish above shows results of plating out from such spots.

B—Inoculated with bacteria and toxin from wildfire culture. Dish above shows organisms present on plating out. Inoculation with bacteria alone requires longer to produce symptoms.

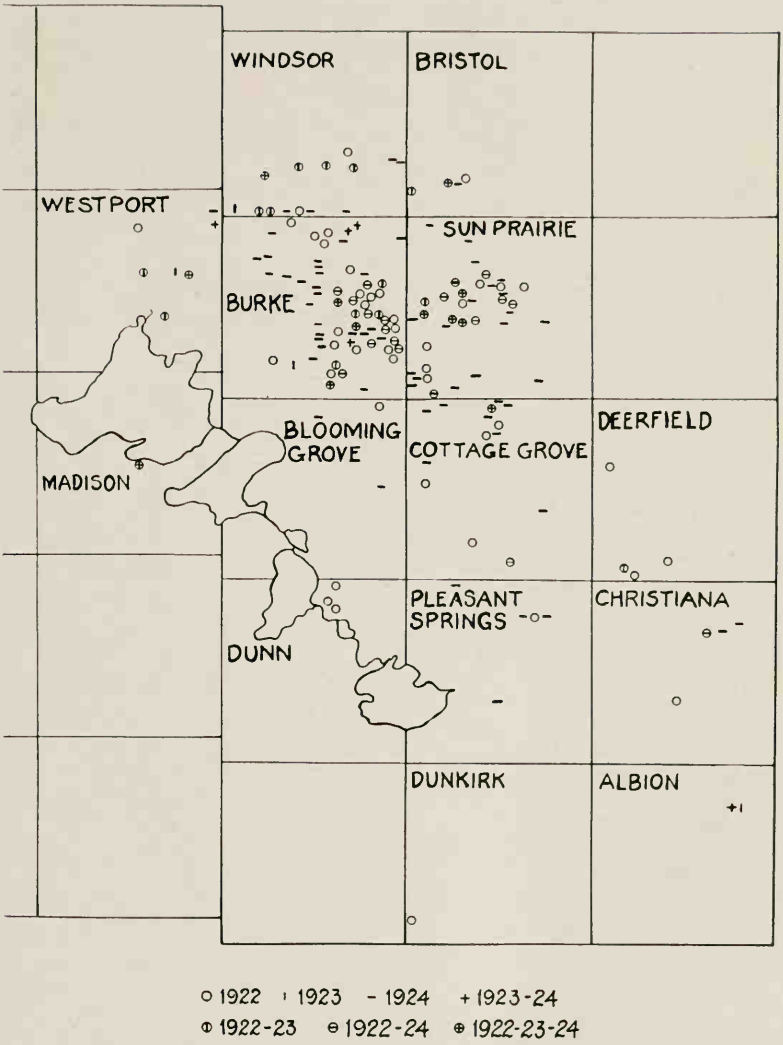


Plate VII

Spread of tobacco wildfire in Dane County, Wisconsin. The cases of infection on farms in 1922, 1923, and 1924 are shown. It will be noted that on some farms the disease re-occurred three years in succession while on other farms no infection occurred after 1922. A marked spread of the disease can be noted in 1924. The survey was made in detail in only the townships of Burke and Sun Prairie in 1924. The survey from which this map was made was supported by the Wisconsin State Department of Agriculture.

month. The wildfire organism may lose all or part of its virulence under relatively favorable conditions for the growth and storage of the organism. The fact that it has favorable conditions for multiplication outside of the host is not necessarily conducive to continued pathogenicity. That the organism can and does retain life and pathogenicity upon such materials as seed or leaves in the dry and latent state is evident from the overwintering tests. Under other conditions, whether the material which harbors it is dry or sufficiently moist to favor growth, it may rapidly die out or lose its virulence as evidenced by the results with soil, cloth and wood. It seems evident, however, that whether the wildfire organism is in all cases really killed or merely loses its virulence has not been actually determined in overwintering experiments conducted thus far. Studies along this line may explain some of the peculiar cases of behavior in overwintering studies.

The Wildfire Toxin

The common and characteristic halo surrounding the ordinary wildfire lesion, the less common chlorosis of bud leaves which sometimes occurs, with few, if any, organisms in the chlorotic area is evidence that a toxic substance is produced by the wildfire organism which is apparently soluble.

To obtain further information on this point, cultures of the wildfire organism on potato agar were suspended in water and filtered through a small Berkfeld filter. The filtrate proved sterile on plating and was used for inoculations on tobacco in the ordinary way. Typical halos were produced by the sterile filtrate in one to two days. Platings from these spots showed that they were sterile (Plate VI). These tests, with other modifications, were repeated five times with similar results. The wildfire organism produces toxin which, though greatly diluted, is very effective in rapidly producing chlorosis in plants. The wildfire bacteria when washed free of this toxin required several days longer to produce typical lesions than did the toxin alone. This observation is of considerable significance in work with the wildfire organism and led us to reconsider some of the previous experimental work here as well as that of others, and explained some observations previously not understood. It was always noted, for instance, that the wildfire organism required several days to produce any signs of infection from some of the overwintered materials, whereas 24-48 hours sufficed for cultures. It is evident that the presence or absence of already formed toxin had much to do with this result.

Most of the determinations on overwintering and influence of other environmental conditions on the pathogenicity of the wildfire organism have been based on inoculations of living plants. It is possible, therefore, that these results apply more particularly in some cases to the effect of the toxin than on the organism itself. Anderson, for instance, found that alternate freezing and thawing did not kill the wildfire organism, since inoculations with the exposed cultures produced typical

infection. In experiments here, cultures exposed to alternate freezing and thawing for short periods also gave infection, but these same cultures failed to give growth on other media when transfers were made from them some time after the exposure. It seems evident that the organism was largely, if not wholly, destroyed, but that the toxin was not injured.

The loss of virulence or pathogenicity, as noted in the previous chapter, is no doubt related at least in part to a tendency of the organism to continue to produce toxin in culture. Apparently the observation that old cultures readily produce symptoms on plants, as compared with subsequent transplants, is not the result of any greater virulence of the parasite, but is rather a consequence of the transplanting of the toxin previously produced.

Similarly, the question may be raised as to whether the symptoms produced on a plant, as a result of introducing the toxin through a wound, justify including the plant in the host range of the parasite. In work with the host plants of *B. tabacum* (10) no distinction was made as between the toxin and the organism, in all cases probably inoculating with both. Some of these trials have been repeated sufficiently, however, to justify the belief that in most cases, at least, the organism was actually parasitic, although the initial symptoms may have been produced by the toxin introduced from cultures. The records show, however, that in practically all cases infection was obtained by spraying the inoculum on unwounded plants, as well as by wound inoculations.

Practical Considerations

With the appearance of a new disease of economic importance, the quick demand for control measures often requires the dissemination of such information as may be rapidly gained from limited experimental data, together with deductions from what is known about similar diseases. When the problem is subsequently more thoroughly investigated, it is natural that the relative importance of the earlier recommendations will be altered, possibly some eliminated and others added. This essentially has been the history of the development of wildfire control measures. Control measures can best be applied by adequately understanding a disease and selecting and using control measures that apply best to the case at hand, rather than by blindly following directions.

The results secured from the investigations described in this bulletin do not fundamentally change the principles of control which have been previously recommended by this Station and by workers in other states. They corroborate previous results based on meager data, justify or eliminate certain recommendations which were in doubt, in addition to altering some of the methods.

The fundamental consideration which should be kept in mind in controlling the wildfire disease is that it is of an infectious nature and that measures for its control are, therefore, based largely on efforts to prevent its introduction in the first place into the seed beds, and failing

in this the necessary precautions should be taken to prevent its introduction into the field.

When wildfire has occurred on a farm in the preceding season, it is evident from our experiments that any material which may harbor even extremely small pieces of infected plant tissue may be a possible source of infection to the new plant beds if permitted to reach them, and favorable weather conditions for the infection follow. It is believed, however, that the actual dissemination of infected material from the curing sheds, where the material has remained dry, to the seed beds is one of the most common sources of infection. Seed bed frames or covering, or any other material coming in contact with the seed beds should not be stored in the curing sheds, where infested tobacco hangs, without being thoroughly cleaned and disinfected before using. It is not believed that wood or cloth readily harbors the wildfire organism except as it carries pieces of infected leaf tissue. To further insure sanitary seed bed conditions it is believed advisable to locate the seed beds a considerable distance from the farm buildings or, at least, from the curing shed, since various agencies may easily carry infected material which may be harbored in or about buildings to the plant beds when they lie close at hand. The refuse from the preceding year's crop should be burned or buried to reduce danger of its dissemination.

It is an unsafe practice to sow seed grown in fields infected with wildfire, without adequate disinfection. Corrosive sublimate cannot be used for disinfecting seed when seed is to be sprouted before sowing. Silver nitrate, one part to one thousand parts of water, is a satisfactory disinfecting agent, but two treatments of 5 or 10 minutes each, allowing the seed to dry between treatments, is believed necessary to insure complete disinfection. Some retardation to germination usually occurs. Seed should preferably not be sprouted in the same cloth used in disinfection.

The wildfire organism dies out in a comparatively short time in moist soil. Planting in previously infested fields is believed to be a safe practice, especially if the "stubble" from the preceding crop is plowed under in the fall.

If infection occurs in the seed beds it is hazardous to use even apparently healthy plants from such beds. It is believed to be a good plan to construct small beds, separated by paths, rather than to use large, continuous beds in which infection can spread more readily. In this manner the seed bed areas not infected can be used with greater assurance of safety.

At the first signs of infection in the seed beds, effort should be made to destroy the infected plants together with the immediate surrounding area. After trying out various methods, the conclusion has been reached that the most convenient and cheapest way to do this is simply to cover these areas with three or four inches of soil.

In case a heavy early infection develops in the field, plowing under and replanting with healthy plants should be given serious consideration. If plowing is not done, all infected plants should be removed before re-

planting. Removing the infected plants only or picking off infected leaves is of doubtful value in checking the disease if the disease is scattered throughout the field, even on only a small percentage of the plants.

Working in a wildfire infected field when the plants are wet is conducive to spreading the disease, if the plants are of such a size that the leaves are touched consecutively.

It is believed that the methods of control worked out and suggested for wildfire apply equally well for the similar tobacco disease known as blackfire (Plate II, top).

SUMMARY

1.—Practically every case of wildfire infection in the field can be traced to seed bed infection. This is borne out by three years of observation of the disease in Wisconsin, as well as being supported by reports from various other states. The control of wildfire is, therefore, almost entirely a matter of preventing seed bed infection by the wildfire organism.

2.—Locating the materials which are the common sources of carrying the wildfire organism to the seed beds is, therefore, an important phase of the development of control methods. This involves determining on which of the various materials likely to come in contact with seed beds the bacteria causing the disease are most likely to overwinter.

3.—Other things being equal, the wildfire organism lives over winter most readily in the dry and dormant condition.

4.—The wildfire bacteria readily overwinter on infected tobacco leaves which are cured or dried and which remain dry between growing crops. Small amounts of infected tobacco trash may accidentally reach tobacco seed beds in a number of different ways and this material is believed to be a common source of infection.

5.—To reduce chances of infection from overwintered material in the curing sheds, it is advisable to locate the plant beds a considerable distance from the curing sheds or other places which may have harbored infected dry tobacco over winter.

6.—The wildfire organism can readily live over winter on the seed. In fact, the experiments indicate that it can live in the dormant stage as long as two years on seed. Seed is not, however, apparently a common source of infection, although it may become so. It is not to be regarded as good practice to sow seed from infected fields, unless they have been adequately disinfected.

7.—The wildfire organism does not seem to remain alive as readily on wood or cloth, even when kept dry, as on leaf tissue or seeds, but since these materials may readily harbor infected leaf fragments, especially if stored in the curing sheds, the cleaning and disinfection of seed bed frames and covers may frequently be advisable.

8.—As far as can be experimentally determined the bacteria do not over-winter in moist soil, consequently there is no danger, as far as known, in using land for tobacco which has grown a previously infected crop, especially if the refuse and stubble from the preceding crop are thoroughly plowed under.

9.—Plants from infested beds preferably should not be used for transplanting. If weather conditions are favorable for the disease an extremely small amount of seed bed infection may result in heavy field infection. In a small percentage of cases this seed bed infection may be so small as to escape even careful inspection.

10.—Spread of the disease in the field is almost entirely dependent upon rainfall, especially with strong winds. No satisfactory measures to prevent the spread of the disease in the field are known.

11.—While dusting with copper lime dust or spraying with Bordeaux mixture in the seed beds, reduce the amount of infection, these procedures are not believed to prevent seed bed infection sufficiently to materially reduce the amount of subsequent field infection if conditions for the spread of the disease become favorable.

12.—Seed disinfection with corrosive sublimate cannot be used where seed is to be sprouted before sowing. After trying out a number of seed disinfecting agents, it was found that a solution of silver nitrate (1-1000) gives the best results. It is believed, however, that two 5- or 10-minute treatments (drying the seed between treatments) is required to adequately disinfect infected seed for wildfire control.

13.—The wildfire organism may often lose its virulence in culture in a relatively short time. Many factors seem to be concerned in this loss of virulence, among them being the nature of the culture medium, the frequency of transfer and "strain" differences in the organism.

14.—The wildfire bacteria produce a toxin in host tissue and in cultures which is responsible for the chlorosis produced in plant tissue. This toxin is readily separable from the bacteria by filtration and will in itself produce typical symptoms by inoculation. This fact should be taken into consideration in further studies on such subjects as host relations, overwintering, loss of virulence, and culture studies.

TABLE 1.—COMPARISON OF DIFFERENT MATERIALS AS OVERWINTERING SOURCES. INOCULATED WITH PURE CULTURES AND EXTRACT FROM GREEN INFECTED LEAVES. (SERIES I.—1922.)

No.	Infested material	Source of Inoculum	Percentage[1] of infection after													
			Days						Months							
			1	2	4	8	16	32	2	4	5	6	7	8	9	10
1	Seed	Pure culture	100	100	100	78	62	88	22	90	80	82	....	+	+	+
		Green leaves	22	24	50	22	18	62	6	20	8	40	....	+	.	.
2	Soil (moist)	Pure culture	100	100	100	44	30	0	0	0	0	0	0	0	0	.
		Green leaves	60	100	78	20	48	0	0	0	0	0	0	0	0	.
3	Cloth	Pure culture	14	12	16	40	8	4	0	0	0	0	0	0	0	.
		Green leaves	40	78	36	38	24	34	0	0	0	0	0	0	0	.
4	Boards	Pure culture	70	66	50	78	38	?	0	0	0	0	0	0	0	.
		Green leaves	48	40	44	12	42	?	0	0	0	0	0	0	0	.
5	Dried green leaf pulp	Green leaves	4	6	4	10	16	28	8	0	. . .	. . .	. . .	0	.	.
6	Extract from green leaves used for 1, 2, 3, 4	Green leaves	2	4	4	0	0	0	0	0	. . .	. . .	. . .	.	.	.
7	Water suspension used for 1, 2, 3, 4	Pure culture	2	2	2	0	0	0	0	0	. . .	. . .	. . .	.	.	.
8	Dried leaves	Leaves	....	....	....	....	90	92	92	96	. . .	80	78	+	+	.
9	Cured leaves	Leaves	....	....	....	....	96	94	90	84	. . .	60	70	+	?	+
10	Control		0	0	0	0	0	0	0	0	. . .	0	0	0	0	.
11	Control	Pure culture	94	92	90	96	100	92	96	98	....	88	90	+	+	+
12	Buried leaves with and without soil contact	Green leaves	....	....	....	....	....	....	....	....	....	....	....	....	0	0

[1]The method of inoculation was not the same in all cases. As a whole the percentages up to two months are based on fifty wound inoculations on single plants. The 4, 5 and 7 month inoculations were made from colonies plated from the infected material and the remainder were direct inoculations to seedlings in flats. + = infection but percentage not determined.

TABLE II.—COMPARISON OF DIFFERENT MATERIALS AS OVERWINTERING SOURCES. INOCULATED WITH PURE CULTURES AND EXTRACT FROM GREEN INFECTED LEAVES. (SERIES II.—1922.)

No.	Infested material	Source of inoculum	Percentage[1] of infection after										
			Days							Months			
			2	8	13	16	32	46	2	3	4	7	9
1	Seed	Pure culture	26	14	22	16	40	32	24	+	+	+	+
		Green leaves	52	22	58	12	26	24	42	+	+	+	+
2	Soil (moist)	Pure culture	20	?	4	0	0	0	....	0	0	0	0
		Green leaves	4	?	4	12	0	0	....	0	0	0	0
3	Cloth	Pure culture	4	6	16	6	0	0	0	...	0	0	0
		Green leaves	16	34	82	40	0	0	8(?)	....	0	0	0
4	Boards	Pure culture	18	?	12	10	0	0	?	0	....	0	0
		Green leaves	2	?	6	16	0	0	?	0	....	0	0
5	Tobacco stalks	Pure culture	4	20	38	4	?	....	?	?	....	+	0
		Green leaves	2	8	4	10	?	....	?	?	....	+	0
6	Liquid inoculum used for 1 to 5	Pure culture	2	0	0	0	0	0	....	....	....	0	0
		Green leaves	?	0	0	0	0	0	....	....	....	0	0
7	Green leaf pulp	Green leaves	?	24	28	14	0	0	....	....	....	?	0
8	Control	....	0	0	0	0	0	0	0	0	0	0	0
9	Control	Pure culture	+	+	+	+	+	+	+	+	+	+	+

[1]Where percentages are given they are based largely on fifty wound inoculations on single plants. The 4, 7 and 9 month inoculations were on seedlings in flats. + = infection but percentage was not determined.

TABLE III.—COMPARISON OF DIFFERENT MATERIALS AS POSSIBLE OVERWINTERING AGENTS WHEN INOCULATED WITH DRIED INFECTED LEAVES. (1923.)

Infested material	Condition of storage	Test No. 1		Test No. 2		Test No. 3		Test No. 4		Test No. 5	
		Age in months	Lesions per 100 plants	Age in months	Lesions per 100 plants	Age in months	Lesions per 100 plants	Age in months	Lesions per 100 plants	Age in months	Lesions per 100 plants
Cloth	Dry	1½	42	3¼	13	5	6	6½	4	7½	5
Boards	Dry	1½	74	3¼	84	5	56	6½	26	7½	70
Tobacco stalks	Dry	½	79	2¼	39	4	81	.....	.....	6½	163
Seed	Dry	.....	.....	2	73	.....	.....	5	18	6	71
Soil (as in shed)	Dry	¾	93	2½	119	4¼	134	5¾	88	6¾	174
Soil (as in field)	Moist	1½	0	3¼	0	5	0	6½	0	7½	0
Rotting leaves	Moist	1½	0	3¼	0	5	0	.....	.....	7½	0
Dried leaves	Dry	½	104	.....	.....	4	128	5½	79	6½	167
Cured leaves	Slightly moist	.....	.....	.....	.....	4	8	5½	6	6½	2

TABLE IV.—INFLUENCE OF STORAGE CONDITIONS ON OVERWINTERING ON VARIOUS MATERIALS. (1923.)

Inoculated material	Stored	Test No. 3		Test No. 4		Test No. 5	
		Age in months	Lesions on 100 plants	Age in months	Lesions on 100 plants	Age in months	Lesions on 100 plants
Cloth	Inside	5	6	6½	4	7½	5
	Outside	5	19	6½	5	7½	3
Boards	Inside	5	56	6½	26	7½	70
	Outside	5	50	6½	37	7½	81
Dry Soil	Inside	4¼	134	5¾	88	6¾	174
	Outside	.....	.....	5¾	91	6¾	187
Tobacco stalks	Inside	4	90	5½	74	6½	128
	Outside	4	108	5½	89	6½	144
Dried crushed leaves	Inside	4	128	5½	79	6½	167
	Outside	.....	.....	5½	98	6½	186

TABLE V.—THE INFLUENCE OF SOIL CONDITIONS ON THE WILDFIRE ORGANISM. (1923.)

Condition of inoculated soil	Inoculum used for soil	Test No. 2		Test No. 3		Test No. 4		Test No. 5	
		Age in months	Lesions on 100 plants	Age in months	Lesions on 100 plants	Age in months	Lesions on 100 plants	Age in months	Lesions on 100 plants
Air-dry soil	Infected dried crushed leaves applied wet	3¼	0	5	0	6½	0	7½	0
Moist soil	Infected dried crushed leaves applied wet	3¼	0	5	0	6½	0	7½	0
Air-dry soil	Infected dried crushed leaves applied dry	2½	119	4¼	134	5¾	88	6¾	174
Air-dry sterile soil	Pure culture	1¼	127	3	95	4½	108	5½	222
Moist sterile soil	Pure culture	1¼	186	.....	.....	.....	.....	5½	291
Wet sterile soil	Pure culture	1¼	145	3	152	4½	99	5½	112
Control	Infected dried crushed leaves	.....	.....	4	128	5½	79	6½	167

TABLE VI.—INFLUENCE OF MOISTURE ON OVERWINTERING OF WILDFIRE ORGANISM. (1923.)

Infested material	Condition of storage	Test No. 1		Test No. 3		Test No. 5	
		Age in months	Lesions on 100 plants	Age in months	Lesions on 100 plants	Age in months	Lesions on 100 plants
Rotting green leaves	Moist	1½	0	5	0	7½	0
Rotting dried leaves	Moist	1½	0	5	0	7½	0
Dried crushed leaves	Dry	½	104	5½	79	6½	167
Cloth inoculated with infected green leaves	Dry	1½	1	5	0	7½	0
Cloth inoculated with infected dry leaf suspension	Moist for a few hours then dry	1½	42	5	6	7½	5
Air-dry soil inoculated with dry crushed leaves applied wet	Moist for a few hours then dry	1½	0	5	0	7½	0
Air-dry soil inoculated with dry crushed leaves applied dry	Dry	¾	93	4¼	134	6¾	174

TABLE VII.—DISSEMINATION OF WILDFIRE IN FIELD. PLOTS IN ROW FROM WEST TO EAST. ONE HUNDRED AND TWENTY PLANTS PER PLOT EQUALLY SPACED.

Plot	Planting (June 28)	Average number of leaves infected per plant Sept. 25	Remarks
1	All "slightly diseased"	10.6	Most of the infection in this plot believed due to infestation which was not visible at time of planting
2	All healthy	3.9	Infected from neighboring plots
3	All healthy except 5	10.3	Infection largely from the 5 infected plants in the plot
4	Alternate rows of healthy and slightly diseased	14.5	All about equally diseased
5	Successive rows of healthy, slightly, considerably and badly diseased. Rows north to south	14.2	All about equally diseased
6	Same as 5 but rows east and west	13.3	All about equally diseased
7	All healthy plants after handling diseased plants	14.5	Amount due to handling questionable due to plot lying in direction of general spread
8	All healthy	13.5	Infection all due to spread from other plots

TABLE VIII.—PERCENTAGE OF PLANTS INFECTED AND AVERAGE NUMBER OF INFECTIONS PER PLANT FOLLOWING DUSTING AND SPRAYING FOR WILDFIRE IN GREEN-HOUSE FLATS.

Condition of leaves	Fungicide applied	Fungicide used	No. of applications		Percentage of plants infected			Av. No. infections per plant
			Parasite	Fungicide	Series I	Series II	Av.	
Wounded	Before inoculation	Nu Rexo Dust	3	3	78	75	76.5	2.1
		Bordeaux Mixture Spray	3	3	65	76	70.5	4.0
	After inoculation	Nu Rexo Dust	3	3	81	82	81.5	4.7
			3	3	90	89	89.5	3.3
			3	6	77	63	65.0	2.6
			2	2	87	82	84.5	3.6
		Bordeaux Mixture Spray	3	3	75	88	82.5	5.2
		Fungi-Bordo Dust	3	3	72	69	70.5	2.9
		Commercial Bordeaux Spray	3	3	87	90	88.5	4.9
		None Inoculated control	3	0	97	94	95.5	4.9
			3	0	98	93	95.5	6.8
		None Uninoculated control	0	0	0	0	0	0
Not Wounded	Before inoculation	Nu Rexo Dust	3	3	64	60	62.0	1.8
		Bordeaux Mixture Spray	3	3	42	50	46.0	1.7
	After inoculation	Nu Rexo Dust	3	3	74	77	75.5	3.8
			3	4	59	56	57.5	2.0
			3	5	48	59	53.5	1.5
			2	2	59	57	58.0	2.0
		Bordeaux Mixture Spray	3	3	63	83	73.0	4.1
		Fungi-Bordo Dust	3	3	43	58	50.5	1.8
		Commercial Bordeaux Spray	3	3	90	57	73.5	4.9
		None Inoculated control	3	0	76	71	73.5	3.3
		None Uninoculated control	0	0	0	0	0	0

TABLE X.—AVERAGE PERCENTAGE OF INFECTION WITH *Bacterium Tabacum* CARRIED ON DIFFERENT CULTURE MEDIA FOR 15 MONTHS WITH WEEKLY, MONTHLY AND WITHOUT TRANSFER. AVERAGE OF 11 SERIES OF INOCULATIONS.

Culture Media	Strain I			Strain II			Strain III			Average of I, II and III		
	Weekly	Monthly	None	Weekly	Monthly	None	Weekly	Monthly	None	Weekly	Monthly	None
Potato dextrose agar pH 5.8–6.2	67.7	56.7	41.4	68.0	60.8	44.4	72.3	66.2	49.5	69.3	61.2	44.1
Bouillon pH 6.3–6.6	57.8	52.1	35.1	55.1	62.1	57.6	50.9	54.4	57.8	54.6	56.2	50.2
Beef-peptone agar pH 6.1–6.3	27.5	37.7	39.6	31.2	41.8	28.0	25.4	36.8	69.0	28.0	38.8	45.5

TABLE IX.—PERCENTAGE OF PLANTS INFECTED FOLLOWING DUSTING WITH DIFFERENT MATERIALS FOR THE CONTROL OF WILDFIRE IN GREEN-HOUSE FLATS.

Material used	Percentage of infection		
	Series I	Series II	Average
Sander's Dust	75	63	69.0
Limate	58	79	68.5
None (inoculated control)	84	79	81.5
Fungi-Bordo Dust	64	84	74.0
Calcium caseinate ("Kayso")	49	65	57.0
Soil Dust	96	90	93.0
None (uninoculated control)	0	0	0

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Transmission of Viruses From Apparently Healthy Potatoes

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Transmission of Viruses From Apparently Healthy Potatoes[1]

DURING the course of cross-inoculation studies on certain virus diseases of solanaceous plants, it was noted that symptoms were secured on tobacco from potatoes selected as healthy controls, and that these symptoms did not materially differ from those secured when various virus diseases of the potato were used as a source of inoculum.[2] An investigation of this matter, therefore, was undertaken and it became increasingly evident that extracts from potato plants which are healthy, within the ordinary meaning of this term, are capable of inducing symptoms of disease in tobacco and other solanaceous plants. Furthermore, the ability of inducing this disease is apparently universally present within most, if not all, of the standard varieties of potatoes. Three distinct symptoms have been secured which are associated with at least two and probably three distinct viruses. These viruses behave like those of most virus diseases of plants as regards transmissibility, and have been not only transferred repeatedly through several generations of tobacco, but have also been used to infect a wide range of other solanaceous plants. In fact, one of these virus diseases when inoculated back into the potato, after having existed in tobacco for one or more generations, produces under the proper environmental conditions a most malignant disease.

Many problems have naturally developed during the course of these investigations, centering around an explanation of these results. As far as can be judged at present only two theories appear to be logical. Either potatoes are almost universally infested with viruses or they are capable of initiating virus diseases in other plants. Whether actual proof of one or the other of these theories can be established remains to be determined by further experimentation. In the meantime it has been thought advisable to present the data secured up to this time in summarized form, with brief discussion of some of the more important features of the problem.

Experimental Methods

The potatoes used in practically all cases have been grown in a low temperature (17-22° C.) green-house suitable for a good development of the potato. In connection with other experiments similar plants have been subjected to a wide variety of environmental conditions, including high temperatures, and in no case have selected healthy potatoes exhibited any symptoms of a disease comparable to those to be described as a result of these changes in environment. The Triumph variety of potatoes was used in all experiments unless otherwise mentioned.

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[1]Cooperative experiments with Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture.

[2]Johnson, James. A virus from potato transmissable to tobacco. Phytopath. (abstract) 15: 46-47, 1925.

The tobacco plants and other host plants used were grown in a high temperature green-house (27-32° C.). The plants were grown in very fertile soil, transplanted to 4-inch pots and usually inoculated when very young, i. e., with only two to four leaves large enough to be inoculated. The potato or other foliage used for inoculum was crushed in a small sterile mortar, the juice strained through cheese-cloth, and inoculated by means of sterile needles wrapped at the end with a small wad of absorbent cotton to more readily carry drops of the inoculum. Twenty to forty punctures per plant were usually made in the leaf blade and midribs, although experiments showed that a fair percentage of infection could be secured by a much smaller number of punctures.

In the early experiments, ten plants were usually used in each series of inoculations. In later experiments only five plants were used. This number gives equally reliable information except in cases of negative results, in which case, however, the experiment has always been repeated with the same source of inoculum. Infection was sometimes evident in six days, although ten to fourteen days were usually required for final counts.

The strain of tobacco used in practically all the experiments was the common commercial variety grown in Wisconsin, Connecticut Havana No. 38. Several other of the more distinct varieties of *Nicotiana tabacum* have been tried sufficiently to warrant the belief that probably no important varietal differences exist as regards susceptibility to these diseases.

Inoculations with Diseased Potato Foliage

In connection with the earlier work, and in later work where mosaic potatoes were used as controls, inoculations have been made from 28 different Triumph potato plants with mosaic symptoms. This involved inoculation to 210 tobacco plants, infection being secured on 154 or 73 per cent of the plants inoculated. The potatoes used were mostly Wisconsin grown, coming, however, from several different farms.

Inoculations have been made from fourteen other diseased potatoes showing yellow-dwarf, spindle tuber, leaf roll, rugose mosaic, and other symptoms not clearly defined. These plants come from tubers grown in such widely separated sections as Maine, New York, Wisconsin, and Oregon, as well as being of different varieties. One hundred and five inoculations were involved, infection being secured on 71 plants or 68 per cent. No consistent differences were noted between the symptoms secured from the different diseased potatoes used as sources of inoculum.

Inoculations with Apparently Healthy Potato Foliage

The potato plants used in this group of inoculations have been largely of the Triumph variety grown in Wisconsin. In connection with a tuber indexing project single eyes have been grown from over 12,000 different tubers, coming from twenty different farms during the past winter. These potatoes grown at a low temperature (17-22° C.) in the green-house in fertile soil have given an exceptional opportunity for the selection of

normal plants as the source of inoculum. From this stock, however, approximately only 170 healthy plants have been used, over fifty of these coming from stock indexed for mosaic the previous season and grown in isolated plots.

In addition healthy plants have been used from tubers of different varieties, coming from Maine, Michigan, Idaho, Oregon, Colorado and Florida. These potatoes have included in addition to the Triumph such varieties as the Green Mountain, Irish Cobbler, Rural New Yorker, Early Ohio, Burbank, People's Russet, Peach Blow, King and Brown Beauty. The plants were usually selected for inoculum purposes when very young, showing usually only four or five leaves, the plants being only three to six inches high. In the case of about 150 of these plants only two or three of the basal leaves were used as the source of inoculum. The plants were then transplanted to 6-inch pots and allowed to grow for an additional two months (Plate IV). During the course of this time not a single one of these plants selected as healthy showed any symptoms of potato mosaic or any other disease.

Inoculations have been made from a total of 170 healthy Triumph potato plants, involving 965 inoculations to tobacco. Infection was secured on 681 plants or 70 per cent of the plants inoculated. In about 2 per cent of the cases the potato plants used failed to give any symptoms in the first trial, but whenever this occurred a second inoculation was made and in every such case positive results were then secured. Consequently, all potatoes tested in this series regardless of the source or variety have yielded the symptoms in question on tobacco. The different experiments naturally have not been equally successful in the percentage of infection obtained. In some experiments four or five infected plants out of five inoculated were the rule, in others only one to three were the rule. These differences are believed to be due in part to environmental differences or to variations in predisposition of the different lots of plants upon which the inoculations were made.

In experiments in which different varieties of potato were used, 20 plants (two of each) were tested. One hundred and twenty plants were inoculated, infection was secured with 66 plants, that is with 55 per cent of the plants inoculated. As a rule, no significiant or consistent differences in expression of symptoms were secured from different varieties. Some varieties, however, seemed to give a higher percentage of infection than others. The Rural variety, especially, was found to be unlikely to give positive results, unless conditions were especially favorable. It should be stated in this connection that we have secured at least two or possibly three distinct types of symptoms from healthy potatoes, although they are not apparently limited to particular varieties. The Rural variety is distinctly different from the other varieties studied in that it is least likely to yield the "mottle" type of disease and most likely to yield the "ring-spot" type. The difference between these symptoms will be described later.

Inoculations from Tubers and Other Organs of Potato

A number of inoculations were made to tobacco directly from the potato tubers themselves, rather than from plants grown from such tubers. For convenience, no distinction was made in this group of inoculations between the use of healthy and mosiac tubers, since in about one-half of the cases the condition was not definitely known, the distinction being in any case apparently immaterial to the problem under consideration. The tubers used in these inoculations came from the various sources previously referred to and included all the varieties previously mentioned. The inoculations were usually made with extract secured by scraping the flesh of a freshly cut tuber and straining through cheese-cloth. Altogether 63 different potato tubers have been used, involving inoculations to 365 plants with a total of 130 plants (38 per cent) infected. The percentage of infection is considerably lower than that secured from potato foliage. Apparently, the infective principle exists in a less virulent or a more diluted form in the tuber than in the foliage, although this conclusion need not necessarily follow from the foregoing results.

In two sets of experiments a considerable number of tubers failed to give infection. It was decided, therefore, that it is simpler for present purposes to use the growing plants as sources of the inoculum, the plant in any case having to be grown to determine whether the tuber used was healthy or diseased. Our data, therefore, show a considerable number of negative results with tubers, but in every such case a plant was grown from the same tuber and positive results secured from this. The infective agent does not appear to be localized in the tuber, interior areas of tuber flesh giving infection as well as the cortical zone. Young white sprouts gave good infection and extract from the stem or root tissues gave as good infection as did that from the green leaves.

Symptoms of the Diseases

The symptoms usually secured on tobacco on inoculation directly from potato is a regular pattern of mottling so obscure or mild that it readily escapes casual observation. On close observation in comparison with healthy controls, however, there is usually no difficulty in recognizing the symptoms, although in some cases it is difficult for even the trained eye to distinguish the diseased from the healthy leaf. On the other hand, in many cases the mottling is especially marked, (Plate 1A), and may in some cases be accompanied by necrosis, (Plate 3E).

The pattern of the mottling has no resemblance to that in ordinary tobacco mosaic. The latter is usually most conspicuous on the youngest bud leaves, whereas in the case of the disease from the potato the bud leaves are normal and symptoms appear only on the older leaves of the young plants (Plate 3B, F). General chlorosis, leaf distortion and stunting are usually absent in the first transfer on tobacco, although in subsequent transfers to tobacco marked necrosis and stunting may occur. The pattern of the mottling and necrosis in these subsequent transfers is quite characteristic though not uniformly so (Plate 1C).

As previously indicated, in inoculations from Rural New Yorker potatoes, it was noted that the symptoms of mottling secured from other varieties were rare or entirely lacking. In a small percentage of the inoculated plants, however, a distinctive necrotic symptom developed (Plate 2B) which resembled a disease which had been occasionally noted in field tobacco, and which has been referred to by some observers as "ring-spot". This symptom has been noted subsequently, however, to occur in inoculations from other varieties than the Rural, and it is apparently associated at times with a preliminary mottling. That the "ring-spot" disease is physiologically distinct from that associated with the "mottling" and "spot-necrosis" types of symptoms secured from potatoes appears evident in subsequent transfers to tobacco and to other differential hosts.

This would seem, therefore, to indicate the existence of at least two distinct types of infectious agents in apparently healthy potatoes. During the course of most of the experiments the necrotic type of symptom commonly associated with mottling on tobacco has been regarded as merely a more virulent form of the latter. Whether this is true or whether this necrotic type indicates still another virus, i. e., a possible third type, in combination with the mottling type, has not been satisfactorily determined. Certain recent experiments lend probability to the idea of three distinct virus types.

While the ring-spot symptom, usually starting with ring-like chlorotic areas, leads to necrotic ring-spots, this development is distinctly different from the necrosis commonly associated with the mottling symptom. The latter form of necrosis frequently bears a relation to the direction of the principal veins in the early stages, (Plate 1B), and the entire leaf may subsequently collapse. At other times only small necrotic areas are formed which may subsequently become sufficiently numerous to cause a gradual death of the entire leaf. This is the form of disease which will later be shown to cause a striking disease when inoculated back to potato. For the purposes of the present discussion, therefore, reference to this is made as "spot-necrosis" in contrast with the other two symptom types, "mottle" and "ring-spot", although further studies may show the first two of these to result from the same virus.

The Infectious Nature and Increasing Virulence of the Viruses

It was at first supposed that the symptoms secured on tobacco from healthy potato might be the result of a toxin or other irritable substances, such as are well known in animal pathology, and consequently not belonging in the category of the virus diseases. Trials soon showed, however, that the transmission of the disease from tobacco to tobacco is more readily accomplished than is the original transfer from potato. In such subsequent tobacco transfers a higher percentage of infection is secured, (Table I), the incubation period is shorter and the symptoms are more marked. If it is considered that the "spot-necrosis" type of symptoms belongs with the "mottle" type, then very striking increase in virulence occurs as a result of passing the virus through one or more

generations of tobacco. Occasionally, however, the "spot-necrosis" form has been obtained on tobacco directly from healthy potatoes, but more often it develops following the transfer of the "mottle" form through one or more generations of tobacco. Increased intensity of the "mottle" and the "ring-spot" form have also been noted, and increased

TABLE I.—SUMMARY OF RESULTS OF INOCULATIONS TO TOBACCO WITH VIRUSES OF THE TYPE SECURED FROM APPARENTLY HEALTHY POTATOES.

Source of inoculum	Number used as inoculum	Number plants inoculated	Number plants infected	Per cent infection
Potato (Triumph) mosaic foliage	28	210	154	73.3
Potato (3 var.) 7 other virus diseases	14	105	71	67.7
Potato (Triumph) healthy foliage	170	965	681	70.5
Potato (10 var.) healthy foliage	20	120	66	55.0
Potato (10 var.) healthy tubers	20	120	30	25.0
Potato (Triumph) healthy and mosaic tubers	43	245	100	40.8
Potato (seedlings) healthy		110	5(?)	4.5(?)
Tobacco—infected with viruses from healthy potatoes		695	556	80.0
Tobacco—tobacco mosaic + virus from healthy potatoes		270	220	81.5
Other plants (18 species) healthy foliage		180	0	0
Controls—no inoculation		360	3(?)	0.8

virulence up to a certain limit is apparently established whether "spot-necrosis" is considered as a separate virus or not. This fact is of special interest, since as far as known such increase of virulence has not been definitely noted in other virus diseases of plants, although it is commonly observed with animal diseases and is also supposed to occur with some bacterial diseases of plants.

In the course of experiments conducted to throw some light on questions of infectivity, increased virulence and other properties of the virus, 695 plants have been inoculated and infection has been secured on 556 or in 80 per cent of the plants inoculated. This percentage of infection compares favorably with that ordinarily secured in experiments with other virus diseases. The virus has been passed through ten successive generations of tobacco, with but little if any changes in its behavior after the second or third generation. The change in virulence may be merely a result of changes in concentration of the virus. It also may develop that the number of transfers through tobacco is of little importance and that similar changes may occur as a result of the continued passage of the virus upward into the new leaves of the host plant, when allowed to develop to larger size than commonly used in our experiments. Neither of these ideas is substantiated by preliminary observation, however.

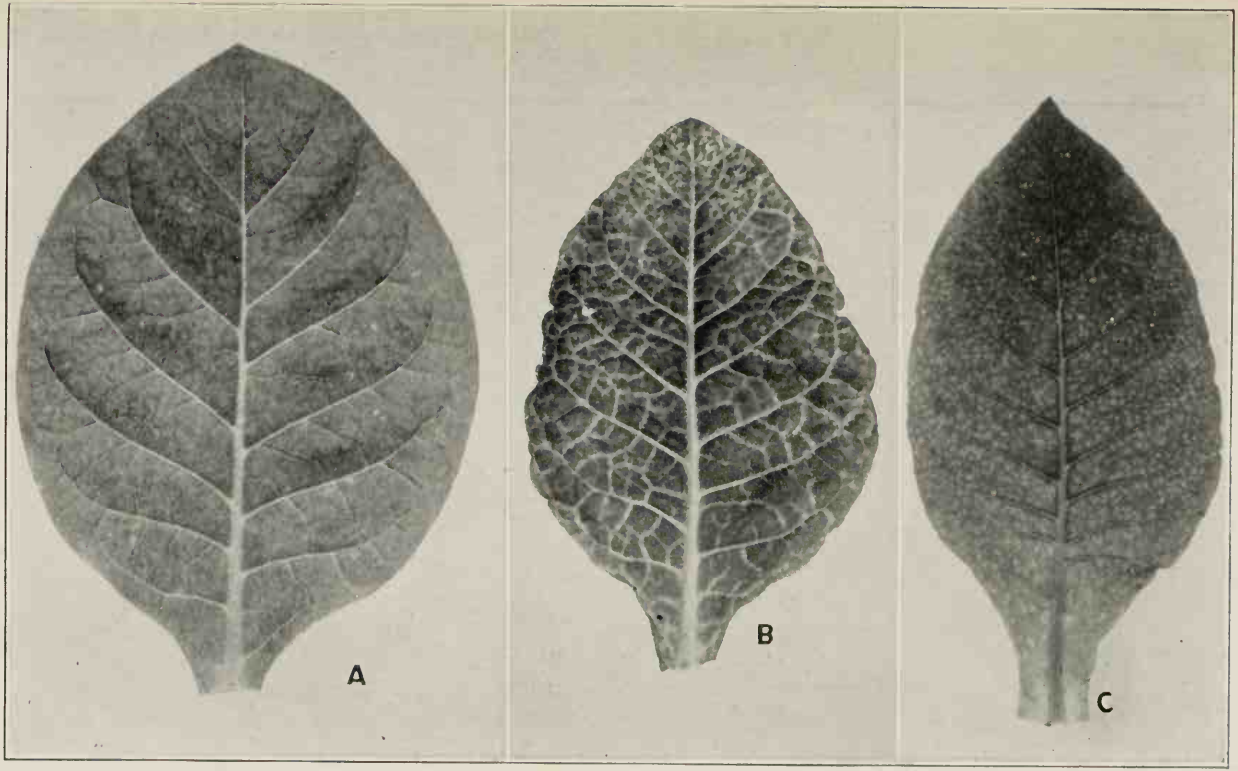

Plate I

Types of symptoms secured when extract from apparently healthy potatoes is inoculated into young tobacco plants.

A. "Motting" type commonly secured on first transfer from potato to tobacco.

B. Early stage of more virulent form commonly secured after one or more transfers through tobacco. Necrosis has started at the tip of the leaf.

C. The "spot-necrosis" type, on older leaf, a few weeks after inoculation. The chlorotic spots usually become necrotic.

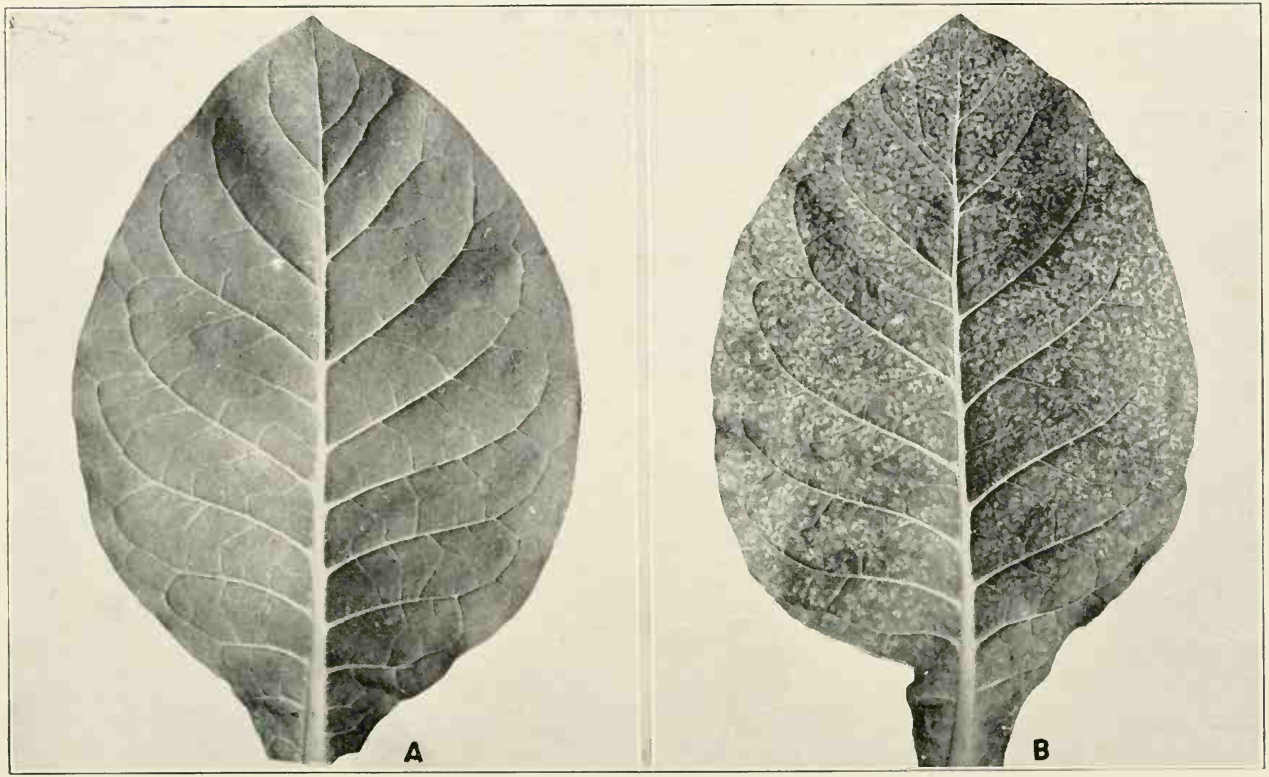

Plate II

The Rural New Yorker variety produces a distinct symptom.
A. A healthy tobacco leaf from a control plant.
B. The "ring-spot" type of disease, apparently more commonly secured when extract from Rural New Yorker potatoes is inoculated into tobacco.

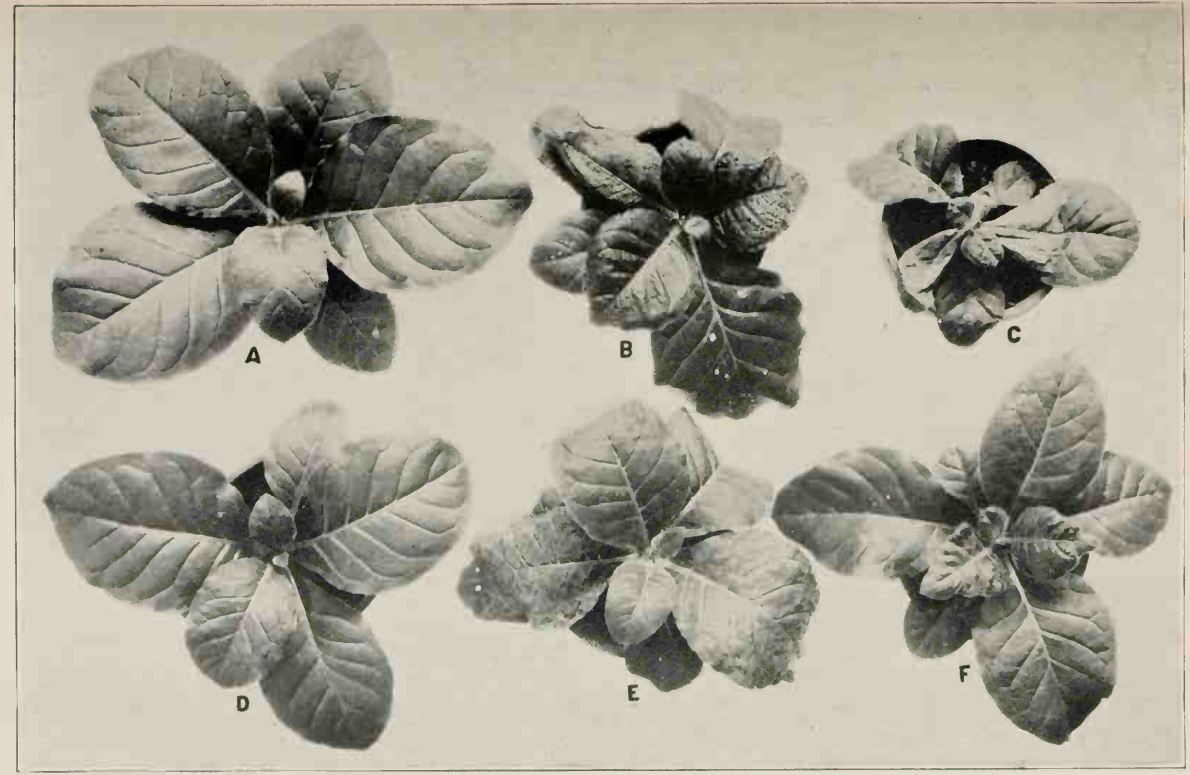

Plate III

Comparison of symptoms on young tobacco plants following inoculation with virus secured from apparently healthy potatoes, with that of ordinary tobacco mosaic.

A. Inoculated with healthy potato foliage. Mottling barely visible in photograph.

B. Inoculated with virus secured from healthy potato after three transfers through tobacco. Note symptoms are most marked on older leaves.

C. Inoculated with a combination of the virus from healthy potato and ordinary tobacco mosaic. This combination produces an especially malignant disease on tobacco and other solanaceous plants.

D. Uninoculated Control.

E. Tobacco inoculated directly with healthy Triumph potato. In a small percentage of cases marked mottling with necrosis occurs in the first transfer from potato as shown on the older leaves of this plant.

F. Inoculated with ordinary tobacco mosaic. Note that the symptoms are especially marked on the younger leaves as contrasted with B and E.

Plate IV

Type of potato vines used for source of inoculum.

A. An apparently healthy plant at the stage usually selected as a source of inoculum. Three or four basal leaves were usually used and the plant transferred to larger pots and allowed to continue growth for about two months.

B, C. Potato plants, several weeks after being used for inoculum, showing the plants remained healthy, as far as can be judged from the foliage.

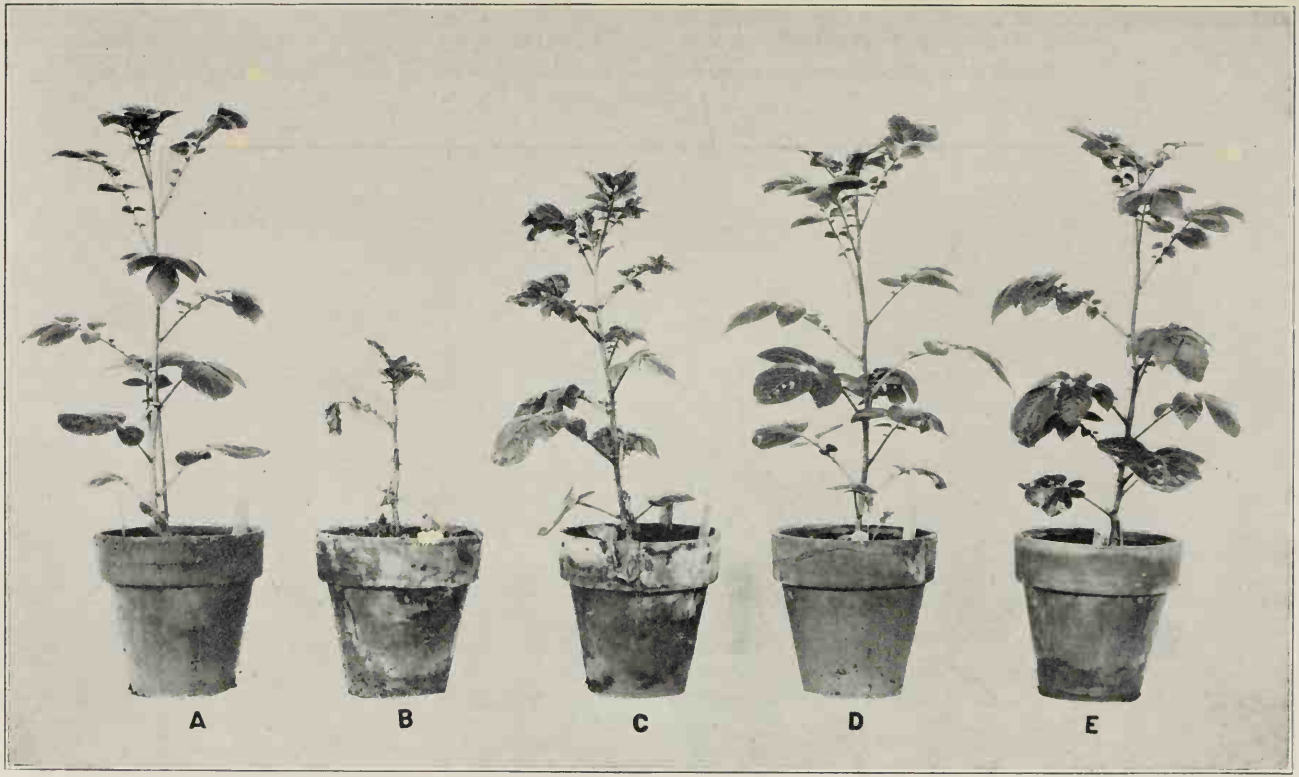

Plate V

Comparison of viruses secured from healthy potato when inoculated back to potatoes.

A. Control, not inoculated.
B. Inoculated with "spot-necrosis" from tobacco.
C. Inoculated with "spot-necrosis" (originally from healthy potatoes to tobacco) from potato after being transferred three generations through potato. Symptoms still marked, though less malignant than when taken directly from tobacco.
D. Inoculated with the "mottle" type from tobacco. No symptoms evident.
E. Inoculated with "ring-spot" from tobacco. No symptoms evident.

Plate VI

The virus secured from healthy potato is not ordinary potato moaic as shown by these inoculations to potato.

A. Control inoculated with extract from healthy potato. No symptons evident.

B. Inoculated with Triumph potato mosaic. Very marked and typical mosaic symptoms above points of inoculation.

C. Inoculated with "spot-necrosis" from tobacco. Although this virus was secured originally from healthy potatoes it is apparently very virulent when transferred back to potato.

Plate VII

Early, medium and late stages of infection on potato following inoculations with the "spot-necrosis" virus from tobacco.

Plate VIII

Tomato plant inoculated with a combination of tobacco mosaic and virus from apparently healthy potatoes showing early stage of disease. Control plant at right.

It should be noted in this connection that there is a decided tendency at times on the part of plants to recover following the first attack of the disease. This has also been noted in connection with other virus diseases of plants. Whether this is merely a form of partial or complete masking as a result of minor changes in environment, or represents a tendency on the part of the plant to develop less predisposition to the disease has not been determined. The latter suggestion is to some extent counteracted by recurring periods of attack by the disease.

The Properties of the Viruses

The properties of the individual viruses have not yet been studied in as great detail as they merit. Some of their more important characteristics, however, should be stated at this time, although it is expected that this subject will form the basis of a later paper. The viruses are apparently not as readily filterable as the virus of the ordinary mosiac disease of tobacco. However, they will pass through the coarser porcelain filters which yield a sterile filtrate. Transmission by aphids has given only very low infection from tobacco to tobacco. On the other hand, the potato aphid has given high percentage of infection of the "spot-necrosis" type from potato to potato or from potato to tobacco. These differences may be due primarily to the species of aphid used in relation to the host plant.

Experiments conducted to study the longevity of the virus outside of the living host, both in the liquid extract of the plant and in the desiccated condition, have given some variation in results, both as regards the particular virus used as well as between the separate experiments. In general these viruses are strikingly different from ordinary tobacco mosaic in that they are short-lived when separated from the living host. In most cases the survival is less than 20 days and frequently as low as 10 days. In general the virus survives longer in the drying leaf than in extracted plant juice.

The viruses seem to be considerably less resistant to heat than is the ordinary tobacco mosaic. The "mottle" and "spot-necrosis" types are apparently destroyed at about 70°C. in 10 minutes.

The "mottle" and "spot necrosis" type have been diluted up to one to five thousand and have still yielded good infection. Further dilution could no doubt be tolerated. Judging from preliminary experiments the viruses in question are relatively resistant to germicides and other chemicals, as compared with bacteria, being more similar to tobacco mosaic in that respect.

Trials with Potato Seedlings and Other Healthy Plants

Repeated attempts to induce the diseases in question in tobacco by inoculating with juice from the foliage of potato plants grown from true seed have failed or yielded only questionable symptoms. The seedlings used have been small for the most part, but in a few instances plants from tubers grown from seed the previous season were used with like results. The potato seedlings can, however, apparently be infected with the viruses, so

that they are not entirely resistant to it. Nothing is known, however, about the source of the seed used in these experiments with respect to variety relationship or to age. This phase of the problem needs further investigation as bearing on the origin of the viruses. It is evident from experience here with the Rural variety that potato varieties may be expected to differ in this respect and because of the heterozygous nature of potato seedlings they may be expected to be very variable in their infectious properties. Furthermore, it has been shown that the viruses obtained from potatoes are not resistant to desiccation, and that if they ever do exist within the seed coat they are probably destroyed by aging. The experiments preferably should be repeated with as young seed as possible secured from the Triumph variety.

Extracts from eighteen different species of healthy plants, mostly of the solanaceous family, have been inoculated into tobacco without yielding any symptoms of disease. So far as known, therefore, the potato is the only plant which produces infection on tobacco and other solanaceous plants when apparently healthy plants are used as a source of inoculum. In this category of "apparently healthy plants" there has not been included, of course, known susceptible hosts of mosaic diseases which may show no apparent symptoms on account of masking or other circumstances.

Other Host Plants

The diseases produced on tobacco from healthy potatoes are not specific for tobacco. It is believed that a number of widely different solanaceous plants might have replaced tobacco in these experiments. Infection, in fact, has been secured with one or more of the viruses in question on all of the solanaceous hosts tried. These include eight distinct varieties of *Nicotiana tabacum,* twenty-two species of Nicotiana and eight other species of the Solanaceae, namely tomato (*Lycopersccum csculcntum*), Physalis, (*Physalis pubcscens*), egg plant (*Solanum melongcna*), black night-shade (*Solanum nigrum*), jimson weed (*Datura stramonium*), pepper (*Capsicum annum*), petunia (*Petunia violaccae*) and buffalo burr (*Solanum rostratum*). A marked variation in symptoms naturally occurs on the different hosts. "Spot-necrosis" is considerably more malignant on certain other hosts than on tobacco, and the "ring-spot" virus apparently shows no typical symptoms on such hosts as tomato or pepper, only mottling or general necrosis occurring.

Transmission Back to Potato

In the earlier experience with the viruses secured from healthy potatoes, there was some reason to believe that the infection secured on tobacco might be potato mosaic, or some similar known virus disease of the potato existing either in the masked state or in a prolonged incubation period. It was consequently important to inoculate these virus diseases back to the potato from tobacco, in comparison with inoculations to potato from ordinary potato mosaic, as it commonly occurs on the Triumph variety.

It was believed at this time that the potato would prove to be very difficult to infect with the virus of ordinary potato mosaic and that a prolonged incubation period would be required, with the result that current symptoms could hardly be expected. Consequently, it was decided at once to try to improve on the technique by varying the environment, with the hope of shortening the process. In this there was apparently success, since in the later trials 90-100 per cent infection was invariably obtained in 10 to 15 days with either ordinary potato mosaic or the new "spot-necrosis" type. The percentages of infection shown in the summarized results (Table II) are greatly reduced by low percentages of infection secured in the early experiments.

The method used in these experiments consisted simply in placing the inoculated plants for a period of 8-10 days at a high temperature (27-32° C.) and then removing them to a lower temperature (17-22° C.) favorable at least for the expression of potato mosaic symptoms, and the general development of the potato itself. No doubt, this method can be further improved.

TABLE II.—SUMMARY OF RESULTS OF INOCULATIONS TO POTATO (TRIUMPH VARIETY) WITH VIRUSES OF TYPE SECURED FROM HEALTHY POTATOES IN COMPARISON WITH OTHER MOSAICS

Source of inoculum	Number of plants inoculated	Number of plants infected	Per cent infection	Symptoms
Tobacco—"spot-necrosis"	80	65	81.2	Mottling and leaf drop, plants frequently killed
Potato—"spot necrosis"	90	73	81.1	Disease similar but not as virulent as above
Tobacco—"mottle"	20	1(?)	...	One plant dead probably accidentally infected with "spot-necrosis"
Tobacco—"ring-spot"	20	0	0	None
Tobacco—tobacco mosaic	30	0	0	Lesions on stems and petioles
Potato (Triumph) potato mosaic	40	25	62.5	Typical potato mosaic
Tobacco—healthy foliage	50	0	0	None
Controls—no inoculation	50	0	0	None

As shown in Table II good artificial infection with ordinary Triumph potato mosaic was secured on the Triumph variety. These inoculations were made simultaneously with the inoculations from the three forms of virus diseases secured from healthy potatoes. With the appearance of the symptoms it was at once strikingly evident that the ordinary potato mosaic was an entirely different disease from any of those secured from healthy (or mosaiced) potatoes on tobacco (Plate VI). The "mottle" and "ring-

spot" types of disease apparently produce no symptoms on the potato, although this may need further vertification (Plate V). The "spot-necrosis" disease when inoculated into the potato from tobacco, however, produces a diseased condition of such a serious nature that not frequently the plant is killed in fifteen to thirty days. Following the first general chlorosis of the basal leaves, leaf-drop, and mottling on the younger leaves, the plant may partially recover, leaving a tuft of curled and mottled leaves at the top. (Plate VII). Tuber formation, however, is almost prevented in most instances when compared with the uninfected controls. There is some similarity in this disease to some of the known virus diseases of the potato, as, for example, late stages of "streak" or "stipple-streak", and probably to "leaf-drop streak", the latter not yet apparently fully described in literature. Whether this disease is identical with any known virus disease of the potato remains to be determined.

Eighty potato plants have been inoculated with "spot-necrosis" from tobacco and infection was secured in 81 per cent of the plants. In the three last experiments involving ten plants each, 100 per cent infection was secured.

If now potato plants are inoculated with the virus secured from potatoes infected with "spot-necrosis", infection is readily secured, usually in a slightly smaller per cent of the cases, and the disease is decidedly less malignant than in the transfer from tobacco to potato. The virus in this case apparently looses some of its virulence while in the potato. This also can be shown by comparative inoculations back to tobacco. In three generations on potato it has not lost all of its virulence, however, and whether or not it will ever do so remains to be ascertained.

Neither healthy tobacco plants nor tobacco plants affected with ordinary tobacco mosaic produced any symptoms on potato, with the interesting exception that tobacco mosaic produces brown or black necrotic lesions on the stems and petioles of potatoes apparently at the points of inoculation. Tobacco mosaic infection was not found to be systemic in potato, however, and it cannot therefore be said to be a typical host of tobacco mosaic.

A Combination Disease

An interesting phenomenon occurs when the viruses secured from healthy potatoes are combined with ordinary tobacco mosaic virus and inoculated into tobacco, tomato or certain other solanaceous species. This is especially true when the "spot-necrosis" form is combined with tobacco mosaic. The combined effect of the two diseases is much more malignant than either disease alone, in simultaneous inoculations. If, for instance, the "mottle" type of virus from potatoes and ordinary tobacco mosaic are combined, marked necrosis, in the form of leaf spotting, may occur on tobacco whereas neither one of these alone produce necrotic symptoms on tobacco.

The combination disease on some hosts like tomato has been noted at times to be so virulent as to kill the entire plant. The relation of environment or other circumstances to this effect is not sufficiently understood.

It is interesting to note that frequently the combination disease on tobacco

proceeds with necrotic effect along the midrib or the principal veins of the leaf. This condition may sometimes be found to a less striking extent, however, with a single virus when it possesses necrotic properties. The plants if not killed exhibit a special tendency to recover from the first effects of the combination disease.

Discussion of Results

The experimental evidence summarized is believed to be sufficient to warrant the conclusion that most potato varieties uniformly possess the property of inducing a disease in tobacco and other solanaceous plants, which is infectious in nature and belongs to the class of filterable viruses. This ability is present regardless of whether the potato is healthy, as this word is generally applied, or affected with one or another of the common virus diseases of the potato. It is not meant to imply, however, that none of the virus diseases now known to occur naturally on potatoes may not be transmitted to tobacco or tomato, although the evidence that such has been done in the case of potato mosaic, as reported by Schultz and Folsom[3] and Quanjer[4], appear doubtful in view of the results secured with healthy potatoes.

In view of the wide host range of these viruses within the solanaceous family it is rather surprising if these do not actually exist in nature occasionally as specific diseases. While it has not yet been definitely proven, it is possible that the "ring-spot" disease of tobacco as it occurs in nature is identical with the "ring-spot" disease secured from potatoes on tobacco under experimental conditions. It would be rather peculiar if the disease referred to as "spot-necrosis" on tobacco, which attacks potatoes with such virulence, is not found to occur in nature on potatoes. It is difficult to understand, on the other hand, how potatoes can so generally harbor this virus, without any apparent symptoms being expressed, assuming that this is actually a virus disease of the potato. In this connection it should be pointed out that the previously existing evidence of true virus carriers (meaning infected plants in which symptoms of any sort are never expressed) is extremely meager, and that which does exist needs further verification. The potato has not yet been put into the category of a "carrier", although it may eventually prove to be an excellent example of this phenomenon.

If, on the other hand, it is assumed that the viruses secured from healthy potatoes are not actually present in the potato as a true virus, but merely as normal (or possibly abnormal) protoplasm another hypothesis, possessing many advantages from the standpoint of explaining what is now known about virus diseases, is at hand. It is not the purpose of this bulletin to present the apparent evidence one way or the other on this subject. Further experimental data must be secured to establish either of

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[3]Schultz, E. S., and Folsom, D. Transmission, Variation and Control of Certain Degeneration Diseases of Irish Potatoes. Jour. Agr. Research 25, p. 43-117 (Plates I-15). 1923.

[4]Quanjer, H. M. General Remarks on Potato Diseases of the Curl Type. Report Internat. Conf. Phytopath. and Ec. Ent. Holland, p. 23-28 (Plates I-IV). 1923.

the hypotheses that have been presented to explain the results obtained with healthy potatoes. By attacking the problem from new angles it is hoped that this may eventually be accomplished.

SUMMARY

1.—The summarized results of inoculations from potatoes which are healthy as far as can be determined are believed to be sufficient to show that at least two different viruses are commonly, if not universally, present in most standard varieties of potatoes.

2.—The diseases produced on tobacco are infectious and are characteristically of three types, which are referred to as "mottle", "spot-necrosis" and "ring-spot." The two former may be different expressions of the same disease. An increase and decrease in virulence of these forms is apparent as they are transferred between hosts.

3.—The properties and nature of the viruses are similar to those of other well-known virus diseases of plants, with respect to filtration, dilution, insect transmission and resistance to desiccation, putrefaction, heat and chemicals.

4.—One or more of the viruses can be transmitted readily to a large number of different species of plants of the solanaceous family.

5.—The "spot necrosis" form can be transmitted back to the potato where it causes a virulent disease, the "mottle" and "ring-spot" forms apparently give no symptoms on potato.

6.—No other species of healthy plant has been found of which the extract will induce symptoms of any kind in tobacco. Potato foliage from true seed has also failed to give any definite infection on tobacco.

7.—Ordinary tobacco mosaic combined with the virus from healthy potatoes results in a combination disease with striking necrotic effects.

8.—The experimental results indicate that potatoes are either "true carriers" of viruses, or that potato protoplasm is actually the causal agency of one or more of the virus diseases of tobacco and other solanaceous plants.

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Pea Disease Survey in Wisconsin

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Contents

Pea Disease Survey in Wisconsin[1]

AVOIDANCE of disease is a major consideration in the production of peas, whether by the canner, the seedsman or the market gardener. Each of these growers when entering new territory has usually found it to his advantage to grow peas repeatedly on the same ground, and each of these, outside of certain irrigated territory, has observed sooner or later that peas failed to grow as successfully as formerly on his older pea fields, and has been obliged to remove the crop to new ground or to adopt a long rotation. The reason for the failure of peas in old pea fields seems always to have been due to pea diseases which have increased rapidly with the intensive culture of the crop. Thus it has come about that pea diseases have largely determined the cropping practice wherever peas have been grown for a considerable time. This is especially true in the production of peas for the canning factories in Wisconsin.

Pea Diseases and the Canning Industry

From the time of establishment of the first canning factory in the state in 1889 until about 1912, nearly every company that entered the business owned land upon which it grew at least a part of the peas which it canned. In certain instances, as many as ten successive crops are said to have been grown on company owned land. However, conspicious crop failures upon land that had grown repeated crops of peas experienced by some companies in 1910 and following years were called to the attention of the State Experiment Station and led to the beginning of an investigation of pea diseases. The opinion was soon expressed that peas could not be grown successfully on the same ground indefinitely as the companies owning the land had hoped. Following the advice of the Experiment Station, intensive growing of peas upon company owned land was generally abandoned by 1915. Since that time, the most of the peas have been grown for the canning companies under contract by farmers with the supervision of the company field agent, though a few companies do their own farming on land leased for two or three years. Since this dispersal of pea growing over a large territory around factories, disastrous crop failures from pea diseases have been less frequent, though they have occurred here and there where farmers have been permitted to repeat the mistake made by the companies earlier. Loss from disease is no longer the inhibiting menace to the industry that it was felt to be in 1912; and the canning business has grown until the number of plants in the state reached 135 in 1924, producing peas on about 102,000 acres.

Studies of Pea Diseases in Wisconsin

From the time of the first alarming failures of peas until the present, the department of Plant Pathology of the Experiment Station has been active in studying the several diseases which have been found contributing to these failures. At first, the conspicuous foliage diseases were regarded as the chief cause of loss, and effective control measures were devised and

[1]By Fred Reuel Jones, Pathologist, Bureau of Plant Industry, United States Department of Agriculture, and Maurice B. Linford, Industrial Fellow in Plant Pathology, University of Wisconsin. The field survey was supported by members of the Wisconsin Pea Packers' Association.

generally adopted. When these foliage diseases subsequently declined in importance, it began to be apparent that in many fields root diseases were present which were not only able to destroy the crop effectually as the foliage diseases, but which were remarkably persistent in the soil. Furthermore, it appeared that trouble of this kind was by no means a local problem, but that it was being encountered by growers in many parts of the United States. Thus it came about that in 1919 the U. S. Department of Agriculture began an intensive study of these root diseases in cooperation with the Wisconsin Experiment Station.

In this later stage in the study of pea diseases several root diseases have been distinguished and described, but one of these appears to be so much more important than the others that it will undoubtedly become generally known as "the rootrot disease" of peas. This disease can be distinguished in the field without great difficulty, and considerable information regarding the behavior of the fungus causing it has been gained. In fact, the study of this disease had reached the stage in 1924 where new and very specific recommendations for its prevention could be made. The new recommendations, however, made it necessary that the field agent of the canning company should be able to recognize this rootrot with certainty, not only in cases of conspicuous crop failure, but in less conspicuous beginnings. Thus the time had come when any assistance which could be given the field representatives of the canning companies in acquiring experience and skill in recognizing the disease was of financial value to the industry.

GENERAL PLAN OF THE 1924 SURVEY

Although the study of the root diseases of peas had reached a stage at which practical suggestions for control measures could be made, it had by no means reached a satisfactory conclusion. The relative importance of the several diseases recognized had not been adequately studied in the field; an important effect of soil type on both the increase and persistence of disease was suspected but not thoroughly examined; and the value of resistant varieties had not been tested. Further study of any of these things required wide field experience. This situation presented to the pea canners of the state early in 1924 led to a field inspection for disease in a part of the territory of 30 canning companies at their own expense[2]. This bulletin is a fuller report of the findings of this survey than that given in a previous circular (11) together with a description of diseases of peas occurring in Wisconsin, and summaries of fragmentary studies by the senior author of some of these diseases.

The first purpose of this survey was to bring assistance to the canning companies in recognizing rootrot in their territory, and especially in detecting it before it had become destructive in order that they might avoid losses which would come from replanting infested soil in the near future. The field studies reported in this paper were thus more or less secondary to this major purpose and were in some respects limited by it.

In the course of the survey, 688 fields were visited comprising 5,416 acres, or approximately one-sixth of the total acreage of thirty-seven factory districts. Nearly all these districts were visited twice in order to

[2]The 1924 Wisconsin pea disease survey was financed by 30 canning companies as listed in a previous circular (11), operating in 35 factory districts. Through W. E. Nicholoy, Business Secretary of the Wisconsin Pea Packers' Association, these companies subscribed from 300 to 600 acres each for inspection, paying fifteen cents per acre for the service. The fund obtained in this way was administered by the University under the general supervision of L. R. Jones in consultation with R. E. Vaughan.

examine both early and late plantings at the stage most favorable for inspection, and a few which contained considerable rootrot were returned to after the close of the canning season to obtain records of yields from diseased fields and to complete other important records.

Field Notes

Individual records were made of each field inspected (fig. 1). In most cases diseases both of foliage and roots were determined and recorded in the field, but in frequent doubtful cases samples were collected for microscopic examination or for making of cultures.

PEA DISEASE SURVEY

Company No.

Farm Owner Address Date

Variety Planted Area

Stage Soil Type

Rootrot

% infestation

FIELD HISTORY 1923	1922	1921
Ascochyta	Fusarium	
Colletotrichum	Pythium	
Septoria	Rhizoctonia	
Bac. Blight	Mosaic	
Downy Mildew	Nodules	
Powdery Mildew	Aphids	

FIG. 1—DATA INCLUDED ON FIELD SURVEY CARDS

The soil type was determined from soil maps of the Wisconsin Soil Survey in mapped counties. In counties not mapped a few of the more common series were readily identified, but in some cases the soil class only was recorded. The classification and nomenclature of the Soil Survey are followed in this bulletin.

Records of Cropping History

The most necessary information to be secured regarding each field visited, and the most difficult information to secure with adequate accuracy was its previous cropping history, and especially the years in which all previous crops of peas had been grown. A cropping history was regarded as satisfactorily complete when the dates of all previous crops of peas were secured, but even this limited field history was often lacking and data of great value lost.

In this connection, it may be pointed out that while the survey aimed to

[3]Data are included in this paper from two factory districts in addition to the thirty-five subscribed.

cover fields in which peas had previously been grown in preference to others, nevertheless records at the end of the season showed that 48 per cent of all fields visited had never grown peas previous to 1924. The percentage of fields visited which were growing their first, up to their sixth crop of peas is shown in Fig. 2. Apparently at least half, probably much more than half, of the peas grown in Wisconsin in 1924 were grown on ground new to peas, and only a very small acreage had ever grown as many as two previous crops. A smaller acreage on land new to peas will necessarily be found in succeeding years.

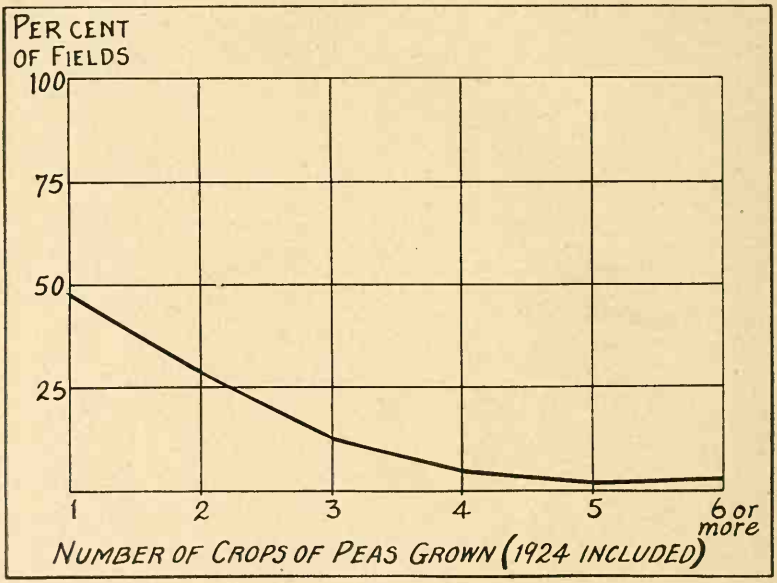

FIG. 2—DISTRIBUTION OF FIELDS ACCORDING TO NUMBER OF CROPS OF PEAS GROWN

Showing the extent to which inspected peas were growing in fields new or relatively new to peas.

ROOTROT CAUSED BY APHANOMYCES

Description of the Disease

Inasmuch as this survey was designed primarily to discover the relation of rotation and soil conditions to the occurrence of rootrot it is necessary to describe in detail the disease and the fungus causing it.

Rootrot is primarily a fungous soft rot of the primary cortex of the roots and epicotyl of the plant (fig. 3), extending one or two inches above ground under humid conditions. Plants are susceptible at all ages, and the disease may begin at any point or at many points simultaneously in the root system. Lesions are at first watersoaked areas yellowish to straw colored, especially on the epicotyl. From any point of entry, the fungus spreads rapidly in all directions through cortical tissue until that tissue has been completely decayed. Although the causal fungus does not

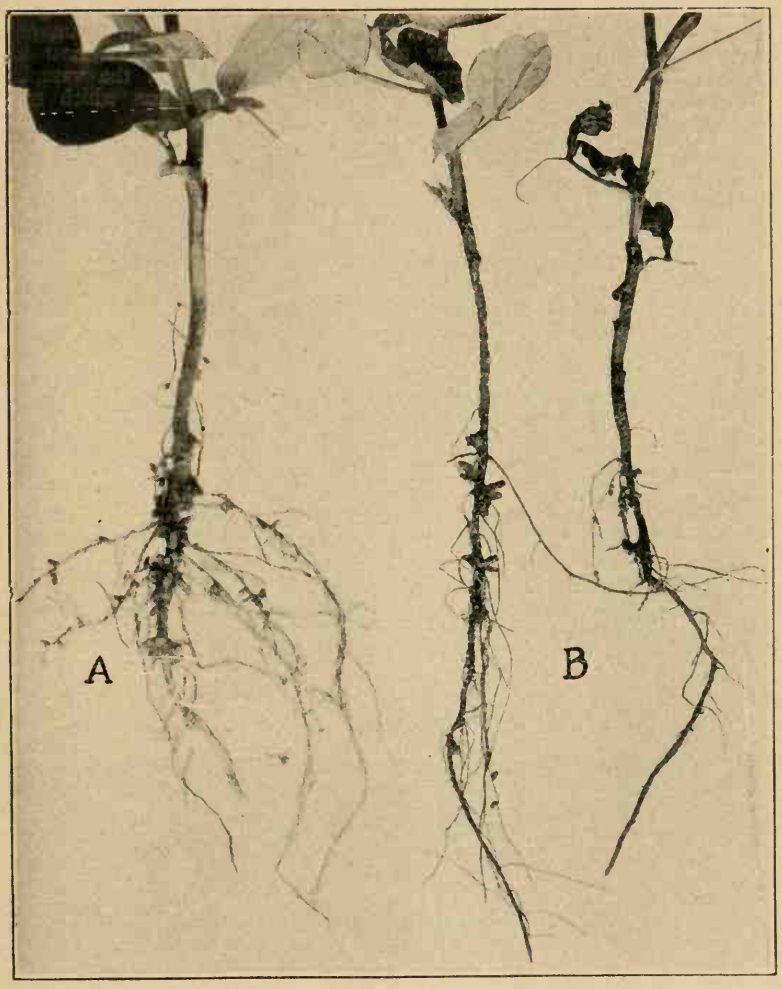

FIG. 3—HEALTHY PLANT (A) IN CONTRAST WITH PLANTS DAMAGED BY ROOTROT (B).

Roots of diseased plants, and stem to just above the surface of the soil, are softened, darkened and shriveled. Stem, roots and nodules of the healthy plant are plump and white. Severe root injury has caused the death of the leaves of the plant at the right. (Photograph courtesy of U. S. Department of Agriculture.)

penetrate the endodermis of the mature root, it does cause the death of the meristematic tissue at root ends. Thus root growth is stopped. In the older roots the decay of the cortex exposes the endodermis to the attack of other soil inhabiting fungi. In a few resistant varieties a protective secondary cortex is formed from a cambium developing in the pericycle; but in most varieties this has not been found to occur, and the endodermis does not appear to be an effective barrier against all invaders. In the epicotyl, unprotected by an endodermis, the vascular tissues are readily entered by several species of Fusarium which may quickly kill the plant.

The effects of the disease upon the plant as a whole are not definitely characteristic, but depend largely upon the stage of development at which infection takes place. If the attack comes early in the development of the plant while the root system is small and incapable of supporting a large vine, the vines are stunted or killed immediately. If severe injury to the roots is delayed until the roots have attained nearly their full extent and if abundant moisture is never lacking from the soil, the plant may appear nearly normal, and mature to the canning stage at least a large fraction of a normal crop. Thus this disease is not always readily discernable in the field. When conspicuous injury to the vine is absent, it is necessary to examine roots to find the disease. When these are dug, the softened shrunken condition of the cortex of roots and base of the stem is usually readily observable. Sometimes when diseased plants are pulled the vascular cylinder of the tap root pulls out readily from the decayed cortex as a long string, while roots of healthy plants almost always break at the attachment of the seed. In cases which are not readily determined from superficial examination it is always possible to discover the characteristic spores (10) of the causal fungus in the decayed cortex by a microscopic examination.

Since the parasitic fungus causing rootrot has been described fully in a recent paper (10) only the more important details of its life history need to be repeated here. The fungus, *Aphanomyces euteiches* Drechsler, is one of the few exceptional species of this genus of the Saprolegneaceae not strictly aquatic in habit. It is, however, dependent upon a period of submergence in water for the production of its asexual spores. It is most readily discernable in the host tissue as subspherical oospores 18 to 25 microns in diameter, surrounded by oogonial walls of unusual thickness. The mycelium in the host tissue is abundant, intracellular for the most part, but ephemeral in a living condition, since the contents are rapidly transferred to the abundant oospores. This mycelium is not readily distinguished from that of other Phycomycetous species, especially of Pythium, which frequently accompany it. The oospores occurring in the host tissue have not been germinated, but those produced in culture germinate readily giving rise to non-spetate mycelium on a moist substrate or in water having sufficient nutrient material. In pure water they give rise more or less directly to zoospores. These zoospores come to rest after a period of motility and germinate giving rise to mycelium. The mycelium when young may function more or less completely without apparent differentiation as sporangia under aquatic conditions giving rise to numerous zoospores.

It has not been possible to follow the life history of the fungus in nature. No evidence of conveyance with pea seed has been found. It is believed that the abundant oospores formed in the host tissue persist for a long time in the soil. Mycelium from germinating oospores is able to penetrate pea roots, and it is possible that zoospores formed when the soil is filled with water may also be a source of infection. It is also possible that the mycelium may persist for a time in the soil as a saprophyte. No other plant than species of Pisum are known to be invaded by this fungus.

Environmental Conditions Controlling Infection

The time of appearance of the disease in the field appears to be controlled chiefly by the temperature of the soil. Although laboratory study shows that the oospores may germinate giving rise to zoospores at a temperature as low as 9 to 10°C., very little infection of pea plants has been found either in controlled experiments or in the field until a temperature of 15° C. has been reached. The disease develops rapidly when soil temperature is between 15° and 30°C.

The effect of soil moisture upon the development of the disease in the field appears to be important. Although greenhouse experiments have

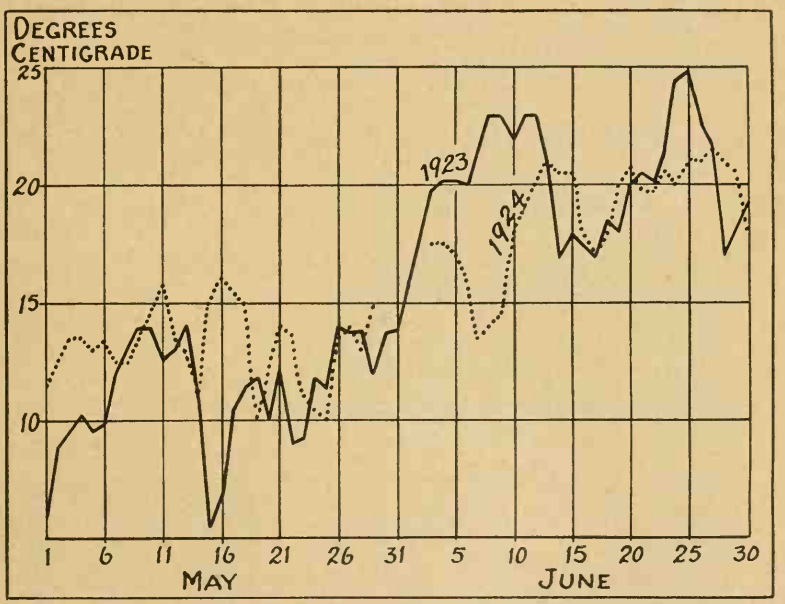

FIG. 4—MEAN DAILY SOIL TEMPERATURE AT A DEPTH OF 2 INCHES
Recorded at Madison, Wisconsin, from May 1 to June 30, 1923 and 1924.

failed to show that there is any necessary relation between soil moisture and the severity of disease, yet in the field infection seems to be more prompt and abundant after heavy rains. Thus in the field the disease can be expected when rains have thoroughly wet the soil after it has attained a daily average temperature of 15°C.

Climatic Conditions of 1924 in Relation to Disease

The climatic conditions during the pea growing season of 1924 presented peculiarities which need to be considered in interpreting the results of this survey.

April, May, and June were cold and cloudy with an unusual number of rainy days. Planting was begun early in April but it was not completed along the shore of Lake Michigan until June 13, and the peas planted early spent an extradordinary length of time between planting

and harvest. July and August were both cool, and in the major pea sections of the state were marked by frequent rains. August 1924 was the wettest August in the climatological history of Wisconsin, and heavy rains in the fore part of the month caused great damage to the peas which were not then harvested. That low soil temperature restrained the development of rootrot until later than usual is indicated by a record of soil temperature kept at Madison during this season. The record for 1924 is charted with a similar record made in 1923 in Fig. 3. This figure shows that while soil temperature in 1924 averaged somewhat higher than in 1923 up to May 24, it was not high enough to permit of much infection by Aphanomyces to that date. After May 24, soil temperature was cooler in 1924 than in 1923. Not until June 10 did the soil become permanently warm enough to favor the development of the disease, whereas, that condition was reached June 1 in 1923. From this and from other meager soil temperature records at hand it appears that the disease was restrained from development in 1924 until later than usual. Continued low temperature through June was unfavorable for other soil fungi which often complete the destruction of plants injured by Aphanomyces. Thus cool weather probably accounts for the less destructive character of rootrot in 1924,—a condition believed to be a fact by several observers.

Occurrence and Importance of Rootrot

During the 1924 survey the rootrot of peas caused by Aphanomyces was found to be far more important than all the other parasitic diseases combined, causing losses amounting to approximately 8 per cent of the yield of the total acreage inspected. Of the 688 fields aggregating 5,416 acres inspected, 222 fields or 32 per cent were found to contain the disease in greater or smaller amounts. The infested territory was by no means distributed uniformly through the several districts. Two of the 37 districts appeared to be free from any traces of it while in one district 62 per cent of 566 acres examined were more or less thoroughly

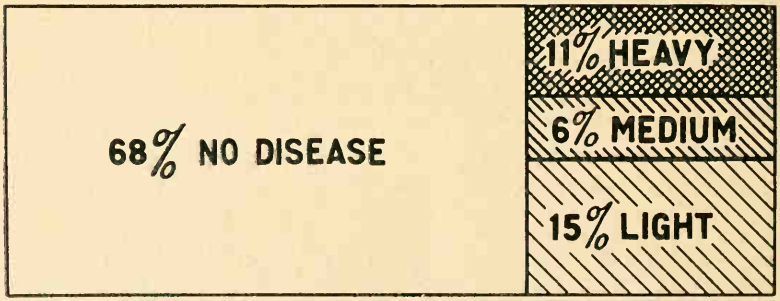

FIG. 5—ROOTROT IN SURVEYED FIELDS

Diagram showing the percentage of all fields examined which contained the amounts of rootrot indicated. (See discussion, page 9).

infested. In general, the younger canning districts were freer from disease than the older, though a few older districts had rotated the crop so carefully or had removed the crop to new ground so completely that little disease was found in them.

Fields recorded as containing rootrot had widely different amounts from a mere handful of plants to complete infestation. In each case an estimate was made of the area infested as a certain percentage of the entire field.

The infested fields have been classified for convenience as follows: fields showing from a trace to 25 per cent of the area infested are regarded as having "light" infestation; those having from 26 to 75 per cent are called "medium", and those with from 76 to 100 per cent are regarded as having "heavy" infestation. The result of this classification is shown praphically in Fig. 5. Of all fields surveyed, 68 per cent had no rootrot, 15 per cent had light infestation, 6 per cent had medium and 11 per cent had heavy infestation.

The fields in which rootrot occurred were not always damaged in direct proportion to the amount of rootrot present for reasons which are discussed later. A more accurate picture of the extent and distribution of losses

TABLE 1.—ROOTROT-INFESTED ACREAGE CLASSIFIED ACCORDING TO ESTIMATED[a] REDUCTION IN YIELD DUE TO ROOTROT.

Reduction in yield	Total acreage	Percentage of total rootrot-infested acreage	Percentage of total inspected acreage
0–5%	1228	57	22.7
6–25%	356	16	6.6
26–50%	186	9	3.4
51–100%	379	18	7.0

[a]The heavier losses were estimated by comparing actual yields from diseased fields with average yields from disease-free fields of the same variety in the same locality.

can be gained by grouping infested fields into classes based on the extent of crop reduction. Such a classification is given in Table 1. From this table it appears that in over half the infested acreage the loss was negligible but in 18 per cent of the infested acreage loss amounted to from 50 to 100 per cent of the crop.

Relation of Rootrot to Number and Frequency of Previous Crops of Peas

That the occurrence of rootrot is closely correlated with the number and frequency of crops of peas in a given field is one of the most widely recognized characteristics of the disease. An exact statement of this correlation, and a study of its characteristics were among the first objects sought in the survey. The correlation is very striking when the survey records as a whole are considered. There was almost no rootrot in fields which had grown no peas before, and a rapid increase in the frequency of its occurrence was found with each succeeding crop whether these crops were in succession or at short intervals.

Increase in percentage of fields diseased.—Neglecting for the moment the interval that had elapsed in some cases between crops of peas—the records show that the interval was rarely long enough to be important—the fields may be divided into groups based on the number of crops of peas which they had produced. When this classification has been made, fields growing the first recorded crop had rootrot in but 8 per cent of their number, while every field growing the fifth crop had rootrot. The percentage of fields showing rootrot in each class is shown in the upper line of Fig. 6. The increase in infestation with each succeeding crop of peas rises regularly almost as a straight line from 8 per cent in the first crop to 100 per cent in the fifth. Since the last two classes are small, it must not be assumed that this curve presents an altogether accurate

picture of the rate of appearance of the disease under all conditions in the state. However, it can probably be regarded as an approximately correct average.

One characteristic of the correlation between rootrot and the number of crops of peas grown in a field which has appeared in the records obtained this year is not shown in this presentation. This important characteristic is best seen when the fields thoroughly infested with rootrot—those in

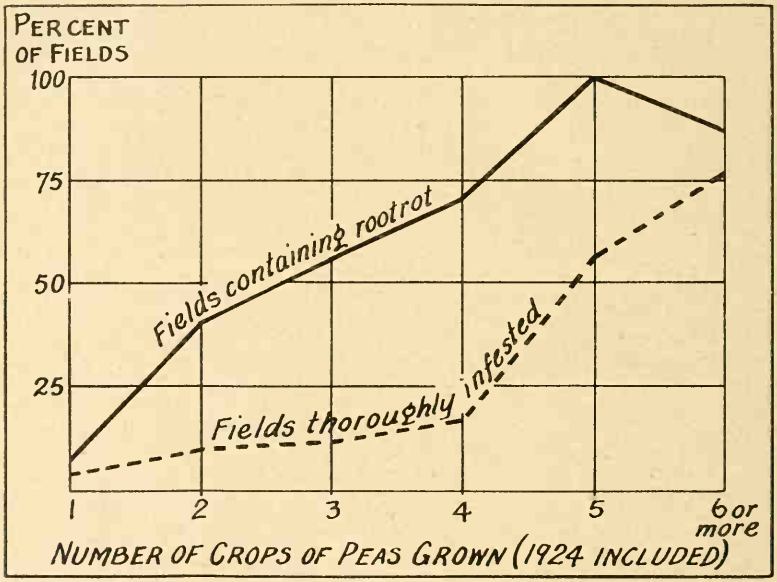

FIG. 6—INCREASE IN PERCENTAGE OF FIELDS CONTAINING ROOTROT, AND FIELDS THOROUGHLY INFESTED, WITH INCREASE IN NUMBER OF CROPS OF PEAS GROWN

which most of the larger losses occurred—are classified and plotted independently (fig. 5, lower line). When the diagram produced in this way, showing the relation of thorough infestation to rotation, rather than the presence of the rootrot disease in any discoverable amount, is examined, its character is found quite different from the former figure. This curve rises very slowly until with the fourth crop of peas only 17 per cent of all the fields are in this class; but in the fifth year the percentage suddenly rises to 56 per cent of all fields in the class. Stated in another way, these figures suggest that under average field conditions in the territory surveyed it is possible to grow on new fields four successive or nearly successive crops of peas without great peril from large loss from rootrot; but that with the fifth crop there is about an even chance that the field will be severely damaged, and that with the sixth crop there is but one chance in four of a profitable yield.

Increase of rootrot in average Wisconsin field.—The field records used in the preparation of the diagram previously discussed may be presented in a different manner that has some interest, even though it is not as clearly and directly significant to the pea grower. If all the fields covered in the survey are classified as previously into groups based on the number of the present crop in the field, and the figures representing

the percentage of rootrot infestation in the fields of each group are average and plotted (fig. 7.), a diagram is produced which may be regarded as showing the percentage of the area of an average Wisconsin field invaded by rootrot with each succeeding crop of peas. This diagram is so similar in character to that of the preceding that it requires no added discussion.

Influence of rotation.—Thus far in this discussion of the rate of appearance of rootrot all the fields covered in the survey have been discussed without regard to any rotation that may have been practiced. This is justified by the fact that only in exceptional cases has rotation long enough to be significant been recorded. It now remains to examine these exceptional cases to determine whether they give any indication that rotation in any form delays or averts the appearance of the disease. From what has been said previously regarding the recent appreciation of the

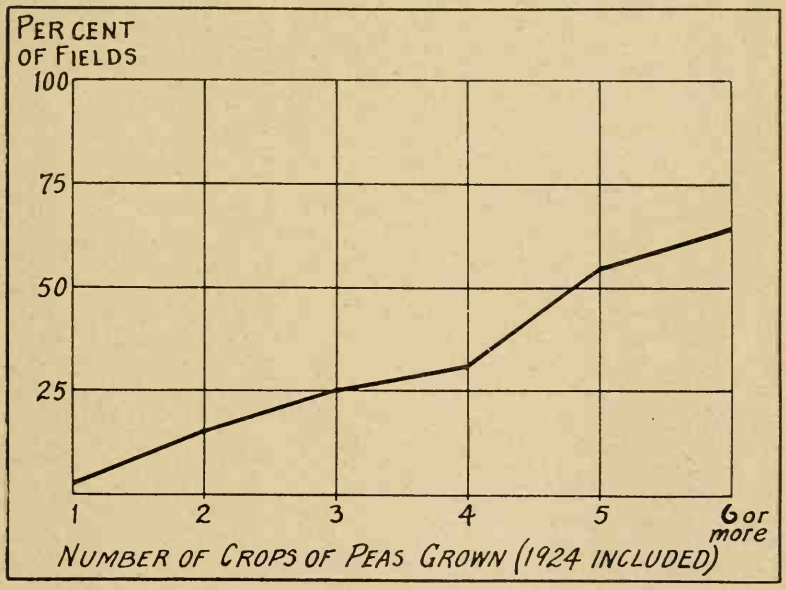

FIG. 7—RATE OF INCREASE OF ROOTROT INFESTATION IN THE AVERAGE WISCONSIN PEA FIELD AS INDICATED IN THE 1924 SURVEY

necessity for rotation in pea growing, the youth of a large number of the canning districts covered, and the lack of adequate records of cropping history, it is not surprising that the number of significant cases found in a single year is too few to form the basis of conclusions.

By way of comparison, it may be stated that only three fields growing their fourth successive crop of peas were found disease-free, while two fields which had been cropped in essentially a three-year rotation were disease-free in their sixth crop. Other instances of three-year rotation showed convincingly, however, that this is not long enough under other conditions to prevent the entry of disease even up to the sixth crop. Only a few instances of four or five-year rotations were found, and these were started so recently that they give no indication yet of their effectiveness over a long period of time.

The extent to which care in rotating peas is avoiding loss from rootrot

TABLE II.—PEA FIELDS SURVEYED IN 1924, CLASSIFIED ACCORDING TO SOIL TYPE,[a] CROPPING HISTORY, AND DEGREE OF INFESTATION[b] WITH ROOTROT.

	No record of rootrot before 1924																								No crop records obtained				"Blighted" peas before 1924			
	First peas				Second peas				Third peas ("some")				Fourth peas				Fifth peas ("many")				Sixth peas or more											
	none	light	medium	heavy	none	light	medium	heavy	none	light	medium	heavy	none	light	medium	heavy	none	light	medium	heavy	none	light	medium	heavy	none	light	medium	heavy	none	light	medium	heavy
Carrington silt loam	78	2	..	1	24	11	..	4	10	5	4	1	1	3	2	1	..	1	3	5	..	2	..	4	10	4	2	..	..	1	..	1
Miami silt loam	68	1	..	2	18	1	1	2	5	3	3	..	4	..	1	2	..	1	1	..	..	..	..	5	12	3	2	..	2	..	..	6
Colby silt loam	11	..	..	..	..	1	..	3	..	2	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..
Superior silt loam	12	..	..	..	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Clyde silt loam	7	..	..	..	1	1	..	..	1	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Waukesha silt loam	..	..	..	..	2	..	..	..	3	2	1	..	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Knox silt loam	1	..	..	..	1	..	..	..	3	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Fox[c] silt loam	3	..	..	..	7	..	..	..	3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Wabash silt loam	2	..	..	..	2	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..
Marathon silt loam	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Unclassified silt loam	7	1	..	1	10	6	..	..	3	4	1	..	..	1	..	..	..	..	1	1	..	..	..	1	3	1	1	2	..	..	..	..
Carrington loam	..	..	..	..	3	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Poygan loam	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Miami loam	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Superior loam	3	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Unclassified loam	5	1	..	1	..	3	..	2	2	..	..	..	1	..	..	..	..	..	..	..	..	2	..	3	3	..	1	1	1	..	..	..
Poygan clay loam	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Clyde clay loam	1	..	..	..	2	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	..	1	..	..	..	..	..
Superior clay loam	2	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Red clay loam	1	..	..	..	1	2	..	..	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	1	..	..	..	..	1	..	..
Unclassified clay loam	8	..	..	1	5	5	1	1	2	1	1	..	..	..	1	..	..	..	..	1	..	..	..	1	13	4	4	3	..	..	..	1
Red clay	10	1	..	..	3	4	..	..	2	1	..	4	..	..	..	..	..	..	..	1	..	..	..	1	4	..	2	..	..	..	..	..
Poygan clay	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Unclassified clay	2	1	..	1	3	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	5	..	2	1	..	..	..	..
Miami fine sandy loam	8	2	1	1	7	2	..	1	..	..	..	2	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Superior fine sandy loam	7	..	..	..	3	1	1	..	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..	1
Carrington sandy loam	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Unclassified sandy loam	19	..	..	1	7	5	..	1	1	..	1	1	2	1	..	..	..	..	..	1	1	..	..	..	3	2	..	..	2	..	..	..
Plainfield fine sand	1	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Coloma fine sand	1	..	..	1	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Unclassified sand	5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..	..	..
Miami gravelly loam	3	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Muck and peat	1	..	1	2	1	..	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	..	..	..	..	..	..	..
Unclassified soils	9	1	..	..	7	1	..	..	2	3	1	1	1	1	..	..	..	..	..	..	..	..	..	2	8	..	1	..	..	..	..	4
Totals	284	11	2	12	112	48	6	19	38	23	14	10	9	11	5	5	..	2	5	9	3	2	..	16	69	15	16	7	5	4	1	13

[a] Fields including two soil types are entered twice in this table.
[b] *Light infestation* includes fields which contained from a trace of rootrot up to 25%. *Medium*, from 26–75%. *Heavy*, from 76–100%.
[c] Fox silt loam includes soils formerly mapped as Plainfield silt loam. This latter name has been dropped by the Wisconsin Soil Survey.

in present practice may best be illustrated by comparing two adjoining factory districts on the same soil types and comparable in every respect. One of these districts operating in its thirteenth year was established by a company which had previous experience with the disease, and which had avoided planting peas repeatedly. In this district only three fields showed rootrot, and that in small amount. Two of these three fields were growing their third crop of peas, and no field in the district was growing more than its third crop. The loss was negligible. In the second district, operating in its twelfth year, repeated planting of peas on the same ground had not been avoided. In this district 18 fields were found with rootrot, half of which were severely infested with heavy losses in five. All of these five fields were said to have grown many crops of peas before, one having produced at least seven crops in ten years.

Relation of Rootrot to Soil Type and Drainage

Earlier observations.—In a previous paper (10) some scattered observations have been recorded showing that in different localities where peas have been grown intensively in a similar manner, there is great difference in the time which has elapsed before the disease has made its appearance in fields, and also in the rate of its spread and increase in destructiveness. These differences appeared to be associated with differences in the capacity of soils to hold water, or with drainage and sub-irrigation. For instance, in Wisconsin the Superior red clay appeared to be more subject to severe injury from rootrot than contiguous loams. Some sandy soils in Maryland underlaid by impervious clays seemed remarkably favorable for the development of disease—an observation which seems to be supported by more recent observations by Drechsler (3). In irrigated districts of the Rocky Mountain States, peas on soils of low moisture-holding capacity rarely suffer from disease unless subirrigated, though on some of them occasional diseased plants can be found. There are several ways in which the water relations of soil might affect the development of disease in peas. The most apparent of these is the favorable environment which abundant water in the soil might provide for the semi-aquatic parasite causing the disease. If, as has been assumed tentatively in this paper, the fungus is widely distributed in soils, it may be originally much more frequent in wet soils than in those which do not retain water.

Studies in 1924.—Whatever cause or causes give rise to the observed variation in the behavior of rootrot, it was clearly of great importance in this survey to determine to what extent the several pea growing soils of Wisconsin do affect the behavior of this disease. Any differences which might be found would not only affect the cropping systems which must be used on the several soils to avoid disease, but might affect the direction of expansion of the industry.

The method of classifying soils found most suitable for this study is that provided by the Wisconsin Soil Survey. To a considerable extent, soil types as distinguished by the soil survey are representative of a certain degree of drainage. Some entire series are characteristically well drained. Others are poorly drained. Within each soil type there are, however, many relatively minor, but still, from the point of view of this study, important differences in drainage.

Fields of uniform soil type.—In the course of the survey, peas were examined on 27 distinct soil types besides seven groups of incompletely classified soils, making in all 34 groups into which the total of 688 fields are divided. There are, therefore, too few fields in many of these groups to afford a satisfactory basis for comparison. A complete summary of the data obtained is given in Table II.

From this complete table, one important conclusion can be drawn. None of the soil types encountered shows any promise of furnishing an environ-

ment where peas may be grown without danger from rootrot. Diseased fields are recorded on all but eight of the types. The eight exceptional types are represented by so few fields that the absence of disease in the location where they were found can not be taken as evidence that they are naturally less liable to disease than others. For instance, of the fifteen fields on Superior silt loam, only three were growing their second crop, and these were in a relatively new canning district in situations where disease would hardly be expected. The 13 fields on Fox silt loam were in a district producing its fifth crop of peas but none of these were growing more than its third crop. Under these circumstances, it may be said that this soil type has a more promising record indicating freedom from disease than any other.

In contrast with the Fox silt loam, the soil type which shows the most unfavorable record with reference to rootrot is the Colby silt loam. Since only 20 fields were encountered on this soil, its present record with reference to rootrot should not be regarded as convicting it of being the most favorable soil for disease in the state. The table shows, however, that of the nine fields found which had grown one or more crops of peas previously, all were diseased to a greater or less extent. This soil is characteristically compact with poor internal drainage and in many cases with faulty surface drainage. In view of the tendency of the canning industry to expand on to this soil, a more comprehensive examination of the behavior of the crop on this soil should be made.

The two leading soil types.—The two soil types upon which sufficient numbers of fields were found for adequate comparison are Miami silt loam and Carrington silt loam. These show no important difference in behavior (Table III). The Carrington silt loam shows a larger percentage of infested fields, but a lower percentage of fields extensively invaded. When these two soils are compared with the total number of clay loams and clays summarized in the same table, it appears that the heavier soils show both greater percentages of total fields infested and of fields thoroughly infested. Since the average cropping histories of the fields on the heavy soils is not markedly different from that on the silt loam, the comparison appears to demonstrate a greater tendency for rootrot to become troublesome on the heavy soils.

TABLE III.—COMPARISON OF CARRINGTON SILT LOAM AND MIAMI SILT LOAM, THE TWO CHIEF PEA-GROWING SOILS OF WISCONSIN, WITH THE TOTAL CLAY LOAMS AND TOTAL CLAYS AS TO THE PERCENTAGE OF FIELDS FOUND INFESTED WITH ROOTROT AND THE PERCENTAGE OF FIELDS SHOWING LIGHT, MEDIUM, OR HEAVY INFESTATION.

Soil	Total fields	Number of fields with rootrot	Per cent fields with rootrot	Percentage of total fields (Infestation)		
				Light	Medium	Heavy
Carrington silt loam	180	57	32	16	6	9
Miami silt loam	146	37	25	8	3	12
Total clay loams	76	33	43	17	12	14
Total clays	51	21	41	16	8	18

Comparison of soil classes.—In an attempt to condense Table II in significant manner preserving the summarized cropping histories of fields, all sands and sandy loams were placed in one group, all loams and silt loams in a second group, and all clays and clay loams in a third. Table IV, prepared in this way, reveals differences in behavior between the medium light and the heavy soils. In the group of clay loams and clays it appears that rootrot makes its entry more promptly and spreads through the field

more rapidly than in the silt loams and loams. All fields on clays and clay loams growing their fourth crop were diseased, while 29 per cent of such fields on loams and silt loams, and 40 per cent on sands and sandy loams were still rootrot free.

An attempt has been made to condense Table 11 by grouping together all fields in the same soil series. The groups thus formed appear to be too small in most cases for satisfactory comparison.

Rootrot in uneven fields.—The comparison of the behavior of soil types presented in the foregoing tables does not emphasize differences so much as examination of individual fields extending over two or more soil types, or fields not uniform in drainage. Fields of one soil type, with poorly drained portions, or of two or more types differing in tendency toward wetness were found in almost every district. In such fields, as a general rule, whatever the cause of the wet spots, whether lack of drainage, or seepage, or the texture of the soil enabling it to hold water, rootrot appeared to have entered the field first in these wet spots. Many of the apparent exceptions were probably due to accidental introduction of the rootrot fungus. In determining the presence of disease on very wet ground, microscopic examination was frequently used to distinguish rootrot from drowning of roots from standing water. These general field observations emphasize more than do the tables the differences between soils types, and at the same time strengthen the suggestion that the observed differences between heavy and light soils may be associated fundamentally with the natural wetness of such soils.

Relation of Soil Reaction and Fertility to Rootrot

No special study of the relation between soil acidity or fertility and the occurrence and destructiveness of rootrot was made in this survey. Neither in previous field experience, nor in that gained in this survey has it been obvious that any important correlation exists between these field conditions and the disease. There are, to be sure, a number of instances cited among growers in which high acidity and low fertility have been correlated with destructive occurrence of disease; but when these have been examined they have not provided convincing evidence that correlation with either of these conditions was the essential factor causing loss. On the other hand, certain canning companies have attempted to render diseased land suitable for pea growing by carefully conducted liming and manuring experiments, but have failed completely.

Rootrot in First Crop of Peas

As indicated in figures 5 and 6 a small percentage of fields was found infested in what appeared, from incomplete cropping records, to be the first crop of peas. Many of these had probably grown some unrecorded crops earlier. There are, however, eleven fields in which adequate records indicate that no peas have been grown before, and in which rootrot infestation was found ranging from a mere trace up to 100 per cent of the field. A number of these fields clearly owed their infestation to inoculation from neighboring diseased fields in the following manner: two from diseased fields on the same farm where the diseases had long been established; one from surface drainage from an adjoining higher field; one from an old barn yard included in the field; one from manuring with uncured vines from the outside of a silage stack; and one with rootrot along the roadside from passing loads of pea vines.

The other five fields which contained rootrot in their first peas were all either poorly drained, wet soils, or contained the disease only in wet pockets. In addition to these there were a number of fields observed diseased in

TABLE IV —RELATION OF ROOTROT TO CROPPING ON LIGHT, MEDIUM, AND HEAVY SOILS. NUMBERS OF FIELDS IN ROMAN TYPE PERCENTAGES IN ITALICS.

	Number of crops of peas grown, including 1924 crop; and the degree of infestation with rootrot, whether none, light,[1] medium, or heavy.																							
	First peas				Second peas				Third peas ("some")				Fourth peas				Fifth peas ("many")				Sixth peas or more			
	none	light	medium	heavy	none	light	medium	heavy	none	light	medium	heavy	none	light	medium	heavy	none	light	medium	heavy	none	light	medium	heavy
Sands and sandy loams	46 *88*	2 *4*	1 *2*	3 *6*	18 *60*	8 *26*	2 *7*	2 *7*	1 *14*	1 *14*	1 *14*	4 *58*	2 *40*	3 *60*	....	....	....	....	....	1 *100*	1 *100*	....	....	
Loams and silt loams	201 *95*	6 *3*	....	5 *2*	72 *64*	27 *24*	2 *2*	11 *10*	30 *52*	16 *28*	11 *17*	1 *3*	6 *29*	7 *33*	4 *19*	4 *19*	....	2 *15*	5 *39*	6 *46*	2 *12*	2 *12*	....	13 *76*
Clay loams and clays	27 *88*	2 *6*	....	2 *6*	14 *43*	12 *36*	2 *6*	5 *15*	5 *42*	2 *17*	1 *8*	4 *33*	....	....	1 *50*	1 *50*	....	....	....	2 *100*	....	....	....	1 *100*
Totals	274 *93.7*	10 *3*	1 *0.3*	10 *3*	94 *57*	47 *28*	6 *4*	18 *11*	36 *47*	19 *24*	13 *17*	9 *12*	8 *28*	10 *36*	5 *18*	5 *18*	....	2 *13*	5 *31*	9 *56*	3 *16*	2 *10*	....	14 *74*

[1]Light infestation, 1–25%; medium infestation, 26–75%; heavy infestation, 76–100%, of the area of the field.

their second crop which were said to have been diseased in their first. In these, as in the preceeding cases, the disease was mostly in wet soil. If *Aphanomyces euteiches*, occuring native in Wisconsin, was the source of infestation in these fields, it appears that it was originally restricted to wet locations.

Persistence of the Parasite in the Soil

Once peas have failed from rootrot and their decaying roots have released into the soil myriads of thick walled oospores, the parasite is able to persist for a remarkably long time. In a previous paper (10) instances were cited in which peas had failed from rootrot when planted on a field in which peas had failed six years earlier. Certain canners have reported experiences which indicate a survival of the parasite for a still longer period.

During 1924 a number of well attested cases were encountered in which peas were growing on fields in which the last previous crop had "blighted" presumably from rootrot from one to ten or more years earlier. Assuming that in all of these cases rootrot was the cause of the earlier blight—an assumption which is undoubtedly true in nearly all of the cases—these fields have been classified in Table V. on the basis of the number of years that have elapsed between the previous blighted crop and 1924. The fifteen fields on which peas had blighted within ten years were found to contain more or less rootrot, the majority of them being still heavily infested. Of nine fields blighted more than ten years ago, only three were still thoroughly infested, while five were, as nearly as could be ascertained by careful search in the field, entirely free from the disease. This indicates that rootrot infestation does actually tend to diminish gradually with time, but the length of time required to free contaminated soil is discouragingly long. It appears unsafe to replant peas on infested soil within a decade.

In this connection it may be added that field observation seems to indicate that the disease persists longer in heavy wet soils than in soils less favorable for the advent of the disease in the first place. This relation is not obvious from the table, however, and requires more careful records for its confirmation.

Resistant Varieties and Date of Planting in Relation to Injury from Rootrot

Although it has been shown in experimental trials that no variety of peas is completely immune to rootrot, and that among the usual commercial varieties there is little difference in resistance, as measured in experimental trials, nevertheless search was made for evidence indicating that the slight differences between varieties is of any importance in averting loss on infested soil. The most resistant varieties that have been found, the Horal and Rice's No. 330, did not occur in surveyed fields. It may be added, however, that in a trial conducted by the Columbus Canning Company in cooperation with the U. S. Department of Agriculture during the summer these two varieties showed far greater resistance than has been shown by any commercial variety in general use.

A study of the resistance in commercial plantings is greatly complicated by the fact that the date of planting is a factor to be considered when comparing the damage sustained by different fields. For instance, the record shows that there was a slightly smaller percentage of fields of Alaskas and Winners diseased and that they seemed on the whole to suffer smaller crop losses than other varieties. However, the peak of the planting season of these two early varieties was from three to

four weeks in advance of that of the sweet varieties, a fact which undoubtedly accounts in large part, if not completely, for their lighter damage. Thus a comparison of a few selected fields furnishes more reliable evidence of varietal resistance than a comparison of the records of varieties as a whole.

From a comparison of suitable fields it appears that the Green Admiral alone showed appreciable resistance. One of the older companies operating in an infested district plants Admirals on all fields suspected of harboring disease. On one farm in this district, a uniform 18 acre field of thoroughly

TABLE V.—PERSISTENCE OF THE ROOTROT FUNGUS IN THE SOIL. FIELDS WHICH ARE KNOWN TO HAVE GROWN "BLIGHTED" PEAS ARRANGED ACCORDING TO THE PERIOD OF YEARS SINCE PEAS WERE GROWN LAST AND ACCORDING TO THE DEGREE OF INFESTATION FOUND IN 1924.

	Interval since "blighted" peas															
	1–2 years				3–4 years				5–9 years				10 or more			
	None	Light	Medium	Heavy	None	Light	Medium	Heavy	None	Light	Medium	Heavy	None	Light	Medium	Heavy
Carrington silt loam										1		1				
Miami silt loam				3				3					2			
Colby silt loam			1													
Wabash silt loam						2										
Unclassified loam													1			
Red clay loam														1		
Clay loam																1
Superior fine sandy loam										1		1				
Sandy loam													2			
Unclassified								2								2
Total			1	3		2		5		2		2	5	1		3

infested Miami silt loam was planted to Alaskas and Admirals on the same day. When first observed on June 30 both varieties were thoroughly infested to the extent of about 90 per cent of all of the plants. The Alaskas were beginning to die, but the Admirals showed no evidence of injury above ground. On July 22, the Admirals were ready to harvest. The vines were short, pods poorly filled, and leaves dead on the lower half of the vines. However, the six acres of Alaskas yielded 166 pounds of peas per acre, while the Admirals produced 2,111 pounds per acre, though quality was not of the best. Here, the Admiral seemed to demonstrate marked resistance.

In another similar field suitable for comparison the same varieties were planted on different dates—the Alaskas on April 11, and the Admirals 11 days later. On June 30 the root destruction had advanced far in both cases. The Alaskas were filling pods, though about 25 per cent of the plants were almost dead, while the Admirals were but 12 inches tall appearing perfectly healthy. Alaskas yielded 1,800 pounds per acre while Admirals yielded 1,035 pounds. The low yield of the Admirals in this case appears to be due to the later date at which they were planted, permitting the disease to attack them at an earlier stage of development.

A number of other less closely comparable instances add evidence in favor of the view that under certain conditions the Green Admiral pea has some degree of resistance, enabling it to produce a fair yield under conditions which damage other varieties much more severely. Until the

newer resistant varieties become available the Green Admiral appears to be the only pea showing a sufficient degree of resistance to warrant its use on infested soil, although this variety may fail utterly under severe conditions of disease.

The Control of Rootrot

The findings of this survey suggest very clearly the control measure which must be employed to avoid rootrot—control measures which have been for the most part stated previously. In districts where pea culture on a large scale has been introduced recently and where there are few diseased fields already established, increase in disease can be avoided readily. First, poorly drained soil should be avoided for pea planting. The adoption of a long rotation on suitable soil should defer the appearance of disease for many years, perhaps indefinitely. The length of rotation required to prevent serious development of the disease appears to be dependent to some degree on the soil type, being longer on clay soils than on loams. A rotation of five or six years duration is suggested as probably adequate on most Wisconsin soils. If it appears advisable for commercial reasons to plant peas as long as possible on the same ground, the field records collected here show that under average conditions it is possible to do this for three years before serious loss from rootrot need be anticipated. Occasionally they may be planted for a longer term of years. Generally, the disease appears in such fields for one or two years before it becomes destructive; and thus a careful examination of fields for disease can readily determine when such fields have become unsafe for further planting. No serious loss from disease need be incurred from such practice if intelligent supervision is employed.

In districts where the disease is already well established avoidance of disease is not so easily accomplished. Fields in which peas have failed from rootrot are not safe for replanting for ten years after the failure on most soil types. Soil from such fields can serve to carry disease to other fields during this period of time, and thus much new land that has never grown peas in infested districts is unsafe for peas. Since it will be impossible in most cases to determine in advance just where such injured areas are, it will be impossible to avoid loss in all cases, even where a suitable rotation is adopted. As soon as infested tracts are located, they must be abandoned for pea culture. Transfer of soil from diseased fields should be avoided. Uncured silage from pea vines should never be fed or returned to fields as manure.

The use of resistant varieties of peas may become profitable on infested soils under some conditions. Such varieties should be planted as early as possible. Under conditions favorable for the development of the disease even the most resistant varieties known at present may be damaged greatly, and in any case their growth increases soil infestation quite as much as those varieties which are readily destroyed.

LESS IMPORTANT PEA DISEASES

Fusarium Stem and Rootrot

A stem and rootrot of peas caused by *Fusarium martii* App. & Wr. var. *pisi* F. R. Jones has been described (8) as occurring in Wisconsin and several other states. This Fusarium which was the only important parasite among several species and varieties tested produced typically its initial and most significant invasion at the base of the stem at or immediately above the point of attachment of the cotyledons. The resultant lesion becomes elongate,

extending up the stem as a wedge-shaped, dark brown or chocolate colored lesion, not appreciably shrunken until well advanced. This cortical rot may deepen and pentrate or even sever the vascular cylinder, after which, at higher soil temperatures the fungus invades the xylem for a short distance, producing a bright orange red or brown discoloration which may extend as far as the first node. Extensive vascular invasion is not a characteristic development. Rootlets may be attacked, in which case the symptoms are not visually distinct from the effects of several minor parasites.

When this disease occurs alone as a stemrot it has been considered to be of easily recognized character. It has, however, almost always been found in association with rootrot where its presence rarely can be discovered except by the isolation of the fungus. In the course of the survey only a few instances of the type of stemrot caused by Fusarium were discovered, and in all of these cases laboratory study showed the cause to be a phoma-like fungus which is mentioned below. Thus it appears that the Fusarium stem and rootrot of peas did not occur in Wisconsin this year as an independently recognizable disease. Laboratory study was not made to determine whether it occurred in association with rootrot.

The absence of this disease this year may not have been due to the absence of the parasite. A study of conditions which make possible the development of this disease has shown that a mean soil temperature of 18°C. is necessary before conspicuous lesions on stems appear, and that a soil temperature of approximately 24° must be reached before plants are killed or conspicuously injured. Reference to the record of soil temperature prevailing this year discussed previously will show at once that this disease must have been delayed in development even more than rootrot, and that there was little opportunity for it to become destructive. Thus it is possible that in a warmer season this disease may appear again, though its seems unlikely that it will be important under Wisconsin conditions apart from its association with rootrot.

Footrot, a Disease Resembling Fusarium Stem and Rootrot

Early in June, 1924, before soil temperatures were favorable to the independent parasitism of *Fusarium martii pisi*, plants were found showing lesions typical of Fusarium stem and rootrot. Such lesions, when plated out, yielded cultures, not of Fusarium, but of a Phoma or phoma-like fungus which the senior author has isolated from pea root and stem lesions many times before. Inoculation experiments in the greenhouse have demonstrated that this fungus is capable of producing lesions, which resemble very closely those produced by Fusarium.

Haenseler (5) has reported frequent isolations of Phoma species from peas in New Jersey. Footrot symptoms were encountered widely but sparingly in Wisconsin in 1924. Probably the disease will not prove of great importance.

Seedling Injury Caused by Rhizoctonia

The sterile or Rhizoctonia stage of *Corticium vagum* B. & C. is another fungus capable of damaging the underground portions of the pea plant. Of wide occurrence in cultivated soils, this fungus is frequently encountered in pea fields where under some conditions it may assume considerable importance. Generally, however, it is of minor importance as a parasite of peas.

Rhizoctonia may attack any underground portion of the pea plant, but it causes greatest injury when invading very young tissues. It may enter germinating seeds killing the embro or destroying the cotyledons,

removing the food reserve of the developing seedling. It may attack seedlings before emergence from the soil, injuring or completely destroying the growing points of roots and stem. When the stem tip is thus destroyed the pea frequently produces secondary shoots, one or more of which may escape similar destruction. Root tip injury may continue even after the plant is well established. This fungus may also produce lateral lesions on stems and roots of a type characteristic of this fungus on other hosts, being brownish, sunken and eroded, oval or oblong cankers. Coarse brown hyphae of the fungus frequently found on and around such lesions are helpful in diagnosis, but in general the injury caused by this fungus, particularly upon roots, is not always readily distinguished under field conditions from that of some other parasites.

Richards (13) has shown that the soil temperature most favoring the parasitism of Rhizoctonia on the pea is 18°C., but that it is able to operate in a less important way through a wide range of temperatures, beginning as low as 9° and continuing up to 29°. The minimum temperature is thus below that of the major pea root parasites, and consequently Rhizoctonia injury occurs earlier than the more important root diseases. Late planted peas suffer greater injury than those planted early in cold soil.

In the 1924 survey it was not possible in all cases to distinguish the injury produced by Rhizoctonia under field conditions. Injury attributed to this fungus was noted in 35 fields ranging from the killing of 30 per cent of the plants in rare cases to reductions of stand that were negligible, and from reduction of vigor that would amount to 25 per cent of the crop to that which would escape detection.

Rhizoctonia injury was noted on soils ranging from light sandy loams to mucky clay loams, but the greater reductions of stand and vigor were limited to a few fields of sandy loams, Carrington silt loam and Miami silt loam

Seedling and Root Injury Caused by Species of Pythium

When pea plants suffering from rootrot are examined in the laboratory, the species of Pythium long known as a destructive seedling parasite will often be found present in the diseased tissue of many or all of the plants. Inoculation with some of the cultures of Pythium obtained in this way has shown that the fungus is capable of preventing germination of pea seed or of destroying many of the seedlings before they emerge from the ground, and occasionally some degree of stem and rootrot is produced. Some preliminary work with this fungus earlier led the senior author (6) to express the opinion that it was the most important cause of pea rootrot—an opinion which was not substantiated by further work, and which has since been corrected (10). However, more recently Stone (16) in Ontario has called attention to the association of Pythium with disease, ascribing to it a rotting of pea plants near the surface of the soil.

Some attention has been given to Pythium species in relation to pea disease during several years and though the study of the relation of species of this genus to root injury is far from complete, a few notes on the progress of the work may be presented. Although it will be shown in the following tables that species of Pythium capable of causing severe seedling injury under favoring conditions are present in abundance in some agricultural soils, yet the survey records no instance of important injury from these species. The most obvious explanation for the failure of this group of fungi to produce injury is found in the comparatively high soil temperature required for their activity. An incomplete study of the more actively parasitic species indicates that a soil temperature of 16°C. is necessary before much seedling injury occurs. Most peas have passed the stage at which seedling injury is possible before the mean soil temperature has reached this point.

The study of the relation of Pythium to root and stemrot has been greatly retarded by the fact that the cultures obtained from peas in the field have been found to belong to several species differing somewhat in pathogenicity and frequency of occurrence; and furthermore it is not at all certain that present methods used in making isolations from plants secure cultures of all species present. Thus a vast deal of work will be required before the relation of Pythium species to stem and root injury of mature plants will be fully known. It may be stated, however, that inoculations made under controlled conditions have shown very slight ability in any species studied thus far to produce either stem or rootrot under usual field conditions.

During the summer, isolations were frequently made to secure cultures of any species of Pythium that might be present. Since previous experience had shown that some species are almost as frequently found in association with roots apparently healthy as with those diseased, cultures were made from both. In making cultures, no sterilizing agents were applied to the surface of decaying tissue because they penetrate rapidly and destroy all Phycomycetous mycelium quickly. Thus mycelium adhering to the outside of roots may give rise to a culture, a difficulty which can hardly be avoided. The results of 76 isolations are summarized in Table VI. This table corroborates previous experience that cultures of Pythium are obtained with approximately the same frequency from healthy as from diseased plants. It also shows that in about one-third of the isolations two species were obtained, though in such cases no two species seem to be found more frequently associated than others.

The cultures obtained in this way have been partially classified into species groups which are designated by letter in Table VII. Species A, B, D, and perhaps E, seem to have been included by pathologists under the name *Pythium debaryanum;* and this group contains the more aggressive parasites. From this table it appears that the several species were obtained with approximately the same frequency from healthy plants as from those showing disease. In fact a small number of isolations from clover roots made at the same time with those from peas have given equal success in obtaining cultures of Pythium, the frequency of occurrence of the several species being somewhat different.

From the observations made thus far it appears that some of these species of Pythium occurring abundantly in the soil may at times be responsible for the death of root ends, and for some rootlet injury; but only rarely for root and stemrot as it is usually known. It is possible that almost universal invasion of the root cortex of peas and clover by a mycorrhizal fungus (9) renders this tissue especially accessible to these species, and that it is from this superficial invasion that the fungus is most frequently obtained in culture.

TABLE VI.—FREQUENCY OF OCCURRENCE OF ONE OR MORE SPECIES OF PYTHIUM IN ISOLATIONS FROM HEALTHY AND FROM DECAYING PEA ROOTS AND STEMS.

	No culture of Pythium obtained	1 species of Pythium obtained	2 species of Pythium obtained
Healthy pea roots	10	22	14
Decaying pea roots and stems	6	16	8

An Undescribed Wilt Disease

During the survey a disease was observed which in its effects upon the vines superficially resembles rootrot injury, but which seems etiologically distinct from any of the known pea diseases in Wisconsin. It was character-

ized generally by rapid and complete withering of the vine without conspicuous rotting or discoloration of the cortex of roots and basal stem such as are typical of the better known diseases. Root tip injury was frequently found associated with it but not to a sufficient extent to account for the death of the plants.

Fifty fields were encountered in which what appeared to be this disease was present. Infestation varied from small patches to 100 per cent of the field; in the latter case crop destruction, especially of the sweet varieties of peas, was almost complete. In several factory districts this

Table VII.—Frequency of Occurrence of Several Species of Pythium in Healthy and Diseased Pea Roots as Represented by Isolation.

	Species A	Species B	Species C	Species D and E	Unclassified
Healthy pea roots	6	20	4	14	6
Decaying pea roots and stems	3	9	2	11	7

disease caused greater losses than did rootrot, and in the total area surveyed it ranked second in destructiveness only to the disease caused by Aphanomyces.

Slightly over half of the infested fields lay in Fond du Lac County, and nearly all of the remainder were in adjoining counties. Three-fourth of all such fields were on black soils of which Carrington silt loam was dominant.

This disease appears to be correlated with the previous growth of peas, much the same as is rootrot.

Leaf and Podspot or "Blight" Caused by Ascochyta

The most widely known of the foliage diseases of peas is the leaf and podspot caused by *Ascochyta pisi* Lib., the conidial stage of *Mycosphaerella pinodes* (Berk. & Blox.) Stone. So abundant and important was this disease in Wisconsin in 1911 and following years that it was regarded as the chief cause of "blight" of peas. Recommendations made for its control assisted perhaps by climatic conditions during the past few years have brought about its almost complete disappearance.

This fungus attacks all varieties of canning peas, and occurs on vetches. On pods, the lesions are rounded, somewhat sunken, light brown at first becoming darker with a light brown border, and with brown pycnidia in the center. On leaves the lesions are irregularly rounded with yellowish brown or ashy centers and dark borders. On stems the lesions are elongate. If lesions are abundant they may become confluent killing a large part of the foliage, or even the entire plant. Lesions near the surface of the ground may spread over a considerable portion of the underground stem, which in the past has given rise to the impression that this fungus was the cause of much of the injury to roots that has later been found due to Aphanomyces.

It has been demonstrated by Stone (15) and Vaugham (17) that this fungus lives over winter on diseased vines producing the ascigerous stage in the spring from the spores of which plants may be infected. The fungus has long been known to be carried in the seed. Several years ago when this fungus was very abundant the senior writer found nearly 10 per cent of peas in one sample of commercial seed carrying this fungus, though among many samples examined, only a few were found to contain infected peas. The fungus usually enters the seed coat and

cotyledons close to the plumule. When the seed germinates, the ends of the embryonic leaves in the plumule become invaded by the fungus. When the leaves are carried above ground and expand, the fungus carried in them produces lesions promptly, usually at the margins of these leaves. Pycnidia are soon formed, the spores are scattered over the foliage of other young plants by splashing rains, and the disease spreads from such centers of infection. Since the fungus penetrates deeply in the seed, no method of seed treatment thus far tried has been able to destroy it without damaging the seed.

Leaf and pod spot caused by Ascochyta was of no importance in Wisconsin in 1924. Only 18 fields situated in 12 different districts were found to contain traces of the disease, and none of these were appreciably injured. The disease was first observed as early as June 2 where it was developing apparently from infected seed on plants only five inches tall. The disease was not found again until July 15. Its frequency of occurrence seemed to increase up to the end of the season, but no factory district was observed to contain more than three fields which showed traces of infection.

Leafblotch Caused by Septoria

Leafblotch of peas caused by *Septoria pisi* West is frequently associated with and confused with the leaf and podspot caused by Ascochyta. This disease was formerly considered an important factor in pea "blight." Melhus has shown (12) that it is rarely difficult to distinguish these two diseases except perhaps in their later stages. The disease caused by Septoria is typically a blotch rather than a spot. Its margins are irregular, sometimes angular when restricted by the larger veins of the leaf, and without any distinct marginal band. They are yellowish green at first, turning brown upon the death of the tissue. Such blotches increase in size indefinitely, sometimes extending down the petiole and infecting the stem. Infected tissues produce numerous pycnidia, yellowish brown at first, becoming darker with maturity.

The overwintering of the fungus has never been traced in a satisfactory manner. Melhus found the pycnospore short-lived and found no seed infection. The senior writer, however, found one collection of this fungus, kept in a sheltered location out of doors, that maintained viable pycnospores for at least a year. Seed infection is not infrequent. Infection usually occurs at the hilum which exhibits a very characteristics pink discoloration, involving more or less surrounding area. Although the fungus can be isolated readily from such seeds, no infested seedings have yet been observed from their germination.

In 1924, Septoria leafblotch was more abundant than leaf and podspot, but still was of very little importance in the state as a whole. It was observed first June 6, and was collected at intervals during the summer without showing any marked increase with late summer rains. It was observed in only 29 fields in twelve districts. Most of these fields contained no more than a trace, while a few were damaged to the extent of about 10 per cent. Septoria was most prevalent in the northern districts, all of the heavily infested fields being situated north of the latitude of the north end of Lake Winnebago.

A Septoria Leafspot New to Wisconsin

In one field in Dodge County was found an unfamiliar leafspot caused by a fungus identified by Dr. J. J. Davis as *Septoria flagellifera* E. & E. Both the spots and the pycnospores of the fungus are distinct from those of *Septoria pisi*. Ellis and Everhart (4, p. 57) have described the spots and the fungus from material gathered in South Dakota as follows:

"Amphigenous, spots suborbicular, 0.25-1 cm., diameter, subzonate, with a slightly raised border, rusty-brown at first, whitening out in the center: perithecia hemispheric —prominent or subconical, dark amber color, 75-124 microns in diameter, sporules filiform, hyaline, nucleolate, only slightly curved, 80-120 x 2-2.5 microns.

"Differs from **S. pisi** West, in the different character of the spots and the much longer sporules."

This fungus occurred sparsely in the one field where noted and showed no evidence of possible importance as a parasite.

Anthracnose

The anthracnose of peas caused by *Colletotrichum pisi* Pat. was recently described in Wisconsin by Jones and Vaughan (7). This disease closely resembles that caused by Ascochyta and may easily be confused with it in casual examination. Occurring on all aerial parts of the plant, it produces lesions that on pods are circular and sunken; on leaves, irregular in outline; and on stems, elongate. These spots are generally brownish with somewhat darker brown borders. Stem lesions when covered with spores from the numerous acervuli are ashen when dry, and copper colored when wet. In later stages, small black sclerotial bodies, which are helpful in identifying the disease, develop in the lesions. The life history of the fungus has not been followed through the winter; although the fungus attacks pods freely, it has not been observed on the seed.

Pea anthracnose has been reported in the United States only from Wisconsin thus far. Although recognized as a destructive parasite, its distribution has been considered so limited that its importance in the state as a whole was minor.

In 1924, both in severity of the disease in individual fields and in the total injury observed during the survey, anthracnose was far more important than any other foliage disease. It was found in 42 fields in 13 districts lying in 6 counties. The majority of these fields were infected very lightly or were infected so late in the development of the crop that injury was slight, but there were a number of fields in which losses were severe, amounting to as much as 50 per cent of the crop in one 28 acre field. A few fields showed severe defoliation relatively early, but in the main heavy infection did not occur until near the end of the season when heavy rains caused rapid increase of the disease where it occurred. In a number of fields anthracnose was associated with bacterial blight in causing important foliage destruction late in the summer.

Downy Mildew

Downy mildew of peas caused by *Peronospora viciae* (Berk.) DeBy. is found widely but sparsely distributed almost wherever peas are grown. Rarely does it become conspicuous. Frequently a few plants may be found which have been completely overrun by the mildew in an apparently systemmic infection. Such plants are dwarfed beyond recovery, but constitute at most a small fraction of one per cent. Typically the mildew occurs as irregular downy patches of violet gray color on the under side of leaves. Such leaves are yellowish above and are usually recurved. The occurrence of downy mildew in 1924 was limited to a very few fields, in none of which did it cause important injury.

Bacterial Blight

Sackett (14) has described a bacterial disease of peas caused by *Pseudomonas pisi* Sackett which caused much loss in Colorado in 1915 and

following years. A similar if not identical disease occurs sporadically in Wisconsin, rarely causing important crop reduction.

The disease is characterized by the production of water-soaked lesions of olive green to olive brown color which may remain small spots, or, under favoring conditions, may spread rapidly to include large portions of leaves and stem. Such lesions become darker as they dry. In wet weather bacterial ooze may appear on lesions. Infection is through stomata and wounds. Lesions may develop on pods, and mature peas beneath lesions are sometimes found to bear flakes of what appears to be the dried bacterial slime. Although it has been assumed that the bacteria may be carried alive in this way and thus infect seedlings, such seed transmission of the disease has not actually been demonstrated.

Early spring infection occurring while the seedlings are still young may result in important reductions of stand. Severe infection at any time later is able to weaken the plants seriously, at times destroying practically the entire leaf surface.

Bacterial blight was seen in 1924 both early and late in 23 fields in various districts. Of the infested fields 74 per cent had never grown peas before, or at least not in recent years, a fact which seems to indicate no important correlation between the occurrence of this disease and the previous growth of peas. Likewise no correlation was found between bacterial blight and source of seed.

Early attacks were observed to weaken the plants and to cause uneven development. Usually such early infection was outgrown and did not lead to subsequent increase of the disease. A few fields were observed to show signs of recent infection during late June and early July, but the most severe cases were observed after the middle of July. At this time a few fields were seen badly damaged, in one of which the disease had developed freely over leaves, stems, and pods.

Mosaic

Pea mosaic, observed in experimental plantings at Madison in 1923, was first recorded as occurring in commercial plantings in Wisconsin in 1924, when it was found widely but sparingly distributed. In spite of its early appearance in 1924 at Madison it was not conspicuous in commercial fields until July 12, but after this date at least traces of mosaic were found in practically every factory district visited. In all, it was recorded in 63 fields in 10 counties. Most infested fields contained a mere trace, while the heaviest estimated infestation was 20 per cent. A considerable number of fields showed from 5 to 15 per cent of the plants infected.

Varieties found diseased with the number of fields of each are: Green Admiral, 20; Alaska, 14; Horsford, 13; Perfection, 10; Winner, 3; Advancer, 2. Not only did Admirals show the greatest number of infested fields, but also the heaviest infestation and the most evidence of injury.

Injury from mosaic appeared to be negligible except in a few fields where infestation was heavy. In some of these fields, it appeared that mosaic plants were somewhat dwarfed and failed to fill as many pods as healthy plants. Judged by its behavior under conditions prevailing in Wisconsin in 1924 mosaic of peas can hardly be regarded as such a menance as mosaic diseases of some other crops have been.

The origin of mosaic in these pea fields can be only conjectured at present. Dickson (1) has reported the appearance of the disease in several varieties of field peas from seed transmission. On the other hand, Doolittle (2), using seed from mosaic plants in experimental plantings at Madison, Wisconsin, and McMillian, Michigan, has planted nearly 1,000 seed from Alaska peas and smaller numbers from other varieties under controlled conditions without obtaining a single mosaic plant.

The field occurrence of the disease in 1924 did not suggest seed trans-

mission, for different varieties and peas from seed from different sources appeared to develop the disease almost simultaneously in certain districts. In one, for example, mosaic was found in only four fields representing three varieties from different sources.

On the other hand Doolittle (2) has produced mosaic in pea plants from mosaic red clover by transfer of aphids and by artificial inoculation. Inasmuch as many pea aphids migrate to peas from red clover, on which they winter, it seems likely that mosaic clover plants which are abundant locally are the source of the disease in commercial plantings.

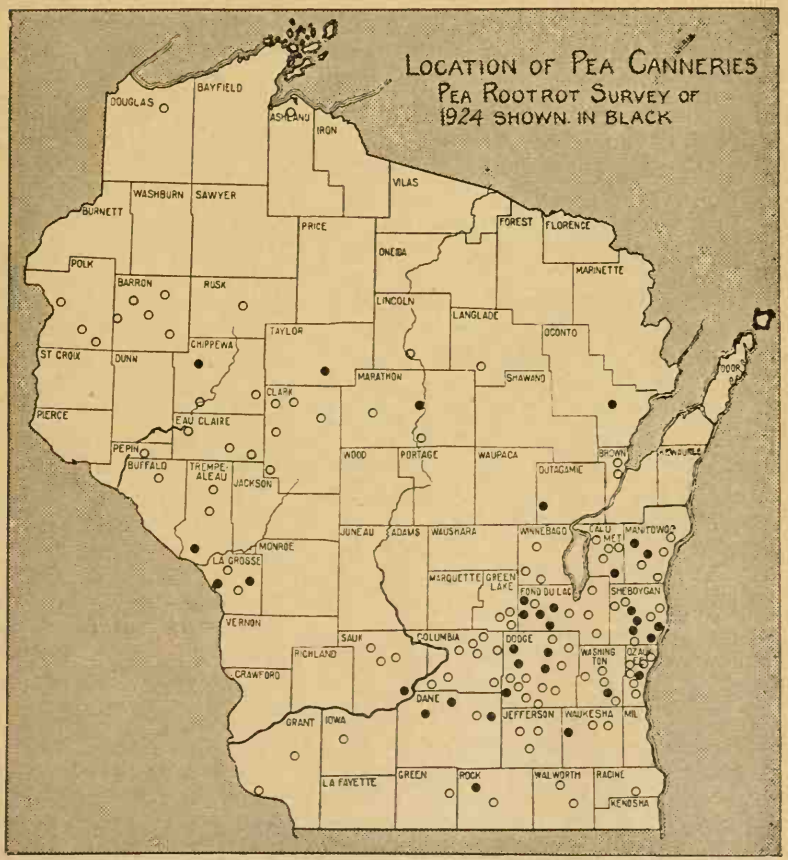

FIG. 8.—LOCATION OF PEA CANNERIES IN WISCONSIN

Circles indicate the location of the pea canning factories in Wisconsin. Black dots designate the factory districts surveyed for disease in 1924.

SUMMARY

1.—The Wisconsin pea crop of 1924 represented a total farm value of over $7,000,000. Pea diseases play a major part in determining the systems of pea culture employed and in reducing profits to both growers and canners.

2.—In 1924 a detailed survey was made of 688 fields comprising 5,416 acres representatively distributed in the pea growing sections of Wisconsin to determine the importance of the various pea diseases and especially to study the development of rootrot in relation to cropping practices, soil types, and other factors which appeared to influence its occurrence and destructiveness.

3.—This bulletin is a summary of the findings of this survey, supplemented with notes from pea disease investigation conducted in this state by the U. S. Department of Agriculture in cooperation with the Wisconsin Experiment Station.

4.—The rootrot chiefly considered in this survey is that caused by the fungus *Aphanomyces euteiches* Drechsler. This fungus is assumed to be indigenous is Wisconsin soils, occurring especially in wet locations. It increases rapidly in the soil with culture of peas.

5.—The season of 1924 was so cool and favorable for the development of peas that fields infested with rootrot did not appear to suffer as great damage as in other years.

6.—The rootrot caused by Aphanomyces was more destructive in 1924 than all other fungous and bacterial diseases of peas combined, considering the state as a whole. In some localities a newly observed "wilt" disease was more destructive, and in the state it ranked second to the Aphanomyces rootrot. Anthracnose caused by *Colletotrichum pisi* Pat. was the most destructive of the foliage diseases, causing important losses in several districts.

7.—Rootrot was found in 32 per cent of all fields examined. Eleven per cent of all fields were severely infested. The total loss in inspected fields is estimated at 8 per cent of the total yield. Since diseased fields were especially sought in the survey it is believed that the pea crop in the state as a whole did not suffer as great a loss as this. Even if the loss in the entire state amounted to only half this amount or 4 per cent of the total yield it would represent a loss to the growers of about $300,000 in addition to losses incurred by the canning companies.

8.—Of the fields inspected 48 per cent were growing their first crop of peas. Fields which had been planted more than once to peas were found to have on the average a rotation period of about two and one-half years.

9.—Rootrot increases both in frequency of occurrence and in severity with the number of crops grown. Rootrot occurred but rarely in fields growing the first crop of peas, while all fields growing the fifth crop were more or less infested. The occurrence of severe infestation does not increase rapidly during the

first four crops; but it rose to 56 per cent of the fields growing the fifth crop.

10.—Peas were found growing on 27 soil types and seven groups of incompletely classified soils, thus making the number of fields on most types too small for comparison. No soil type showed prospects of providing environment in which rootrot cannot develop. The two soil types which include nearly half of all the fields examined—Miami silt loam and Carrington silt loam—show little difference in behavior. In general, with similar cropping, clays and clay loams have a larger percentage of severely infested fields than loams and silt loams or lighter soils. In fields including more than one soil type, disease usually appears first in the soil with greater moisture holding capacity, or in poorly drained spots. Greater precautions to avoid rootrot are needed on heavy or wet soils than on well drained, medium, or light soils.

11.—Rootrot was found to persist in some Wisconsin soils for 10 years after it had caused crop failure. After such failure no fields were found entirely free from rootrot in less than 10 years.

12.—The only commercial variety of pea that showed an important degree of resistance was the Green Admiral; and even this variety was greatly damaged when not planted early.

13.—A five or six year rotation is suggested as a method of control of this disease which should prevent its appearance on most Wisconsin soils not already infested. When a shorter rotation seems advisable, careful inspection of fields can detect its development before it becomes destructive. Resistant varieties are being tested which may be of value in some situations.

14.—Other diseases discussed in this bulletin are as follows:

Stem and rootrot caused primarily by species of Fusarium was not found in 1924.

A new but apparently relatively unimportant footrot caused by a species of Phoma was noted.

Seedling injury caused by *Rhizoctonia solani* Kuhn was noted in 35 fields, but for the most part was not important.

The relation of species of Pythium to seedling and root injury is discussed briefly.

A new wilt disease was found in 50 fields, in some localities causing greater losses than Aphanomyces rootrot. The cause has not yet been determined.

Leaf and podspot caused by *Ascochyta pisi* Lib. was rare and unimportant.

Leafblotch caused by *Septoria pisi* West. was not abundant but was important in a few fields in northern districts.

A leafspot new to Wisconsin caused by *Septoria flagellifera* E. and E. was noted.

Anthracnose caused by *Colletotrichum pisi* Pat. was the most important foliage disease encountered, causing considerable damage late in the season.

Downy mildew caused by *Peronospora viciae* Berk. was rare and unimportant.

Bacterial blight caused by *Pseudomonas pisi* Sackett was encountered occasionally both on early planted peas on wet soil, and on foliage of mature plants late in the season.

Mosaic was encountered frequently late in the season, but rarely appeared to reduce yields.

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Studies of the Epidemiology and Control of Apple Scab

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CONTENTS

Studies of the Epidemiology and Control of Apple Scab

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APPLE SCAB, caused by *Venturia inaequalis* (Cke.) Wint., is one of the most widespread and destructive fruit diseases. The range of its occurrence closely parallels that of apple culture throughout the world, save in comparatively limited areas, as in certain arid sections and in climates which approach the higher temperature limits of apple production. In most of the chief apple producing sections of the world, it occurs in sufficient severity to make its control essential to commercial apple culture. The disease occurs at its worst where the climate is humid and cool in spring and early summer. It is, therefore, very destructive in the more humid sections of the Pacific Northwest of North America and in the north-central and northeastern apple belt of the United States and Canada. It is very severe in its outbreaks in Wisconsin, especially in those sections where the climate is most influenced by the Great Lakes, as on the Door County Peninsula.

The widespread occurrence and great economic importance of apple scab have contributed toward making it the subject of many investigations. Most of the voluminous literature that has resulted, except the more recent contributions, is readily accessible through the works of Aderhold (1896, 1900), Clinton (1901), Wallace (1913), and Morris (1914), which include extensive bibliographies and reviews. Consequently, a general review of literature in the present paper appears to be unwarranted. Such discussions of previous work as seem necessary appear in the appropriate connections in the body of the paper.

Studies of apple scab at the Wisconsin Agricultural Experiment Station were begun by Trelease (1884), who gave one of the best early accounts of the nature and cause of the disease and a discussion of its occurrence and prevention in this state. Several years later, the work was taken up by Goff,[1] who played a leading part in adapting recently developed spraying methods to apple scab control. As a result of Goff's pioneer work, spraying with Bordeaux mixture came to be an accepted practice for scab control in Wisconsin orchards. Subsequently, the Department of Horticulture has conducted numerous

[1]For an annotated bibliography of Goff's work on apple scab, see Clinton (1901, p. 130-131).

tests of the more promising materials and programs which have come into use for the control of this disease. In 1916 and 1917, at the request of R. H. Roberts,[1] who was then in charge of this work, the senior author cooperated in planning the tests and recording results, and made such observations as were feasible upon the development of the causal fungus and of the disease in relation to control measures. This work, which was conducted in the orchard of the Northern State Hospital, at State Hospital, Wisconsin, included comparative trials of commercial liquid lime-sulphur in various concentrations, Bordeaux mixture of various concentrations and ratios of lime to copper sulphate, a mixed program of lime-sulphur and Bordeaux mixture, barium tetrasulphide, and sulphur-arsenate dust (90-10). Arsenate of lead (powder), at the rate of 1 pound in 50 gallons, was included in all spray treatments. Each program was applied according to a four-treatment schedule which, with minor modifications, was then generally accepted as standard for apple scab control throughout the north-central and northeastern apple belt of the United States. The treatments were made (1) just before the blossoms opened, and if possible when the blossom buds were well separated in the clusters, (2) when about three-fourths of the petals were off, (3) about 10 days later, and (4) at a time most advantageous for combating the second brood of codling moth. Because of limitations of space, the detailed data from these trials will not be given. Their most outstanding feature was the failure of any of these programs to control the disease consistently. In 1916, a year of moderate scab development, the disease was easily controlled, except in the case of a very susceptible unidentified variety, which was severely scabbed. On this variety none of the programs tested controlled the disease satisfactorily. In 1917, a year of severe scab development, none of the programs tested gave satisfactory control even on moderately susceptible varieties. In view of these results, it seemed desirable to undertake a more critical study of the disease and its control. After an interruption occasioned by the world war, such studies were begun by the present writers in 1919 and are still in progress. Field laboratory[1] and field studies, the latter in cooperation with apple growers, have been conducted at Sturgeon Bay, Wisconsin. For the five seasons, 1920-1924, the junior author lived in quarters adjacent to the field laboratory, thereby gaining an excellent opportunity for making adequate observations and records. Field, laboratory, and greenhouse studies have been conducted at Madison.

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[1]Grateful acknowledgments are made:

To Prof. R. H. Roberts and the Department of Horticulture of the University of Wisconsin for their cordial and efficient cooperation in the work of 1916 and 1917.

To Messrs. E. A. Stokdyk (in 1919), R. B. Streets (in 1920 and 1921), and E. E. Wilson (in 1923 and 1924) for valued assistance in conducting the field work.

To Messrs. W. I. Lawrence, M. B. Goff, S. T. Learned, and B. W. Sackett for the cordial cooperation which has made the field studies possible.

EPIDEMIOLOGY IN RELATION TO CONTROL[1]

Apple scab varies greatly both in severity of occurrence and difficulty of control. Almost any spraying or dusting program commonly recommended to combat this disease may seem efficient in a season of moderate scab development and easy control: yet, the best methods now in general use may fail to give satisfactory results in a year of severe epidemic outbreak and difficult conditions for control. What are the reasons for these variations, and how may more adequate control measures be developed? When the present work was begun, it was apparent that these questions could not be answered satisfactorily on the basis of the knowledge then available. Many cardinal points in the cycles of development and control of the disease were well understood, but insufficient study had been given to intervening gaps or to variability in these cycles. It seemed necessary, therefore, (1) to seek a more adequate understanding of the cycles of development and control of the disease and (2) to inquire into the extent, causes, and effects of their variability. Consequently, work has been directed along two correlated lines: (1) field studies of the development and control of the disease in relation to the natural environment, and (2) laboratory and greenhouse studies of the development and prevention of the disease under conditions in which certain factors of the environment were varied under control. While many phases of this work are unfinished and are being continued, it has seemed desirable to incorporate in the present paper a report of the progress which has been made.

Field Studies

Field studies were conducted with a four-fold purpose: (1) to procure detailed records pertaining to the development and control of the disease in relation to certain factors of the natural environment, (2) to solve certain urgent practical problems without the delay incident to the development of apparatus and technique for work under controlled conditions, (3) to define problems for laboratory and greenhouse study, and (4) to check against the results of experiments in which certain factors of the environment were controlled.

Seasonal Development Records

The following methods were devised for studying certain significant aspects of the development of host, parasite, disease, and the effectiveness of control measures in relation to certain factors of the environment.

Of the host. The development of two or three leading varieties was followed throughout the season. In order to gain an approximate

[1]A condensed report on certain aspects of these studies has been made by the senior author (1926).

idea of the time at which the foliage expanded in relation to fungicidal treatments and disease development, records were made of the development of at least five twigs of the current year's growth and five blossoming fruit spurs on one or more representative unsprayed trees of each variety studied. Beginning as soon as the leaves were large enough to be observed conveniently, counts of the number of leaves were made at two- to three-day intervals throughout the period of twig growth. In the earlier years measurements were made of the expansion of leaf surface, but these proved to be more time-consuming than was warranted by the purposes served. The earlier stages of bud and leaf development were followed by means of notes and photographs. Fruit development was followed on fifty apples on each variety studied. As soon as the fruit "set," these were chosen from various parts of at least two representative trees of each variety. The diameters of these apples were recorded at suitable intervals throughout the season. Averages from these data from Lubsk's Queen are expressed graphically in Figures 2-5, and from Dudley in Figure 6.

Of the fungus. The only detailed seasonal development record of the fungus, apart from that included in disease development, is of ascospore discharge. The record of seasonal development of the disease, however, is a fairly adequate index of the parasitic development of the fungus and the production of conidia. The earlier records of ascospore discharge were made by a modification of the methods described by Wallace (1913) and Childs (1917). Two thin strips of wood (pot labels) were laid parallel to each other and about two centimeters apart upon each of five representative leaves bearing abundant perithecia of *V. inaequalis*. A strip of blotting paper was attached to the top of each of these pieces of wood, and a portion of a glass slide about one by three centimeters, coated lightly with glycerine jelly, was laid across each pair of wooden supports so as to rest about two millimeters above the surface of the leaf. These strips of blotting paper minimized the chance that condensation water which might form on the glass would run down upon the leaf surface. The glass was used in narrow strips to minimize its sheltering effect in light rains. The glass strips were replaced each morning at 8:00 o'clock, and the approximate number of spores caught was determined by counts under the microscope. If the number of spores caught was small or *nil*, one square centimeter of the exposed surface of each slide was examined. This was accomplished and duplication avoided by using a mechanical stage and a micrometer. If spores were numerous, counts were made in 30 fields taken in representative parts of the slide without duplication, and the average number of ascospores per square millimeter of glass surface was computed. Averages of the results from the five leaves in each experiment appear in Figures 1-6. It should be clearly recognized that these data are only semi-quantitative and approximately representative of the seasonal discharge. Quan-

titative data on the frequencies of ascospores in orchard air were obtained by another method (see p. 43-44 and Table I). Supplementary studies of a wide range of leaf material were made in order to gain a more satisfactory record of the maturity and early discharges of ascospores.

Of the disease. The number of lesions on each leaf and fruit used in following the seasonal development of the host was recorded at two- to three-day intervals throughout the season. In certain instances where there was an excessive drop of fruit, other apples were substituted for those which fell. Any fruits or leaves which became so heavily infected as to preclude accurate counts were discarded from the series. The results are presented graphically in Figures 2-6 in terms of average daily increase in number of lesions per leaf and per fruit.

In certain seasons the usual studies of the natural development of the disease were supplemented by inoculation experiments made in the orchards. Of several methods tried the following, which was the most satisfactory, was used exclusively after 1920. The organs to be inoculated were atomized with sterile distilled water and inserted in cylinders of wire netting one to two inches in diameter and two to four inches long. Wet apple leaves bearing abundant mature ascocarps of the scab fungus were wrapped about these cylinders in such position that naturally ejected ascospores would fall upon the surfaces to be inoculated. Wet absorbent cotton was wrapped over these leaves, after which parchment paper bags were drawn over these improvised moist chambers and fastened tightly to the twigs. After three days the moisture holding devices were removed. Before and after inoculation, the inoculated parts were protected from natural infection by bagging. Organs similarly treated, except that no inoculum was applied, served as controls. Incubation periods shown by these experiments appear in Figures 2, 3, 5, and 6.

Because of their comparatively small volume, these data on seasonal development are to be regarded as only approximately representative. In view of the purposes served and the resources available, it has seemed neither necessary nor feasible to increase the volume or scope of these records.

Meteorological Records

A meteorological station equipped with instruments of types used by the United States Weather Bureau was established in the experimental orchards.

Relative humidity and air temperature. The records of relative humidity and air temperature were secured by means of a hygrothermograph which was housed in a standard instrument shelter set about four feet from the ground. This instrument was adjusted at frequent intervals by means of a sling psychrometer and a standardized thermometer.

Rainfall. Prior to 1923 rainfall records were taken by means of a standard rain and snow gauge. Beginning with 1923 hourly rainfall has been recorded by means of a tipping-bucket rain gauge electrically connected with a quadruple register.

Wind velocity and hours of bright sunshine. Beginning with 1923 hourly records of wind velocity and bright sunshine have been made by means of a standard anemometer and a sunshine recorder, which are electrically connected with the quadruple register.

Discussion of Results

Yearly graphic summaries of data from the field studies appear in Figures 1-6. It will at once be observed that data condensed to this degree are lacking in certain important details. A notable example is in the rainfall section. The total precipitation may be of little importance in comparison with its hourly distribution. For details of this nature it is necessary to refer to the hourly records or to a yearly table which gives bi-hourly averages of the meteorological data in correlation with records of the frequencies of ascospores of *V. inaqualis* in the orchard air, as determined experimentally (Table I). These records of the field studies are used as source material and are discussed later in the paper in relation to various topics to which they are pertinent. They have served their purposes very satisfactorily. They have shown, in general agreement with many previous observations by other investigators (e. g., Aderhold, 1900, 1902; Ewert, 1911; Bremer, 1924) that the moisture and temperature factors of the natural environment play a leading rôle in determining the severity of occurrence of the disease and the difficulty of its control. They have defined critical points in epidemiology and control, and have contributed to material increase in the efficiency of the fungicidal program through its more effective orientation to critical periods.

Laboratory and Greenhouse Studies

In the interpretation of field data and a study of the development and the prevention of epidemics, it is desirable to have a detailed knowledge of spore germination and infection, and of the relationships of the more important factors which affect their occurrence in nature. In the hope of contributing to this knowledge the following work was initiated.

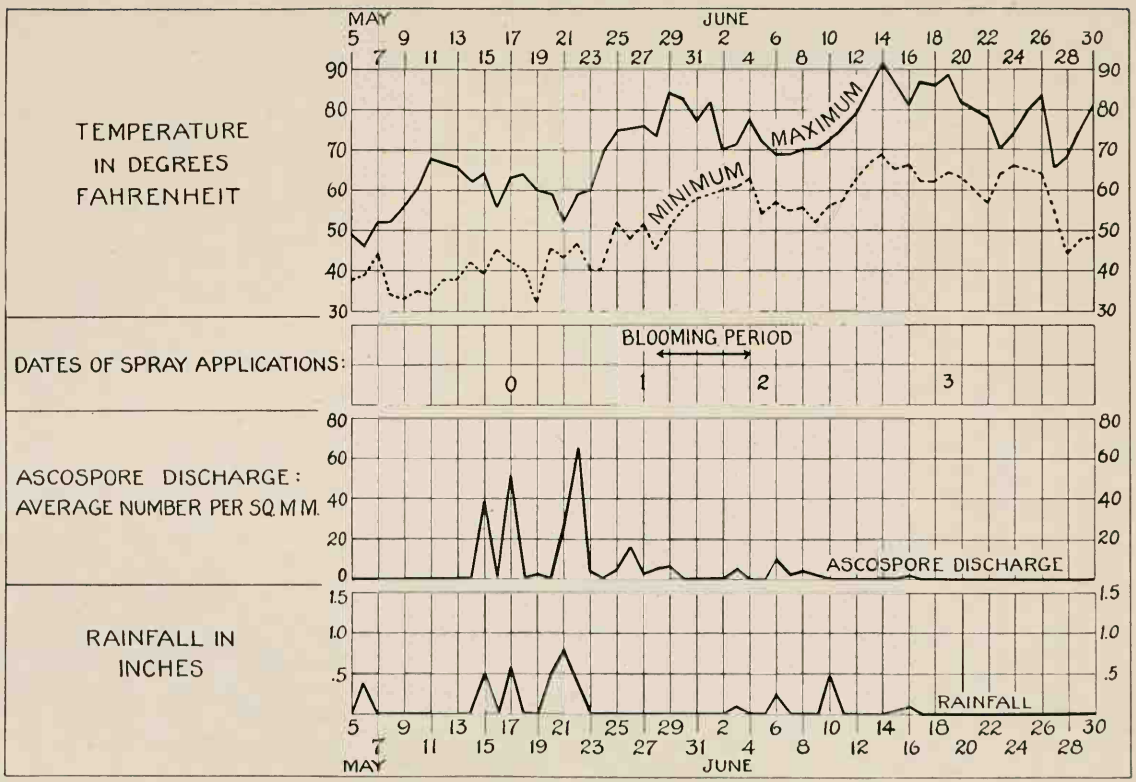

FIG. 1.—A GRAPHIC SUMMARY OF CERTAIN RECORDS RELATING TO THE EPIDEMIOLOGY AND CONTROL OF APPLE SCAB, STURGEON BAY, WIS., 1919 (see p. 3-6).

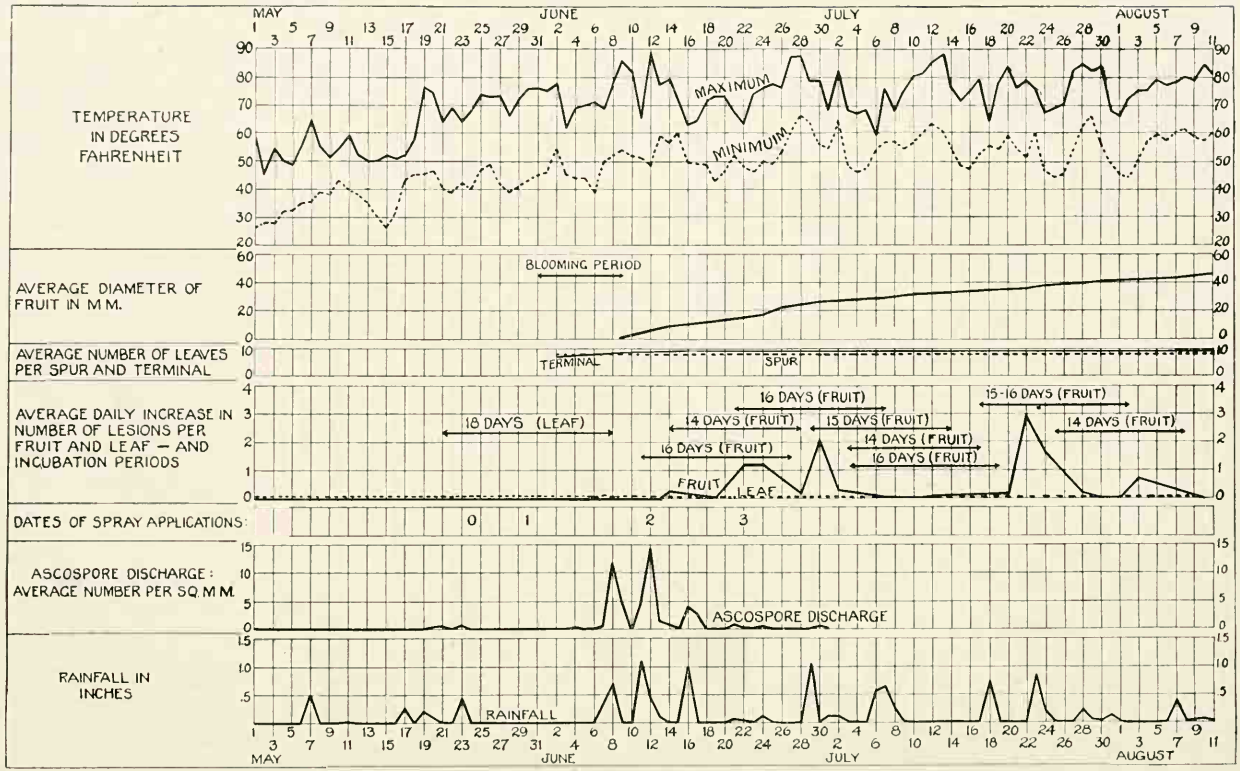

FIG. 2.—A GRAPHIC SUMMARY OF CERTAIN RECORDS RELATING TO THE EPIDEMIOLOGY AND CONTROL OF APPLE SCAB, STURGEON BAY, WIS., 1920 (see p. 3-6)

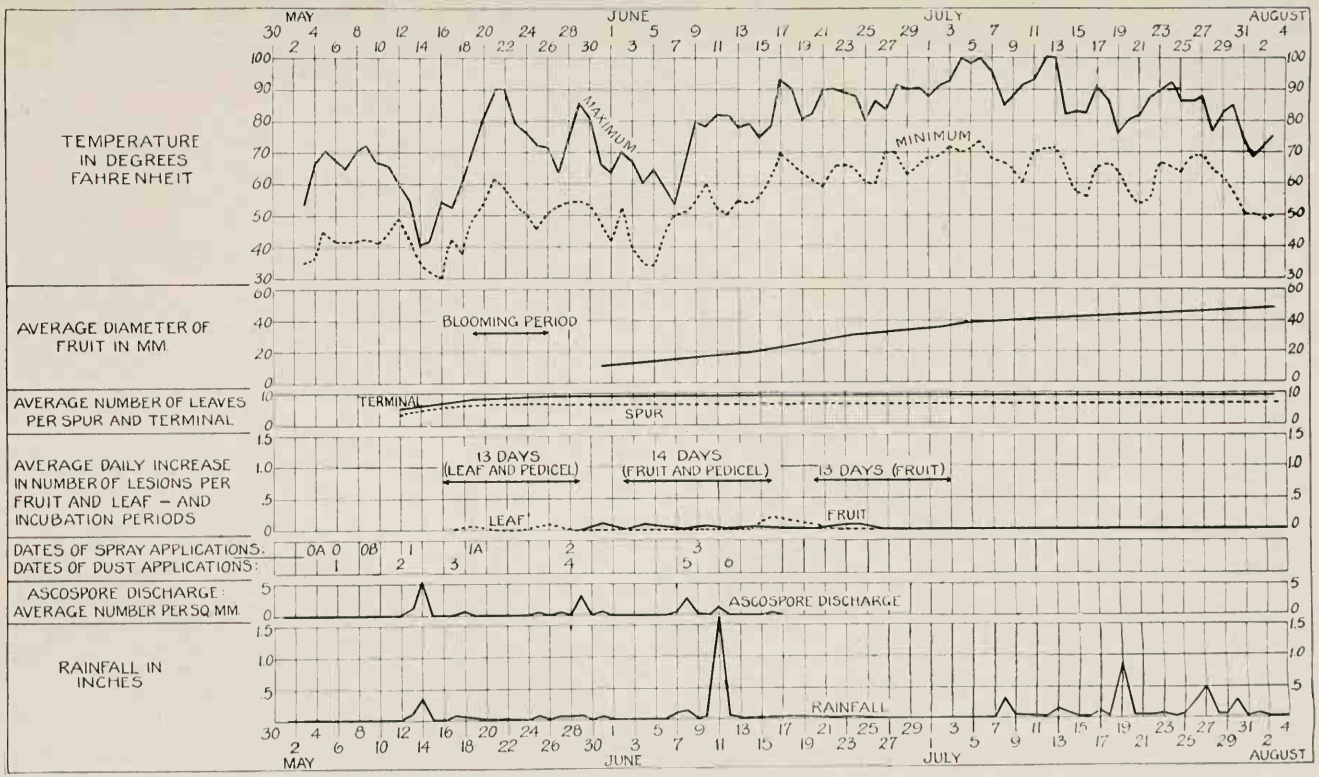

FIG. 3.—A GRAPHIC SUMMARY OF CERTAIN RECORDS RELATING TO THE EPIDEMIOLOGY AND CONTROL OF APPLE SCAB, STURGEON BAY, WIS., 1921 (see p. 3-6)

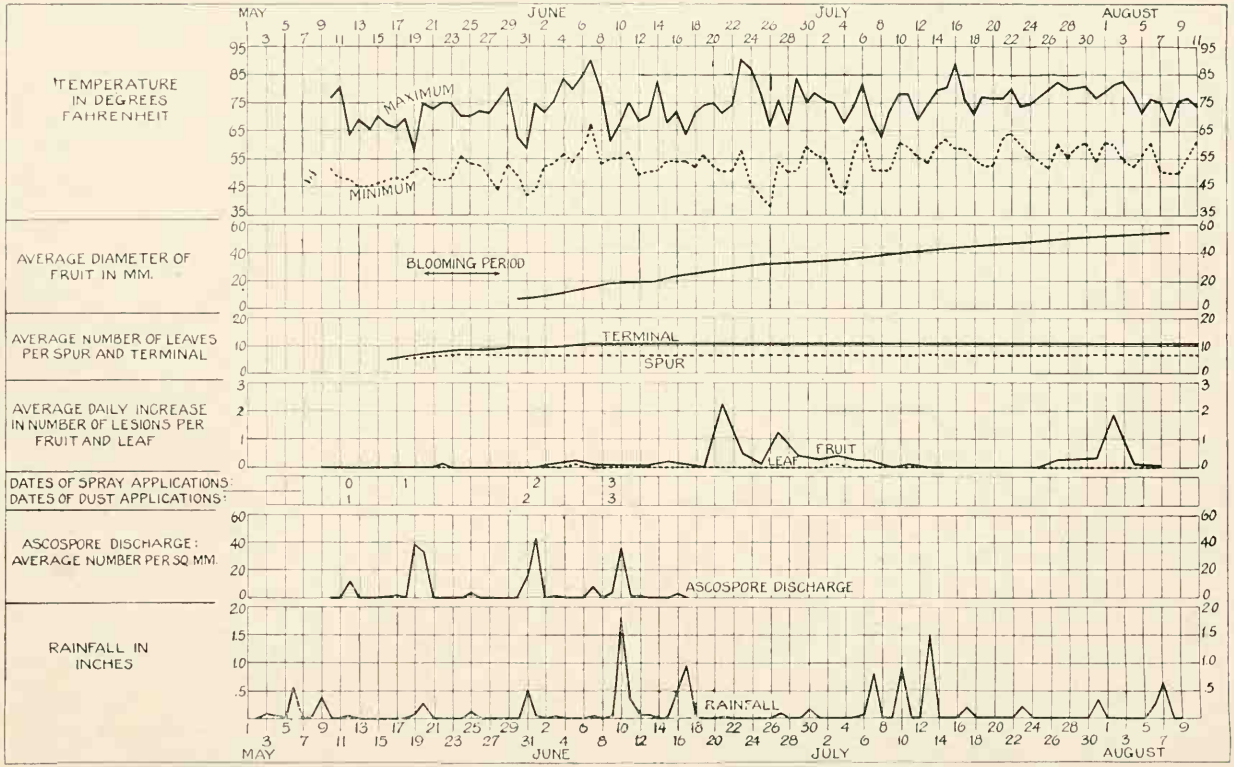

FIG. 4.—A GRAPHIC SUMMARY OF CERTAIN RECORDS RELATING TO THE EPIDEMIOLOGY AND CONTROL OF APPLE SCAB, STURGEON BAY, WIS., 1922 (see p. 3-6)

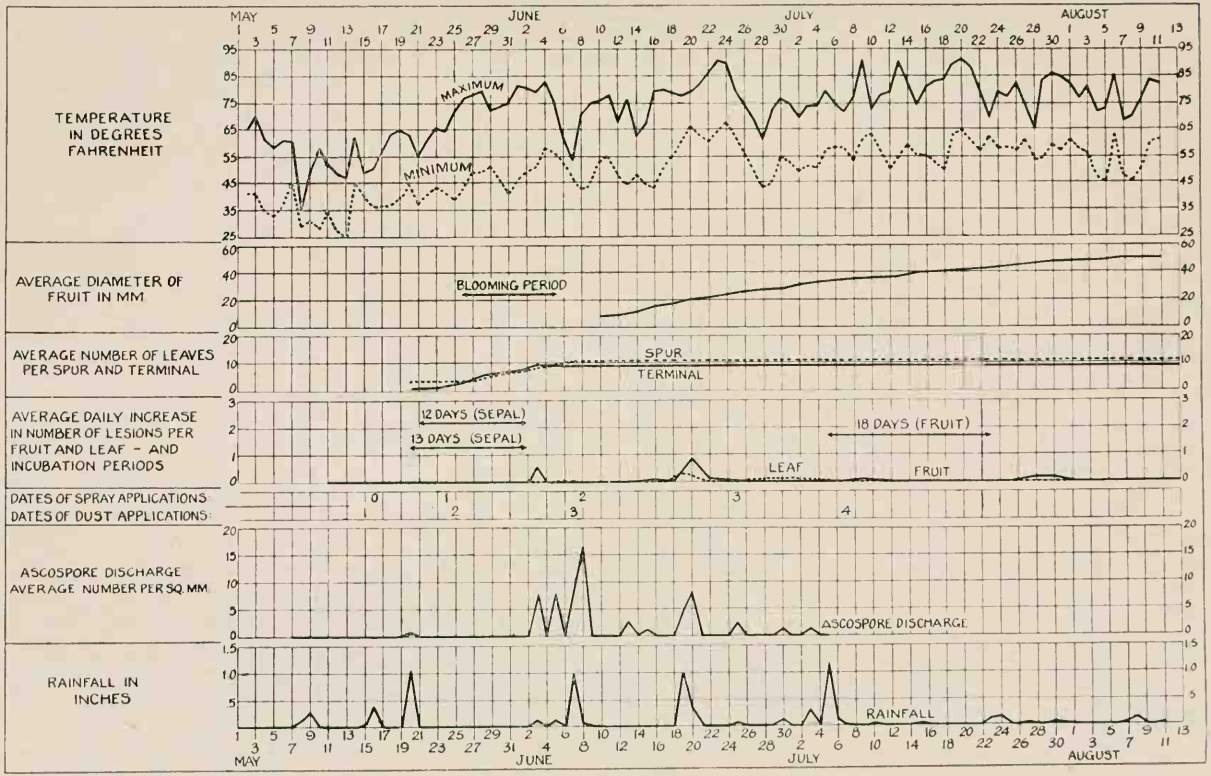

FIG. 5.—A GRAPHIC SUMMARY OF CERTAIN RECORDS RELATING TO THE EPIDEMIOLOGY AND CONTROL OF APPLE SCAB, STURGEON BAY, WIS., 1923 (see p. 3-6)

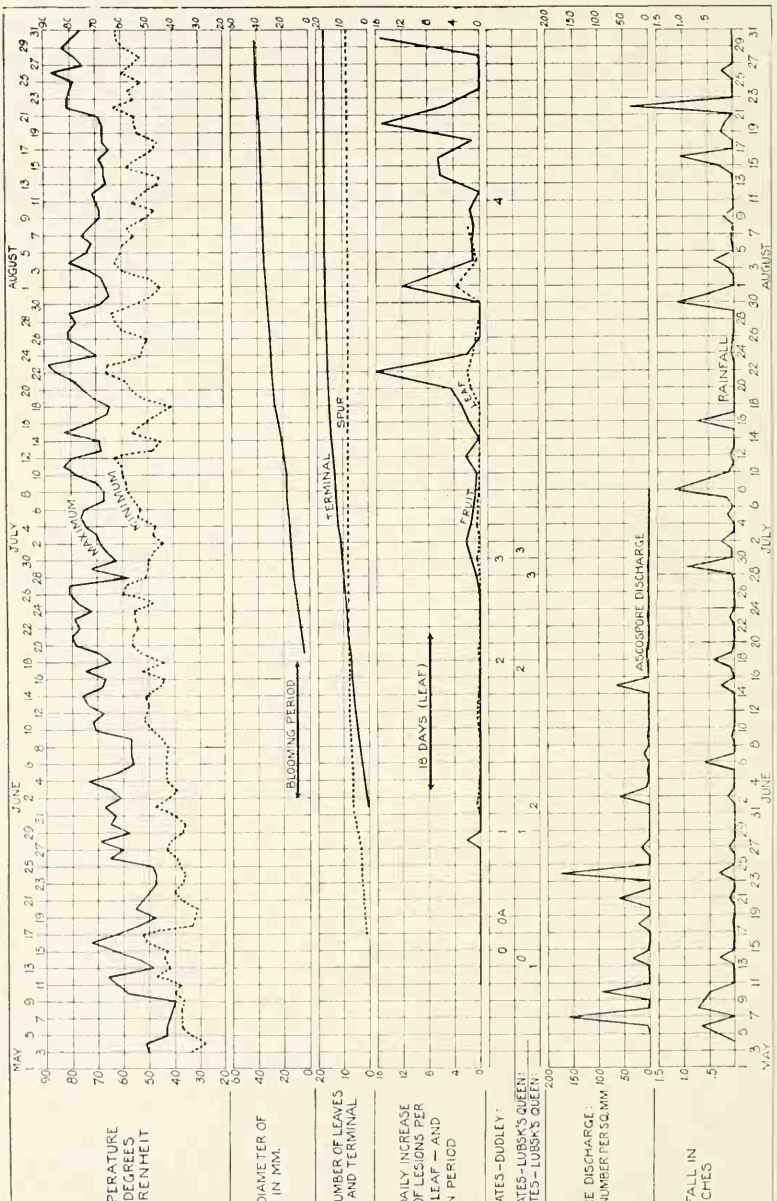

FIG. 6.—A GRAPHIC SUMMARY OF CERTAIN RECORDS RELATING TO THE EPIDEMIOLOGY AND CONTROL OF APPLE SCAB, STURGEON BAY, WIS., 1924 (see p. 3-6)

TABLE I.—FREQUENCIES OF ASCOSPORES OF V. INAEQUALIS IN ORCHARD AIR IN RELATION TO CERTAIN FACTORS OF THE ENVIRONMENT, STURGEON BAY, WIS., 1924[1]

Date	Subject of record[2]	Averaged records for two-hour periods beginning: A. M. 12	2	4	6	8	10	P. M. 12	2	4	6	8	10
May	Rainfall	---	---	---	---	.01	.20	.14	---	---	.05	.22	.33
	Spore Freq.	←		no record									→
5	Temperature	37	41	41	43	41	39	38	40	40	41	40	39
	Sunshine	---	---	---	---	---	---	---	---	---	---	---	---
	Wind Veloc.	5	4	3	5	5	8	9	9	12	15	18	18
	Rainfall	.07	---	.01	---	---	---	---	---	---	---	---	---
	Spore Freq.	←		no record						→	←		
6	Temperature	39	39	38	38	40	42	42	42	43	42	41	40
	Sunshine	---	---	---	---	---	---	---	---	---	---	---	---
	Wind Veloc.	15	14	17	16	19	17	16	15	17	17	16	15
	Rainfall	.01	---	---	.01	---	---	---	---	---	.01	.07	.24
	Spore Freq.			0.6		→	←						
7	Temperature	41	42	42	41	39	39	39	39	39	39	38	38
	Sunshine	---	---	---	---	---	---	---	---	---	---	---	---
	Wind Veloc.	15	15	15	14	15	15	17	18	19	22	24	24
	Rainfall	.14	.11	.13	.09	.08	.06	---	---	---	.04	17	.08
	Spore Freq.			2.2		→	←	1.5		→	←		
8	Temperature	39	39	39	39	38	38	39	41	40	39	37	37
	Sunshine	---	---	---	---	---	---	---	---	---	---	---	---
	Wind Veloc.	---	---	---	---	---	---	21	22	22	19	21	22
	Rainfall	.03	.13	.02	.01	.08	.16	.07	.10	.04	.03	.02	---
	Spore Freq.			0.2	→	← 1.1	→ ←						
9	Temperature	36	36	36	36	37	38	39	39	40	40	40	40
	Sunshine	---	---	---	---	---	---	---	---	---	---	---	---
	Wind Veloc.	22	21	18	15	14	12	11	9	6	3	5	4
	Rainfall	---	---	.01	---	---	---	---	---	---	---	---	---
	Spore Freq.			28		→ ←							
10	Temperature	40	40	41	43	49	55	57	56	55	47	49	47
	Sunshine	---	---	---	---	+	+	+	+	+	+	---	---
	Wind Veloc.	5	4	3	3	3	5	6	7	7	8	6	8
	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Spore Freq.			0		→	←						
11	Temperature	44	40	41	53	55	59	61	59	59	53	48	48
	Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
	Wind Veloc.	4	3	3	3	4	5	8	7	8	6	6	5
	Rainfall	---	---	---	---	---	---	---	---	---	---	.01	.01
	Spore Freq.			0	→	←	0			→	←		
12	Temperature	50	49	50	48	55	64	64	65	63	57	55	51
	Sunshine	---	---	---	---	+	+	+	+	+	---	---	---
	Wind Veloc.	5	3	1	0	1	7	8	10	7	5	3	4
	Rainfall	---	.01	.01	.03	.03	.09	.01	.01	.01	---	.01	.02
	Spore Freq.			2.2	→	← 289		→ ←	2.2	→ ←			
13	Temperature	46	45	43	42	47	46	47	47	46	47	45	43
	Sunshine	---	---	---	---	---	---	---	---	---	---	---	---
	Wind Veloc.	4	5	3	2	5	6	5	5	6	6	7	2
	Rainfall	.06	.05	---	---	---	---	---	.03	.01	-----	-----	-----
	Spore Freq.			16		→ ←		1.7		→	←		
14	Temperature	45	45	45	44	46	46	53	49	51	51	47	47
	Sunshine	---	---	---	---	---	+	+	---	---	---	---	---
	Wind Veloc.	2	3	2	3	5	4	4	6	8	5	5	9
	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Spore Freq.			0	→ ←		0				→	←	
15	Temperature	45	46	44	49	56	62	62	61	61	56	52	51
	Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
	Wind Veloc.	8	6	7	11	11	6	8	11	8	6	6	5
	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Spore Freq.			0	→	←			0			→ ←	
16	Temperature	50	51	53	59	65	70	71	71	66	55	56	58
	Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
	Wind Veloc.	6	8	7	11	16	17	19	17	15	7	10	9
	Rainfall	---	---	---	---	---	.02	---	---	---	---	---	---
	Spore Freq.			0	→	← 0	→ ←		3.0	→	←		
17	Temperature	62	59	58	58	55	54	58	59	60	59	52	47
	Sunshine	---	---	---	---	---	---	+	+	---	---	---	---
	Wind Veloc.	15	13	9	9	5	6	4	4	7	8	10	10

TABLE I.—FREQUENCIES OF ASCOSPORES OF V. INAEQUALIS IN ORCHARD AIR IN RELATION TO CERTAIN FACTORS OF THE ENVIRONMENT, STURGEON BAY, WIS., 1924 —*Continued.*

Date and subject of record[2]	Averaged records for two-hour periods beginning:											
	A. M.						P. M.					
	12	2	4	6	8	10	12	2	4	6	8	10
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.			0		> <							
18 Temperature	44	42	44	46	48	52	52	51	48	43	40	36
Sunshine	---	---	+	+	---	+	+	+	+	+	---	---
Wind Veloc.	6	4	5	9	16	19	24	23	17	12	10	8
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.			0		> <							
19 Temperature	33	32	32	34	36	39	45	45	45	41	37	34
Sunshine	---	---	---	---	---	---	+	+	+	---	---	---
Wind Veloc.	4	2	2	5	7	9	10	15	13	8	4	6
Rainfall	---	---	---	---	---	---	---	---	---	---	.03	.09
Spore Freq.			0		> <				0		> <	
20 Temperature	32	32	34	43	50	54	54	51	49	47	46	41
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	4	5	3	4	6	6	16	20	23	14	8	6
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0.2			> <							
21 Temperature	41	40	37	39	42	46	50	52	52	47	43	42
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	5	10	9	12	13	11	11	11	11	7	5	8
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.			0		> <				0		> <	
22 Temperature	41	41	44	46	46	45	45	48	48	46	42	41
Sunshine	---	---	---	---	---	---	+	+	+	+	---	---
Wind Veloc.	6	6	8	11	12	16	16	12	10	10	9	8
Rainfall	---	---	---	---	---	.08	.18	.08	---	---	---	---
Spore Freq.			0		> <	85	>	< 47 >	< 31 >	< 11 >	<	
23 Temperature	45	47	45	46	45	45	43	42	46	42	39	38
Sunshine	---	---	---	---	---	---	---	---	---	---	---	---
Wind Veloc.	9	9	11	14	16	16	12	9	12	17	12	10
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0.3		> <								
24 Temperature	38	37	37	40	44	46	46	45	44	44	41	40
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	10	10	10	11	13	13	15	16	12	13	10	8
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0			>	<						
25 Temperature	40	39	39	41	46	47	47	47	47	44	41	40
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	9	9	11	10	11	14	15	15	12	7	3	4
Rainfall	---	---	---	---	---	---	---	---	---	---	---	.03
Spore Freq.		0		> <				0			> <	
26 Temperature	39	39	42	48	52	57	60	64	62	57	53	49
Sunshine	---	---	+	+	+	---	+	+	---	---	---	---
Wind Veloc.	5	5	8	9	11	12	14	14	14	14	7	3
Rainfall	.05	.02	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0.1			> <							
27 Temperature	47	47	46	45	49	54	56	57	59	59	52	48
Sunshine	---	---	---	---	+	+	+	+	+	---	---	---
Wind Veloc.	4	6	4	4	3	6	8	9	6	5	7	2
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0				> <						
28 Temperature	43	41	41	48	57	63	67	69	66	55	46	44
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	1	2	3	5	9	13	14	13	10	10	9	5
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		> <								
29 Temperature	40	38	38	44	49	51	55	58	58	49	41	40
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	6	6	5	9	10	11	10	12	7	3	1	1
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		> <								
30 Temperature	40	37	41	47	52	58	60	62	63	57	51	42
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	1	2	4	6	7	8	5	8	10	5	5	7
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		> <								
31 Temperature	41	40	44	53	57	61	62	62	59	53	49	49
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	6	5	3	2	6	7	9	9	9	6	5	6

TABLE 1.—FREQUENCIES OF ASCOSPORES OF V. INAEQUALIS IN ORCHARD AIR IN RELATION TO CERTAIN FACTORS OF THE ENVIRONMENT, STURGEON BAY, WIS., 1924

—Continued.

Date and subject of record[2]	Averaged records for two-hour periods beginning:											
	A. M.						P. M.					
	12	2	4	6	8	10	12	2	4	6	8	10
June												
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0			> <			0			> <	
1 Temperature	49	47	49	57	61	65	62	62	59	56	55	53
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	7	7	6	6	6	5	7	7	4	4	2	2
Rainfall	---	.03	.05	.04	.03	---	---	---	---	---	---	---
Spore Freq.		3.8			> <							
2 Temperature	51	51	49	49	50	55	57	61	61	57	48	45
Sunshine	---	---	---	---	---	+	+	+	+	---	---	---
Wind Veloc.	2	5	8	5	4	6	6	6	5	2	2	3
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0			> <							
3 Temperature	41	40	39	49	58	59	63	63	61	54	48	45
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	2	2	3	3	4	5	6	7	5	4	2	1
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0			>							<
4 Temperature	44	47	49	55	63	71	73	71	67	61	58	55
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	2	5	3	5	7	7	8	11	10	7	7	6
Rainfall	---	---	---	---	---	---	---	---	---	.60	.01	---
Spore Freq.		0			>				<			
5 Temperature	52	46	44	52	58	61	62	60	57	56	57	56
Sunshine	---	---	---	+	+	+	+	---	---	---	---	---
Wind Veloc.	4	4	3	6	11	14	15	12	10	9	5	7
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		19		>	<							
6 Temperature	54	53	51	52	54	53	55	56	54	50	47	45
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	7	8	10	13	14	15	13	12	9	3	3	3
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		2.3			>						<	
7 Temperature	44	44	46	51	55	56	55	54	50	47	45	45
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	4	6	5	6	5	6	9	8	7	4	1	5
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0			>						<	
8 Temperature	45	43	43	45	49	53	55	57	55	52	48	46
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	9	8	8	8	9	10	11	10	10	7	6	7
Rainfall	---	---	.05	---	---	---	---	---	---	---	---	---
Spore Freq.		13		>								<
9 Temperature	46	48	46	47	53	56	57	56	55	54	54	52
Sunshine	---	---	---	+	+	+	+	+	---	---	---	---
Wind Veloc.	6	4	7	8	7	5	4	3	3	4	3	2
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		>								<
10 Temperature	52	50	50	53	60	69	70	70	65	62	60	58
Sunshine	---	---	---	+	+	+	+	+	+	---	---	---
Wind Veloc.	5	2	3	5	6	6	9	10	9	7	6	6
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		>								<
11 Temperature	57	57	58	59	65	65	65	71	71	67	61	57
Sunshine	---	---	---	+	+	+	+	+	+	---	---	---
Wind Veloc.	4	3	4	6	5	5	5	8	8	5	3	3
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		>								<
12 Temperature	56	53	55	59	61	64	64	61	62	57	55	52
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	3	2	3	5	7	8	8	7	5	3	3	3
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		>								<
13 Temperature	50	50	55	62	67	71	72	74	72	63	54	54
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	3	2	3	4	8	6	6	6	5	2	1	2
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		>								
14 Temperature	55	55	55	68	73	75	75	68	62	55	52	54
Sunshine	---	---	---	+	+	+	+	+	+	+	---	---
Wind Veloc.	3	3	2	3	5	6	7	7	7	6	6	6

TABLE I.—FREQUENCIES OF ASCOSPORES OF V. INAEQUALIS IN ORCHARD AIR IN RELATION TO CERTAIN FACTORS OF THE ENVIRONMENT, STURGEON BAY, WIS., 1924 —*Continued.*

Date and subject of record[2]	Averaged records for two-hour periods beginning:											
	A. M.						P. M.					
	12	2	4	6	8	10	12	2	4	6	8	10
Rainfall	---	---	.23	.05	---	---	---	---	---	---	---	---
Spore Freq.	←	3.9				→	←					
15 Temperature	58	59	60	61	63	66	67	66	62	57	52	47
Sunshine	---	---	---	---	---	+	+	+	+	+	---	---
Wind Veloc.	7	7	6	3	3	4	11	13	14	8	6	4
Rainfall	---	---	---	---	---	---	---	---	---	---	---	.01
Spore Freq.		3.5		→								←
16 Temperature	45	45	48	57	62	64	67	66	64	61	57	57
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	5	5	5	6	7	8	10	11	9	7	5	6
Rainfall	.04	---	---	---	---	---	---	---	.44	---	---	---
Spore Freq.		1.0		→					←			
17 Temperature	58	59	60	64	69	73	73	69	64	60	54	53
Sunshine	---	---	+	+	+	+	+	+	---	---	---	---
Wind Veloc.	12	7	9	11	10	8	6	5	5	7	6	7
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		5.3		→						←		
18 Temperature	50	45	46	53	57	59	64	65	63	58	54	54
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	3	3	7	9	11	7	8	9	8	9	8	8
Rainfall	---	.06	---	---	---	---	---	---	---	---	---	---
Spore Freq.		1.5			→							←
19 Temperature	56	54	56	57	62	67	68	68	66	63	62	59
Sunshine	---	---	---	---	+	+	---	---	---	---	---	---
Wind Veloc.	9	9	12	9	14	13	9	9	8	4	2	2
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		→								←
20 Temperature	58	60	63	67	70	73	77	77	73	68	63	62
Sunshine	---	---	---	+	+	---	+	+	+	+	---	---
Wind Veloc.	4	4	4	5	7	7	6	10	10	7	7	8
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		→								←
21 Temperature	60	59	58	65	71	76	79	80	80	74	69	68
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	8	5	3	4	7	9	11	10	9	7	6	7
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		→							←	
22 Temperature	66	64	64	71	76	75	74	73	68	62	55	56
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	7	6	3	4	9	10	11	11	9	6	7	5
Rainfall	10	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		→	←	0	→					←
23 Temperature	58	57	62	68	69	74	78	79	73	66	64	64
Sunshine	---	---		+	+	+	+	+	+	---	---	---
Wind Veloc.	4	5	5	10	10	8	8	5	9	8	5	5
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		→							←	
24 Temperature	62	58	57	62	66	67	70	71	72	69	64	62
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	4	2	2	4	5	5	3	4	4	1	2	6
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		→								←
25 Temperature	55	50	58	66	72	75	78	78	75	63	59	63
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	3	2	2	4	7	8	10	10	7	2	1	4
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		→								←
26 Temperature	62	62	62	68	72	76	80	77	76	70	67	63
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	4	5	2	7	8	7	8	11	10	6	5	3
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0		→							←	
27 Temperature	62	61	61	67	74	78	80	79	77	70	63	59
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	6	6	5	6	5	7	8	9	7	6	9	8
Rainfall	---	---	---	.01	.01	.07	.05	---	.04	.07	.09	.47
Spore Freq.		0		→	←		1.5			→	←	
28 Temperature	57	54	55	55	56	57	58	58	56	55	56	53
Sunshine	---	---	---	---	---	---	---	+	---	---	---	---
Wind Veloc.	8	7	5	10	11	10	12	13	12	14	16	15

TABLE 1.—FREQUENCIES OF ASCOSPORES OF V. INAEQUALIS IN ORCHARD AIR IN RELATION TO CERTAIN FACTORS OF THE ENVIRONMENT, STURGEON BAY, WIS., 1924

—Continued.

Date and subject of record[2]	Averaged records for two-hour periods beginning:											
	A. M.						P. M.					
	12	2	4	6	8	10	12	2	4	6	8	10
Rainfall	.13	.01	---	---	---	---	---	---	---	---	---	---
Spore Freq.	6.5						6.3					
29 Temperature	50	50	52	57	63	68	70	71	65	59	56	55
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	15	10	10	10	12	11	9	5	2	5	3	3
Rainfall	---	.02	---	---	---	---	.02	---	.01	---	---	---
Spore Freq.		0										
30 Temperature	54	52	50	54	59	60	60	57	56	53	52	52
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	6	6	7	9	7	7	5	5	6	8	6	5
July												
Rainfall	---	---	---	---	---	---	---	.24	---	---	---	---
Spore Freq.		0										
1 Temperature	50	49	48	52	57	63	65	63	61	59	51	49
Sunshine	---	---	+	+	+	+	+	---	+	+	---	---
Wind Veloc.	4	6	6	9	9	7	7	4	5	4	3	3
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0					0					
2 Temperature	46	45	50	56	60	65	68	69	66	61	57	52
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	1	1	3	4	5	6	7	6	7	2	3	2
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0										
3 Temperature	49	49	52	60	66	68	69	69	69	63	53	51
Sunshine	---	---	+	+	+	+	+	---	---	---	---	---
Wind Veloc.	1	2	2	2	4	6	7	7	6	4	3	3
Rainfall	---	---	---	---	---	---	---	---	---	---	.08	---
Spore Freq.												
4 Temperature	51	49	52	63	71	74	73	69	65	62	60	59
Sunshine	---	---	+	+	+	+	+	+	---	---	---	---
Wind Veloc.	4	2	3	4	6	7	9	8	5	3	6	2
Rainfall	---	---	.03	.03	.04	---	---	---	---	---	---	---
Spore Freq.		0				0						
5 Temperature	56	55	56	60	68	74	73	72	68	63	58	55
Sunshine	---	---	---	---	+	+	+	+	+	+	---	---
Wind Veloc.	4	3	2	3	5	5	7	7	6	4	3	2
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0										
6 Temperature	54	53	61	69	73	75	73	73	65	61	59	59
Sunshine	---	---	+	+	+	+	+	+	+	---	---	---
Wind Veloc.	3	5	2	4	5	6	7	6	6	3	3	5
Rainfall	---	---	---	.01	---	.14	.18	.05	.14	.30	.11	.06
Spore Freq.												
7 Temperature	58	56	59	66	67	63	60	59	59	58	59	59
Sunshine	---	---	---	---	+	---	---	---	---	---	---	---
Wind Veloc.	4	2	1	2	4	7	4	4	3	6	9	7
Rainfall	---	.01	.09	.19	.25	.09	---	---	---	---	.28	---
Spore Freq.		0										
8 Temperature	59	59	59	58	58	59	63	66	65	64	62	59
Sunshine	---	---	---	---	---	---	+	+	---	+	---	---
Wind Veloc.	6	4	3	7	8	8	9	9	7	6	6	6
Rainfall	---	---	.01	.09	.02	---	.07	---	---	---	---	---
Spore Freq.		0										
9 Temperature	59	60	60	60	61	64	66	68	70	69	64	64
Sunshine	---	---	---	---	---	+	---	+	+	+	---	---
Wind Veloc.	7	7	7	9	10	7	9	15	14	9	4	10
Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Spore Freq.		0										
10 Temperature	61	60	61	63	69	73	76	78	78	72	67	64
Sunshine	---	---	+	+	+	+	+	+	+	+	---	---
Wind Veloc.	9	11	11	12	12	13	11	10	9	7	5	3
11 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Temperature	62	63	65	73	77	80	82	81	78	70	65	64
12 Rainfall	---	.02	.02	---	---	---	---	---	---	---	---	---
Temperature	66	65	65	66	70	73	76	79	78	72	67	64
13 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Temperature	62	61	59	60	63	66	68	65	61	57	52	49
14 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
Temperature	48	46	50	60	65	67	68	69	69	63	58	57

TABLE 1.—FREQUENCIES OF ASCOSPORES OF V. INAEQUALIS IN ORCHARD AIR IN RELATION TO CERTAIN FACTORS OF THE ENVIRONMENT, STURGEON BAY, WIS., 1924

—Continued.

Date and subject of record[2]	Averaged records for two-hour periods beginning:											
	A. M.						P. M.					
	12	2	4	6	8	10	12	2	4	6	8	10
15 Rainfall	---	---	---	---	---	---	---	.01	---	.01	---	.72
15 Temperature	58	58	59	65	72	77	82	80	80	74	68	61
16 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
16 Temperature	60	59	60	67	64	67	70	72	67	61	55	55
17 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
17 Temperature	53	52	51	54	57	61	64	65	61	55	53	48
18 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
18 Temperature	43	43	49	57	58	61	64	65	65	59	52	51
19 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
19 Temperature	53	54	55	60	63	66	69	71	65	57	56	56
20 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
20 Temperature	57	58	59	65	68	73	75	74	70	63	61	61
21 Rainfall	---	---	---	---	---	---	---	---	---	.01	---	---
21 Temperature	59	58	60	67	75	76	77	78	75	72	73	73
22 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
22 Temperature	71	70	67	69	75	80	83	84	83	75	70	69
23 Rainfall	---	---	---	---	---	---	---	---	.03	---	---	---
23 Temperature	69	68	68	74	79	85	87	83	75	72	73	73
24 Rainfall	---	---	---	.01	---	---	---	---	.01	---	---	---
24 Temperature	68	68	67	66	64	63	68	69	64	62	59	55
25 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
25 Temperature	55	55	56	61	70	71	73	73	70	64	60	54
26 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
26 Temperature	51	50	57	65	73	76	76	73	67	64	62	62
27 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
27 Temperature	60	61	65	74	78	80	79	79	81	74	69	68
28 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
28 Temperature	67	65	64	70	75	77	78	78	75	70	67	65
29 Rainfall	---	---	---	---	---	.54	.55	---	.02	---	---	---
29 Temperature	65	66	65	73	79	74	69	70	72	71	70	69
30 Rainfall	---	---	---	---	---	---	---	---	.46	---	---	---
30 Temperature	69	68	67	65	67	68	68	66	63	59	56	56
31 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
31 Temperature	55	55	54	57	59	63	65	65	65	60	53	50
Aug 1 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
1 Temperature	48	47	48	57	62	64	65	66	65	59	52	50
2 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
2 Temperature	49	51	56	59	62	65	67	68	68	65	63	62
3 Rainfall	---	---	---	.11	.36	.01	---	---	---	---	---	---
3 Temperature	61	61	61	61	60	62	70	68	71	71	70	69
4 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
4 Temperature	69	68	67	68	71	76	77	80	79	70	64	63
5 Rainfall	---	---	---	---	---	---	---	---	---	.02	---	---
5 Temperature	64	64	61	65	67	70	73	72	71	70	69	67
6 Rainfall	---	---	---	---	---	---	---	.01	---	---	---	---
6 Temperature	66	66	66	67	68	69	69	72	70	67	65	62
7 Rainfall	.01	---	---	---	---	---	---	---	---	---	---	---
7 Temperature	59	58	57	63	69	72	74	76	75	70	63	63
8 Rainfall	---	---	---	---	---	.11	.01	---	---	---	---	.02
8 Temperature	64	66	65	67	68	66	68	71	70	63	62	60
9 Rainfall	.03	---	---	---	---	---	---	---	---	---	---	---
9 Temperature	57	56	56	57	62	68	69	68	65	61	58	53
10 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
10 Temperature	49	48	50	60	67	67	68	65	63	61	59	57
11 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
11 Temperature	58	59	57	59	62	63	66	67	69	62	57	57
12 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
12 Temperature	58	55	54	59	63	66	70	65	62	60	57	55
13 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
13 Temperature	52	49	48	53	58	60	62	65	65	57	51	48
14 Rainfall	---	.01	---	---	---	---	---	---	---	---	---	.06
14 Temperature	46	45	46	57	63	65	67	66	62	60	58	59
15 Rainfall	---	---	.20	.01	.01	---	.03	.07	.21	.51	.17	.01
15 Temperature	60	60	59	59	63	67	66	64	64	64	63	62
16 Rainfall	.01	---	---	---	---	---	---	---	---	---	---	---
16 Temperature	60	61	58	58	62	64	67	68	65	57	55	54
17 Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
17 Temperature	52	51	51	54	57	61	64	65	60	53	50	49

TABLE 1.—FREQUENCIES OF ASCOSPORES OF V. INAEQUALIS IN ORCHARD AIR IN RELATION TO CERTAIN FACTORS OF THE ENVIRONMENT, STURGEON BAY, WIS., 1924

—*Continued.*

Date	Date and subject of record[2]	Averaged records for two-hour periods beginning: A. M. 12	2	4	6	8	10	P. M. 12	2	4	6	8	10
18	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Temperature	47	46	52	60	64	67	66	65	64	60	55	54
19	Rainfall	.01	.15	.04	.02	---	---	---	---	---	.10	.06	---
	Temperature	54	55	56	57	57	57	61	66	64	62	61	61
20	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Temperature	61	61	60	61	63	65	66	66	63	61	57	56
21	Rainfall	---	---	---	---	---	---	---	---	---	---	.58	1.3
	Temperature	58	58	59	59	60	61	62	67	69	69	69	67
22	Rainfall	.19	.06	---	---	---	---	---	---	---	---	---	---
	Temperature	64	63	64	69	73	76	80	81	80	72	65	64
23	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Temperature	62	58	56	65	74	78	80	81	76	72	65	63
24	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Temperature	62	61	61	65	68	72	77	80	76	64	59	58
25	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Temperature	53	52	54	63	70	75	77	77	73	65	62	62
26	Rainfall	---	.16	.04	---	---	---	---	---	---	---	---	---
	Temperature	63	62	61	62	74	81	85	87	86	79	78	77
27	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Temperature	73	69	66	65	68	71	74	75	74	67	61	57
28	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Temperature	54	53	52	63	71	74	77	75	70	64	64	62
29	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Temperature	60	60	60	70	77	81	81	80	75	70	68	67
30	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Temperature	67	65	64	64	69	75	76	79	78	74	71	74
31	Rainfall	---	---	---	---	---	---	---	---	---	---	---	---
	Temperature	75	76	72	68	67	70	74	75	73	67	63	64

[1] See pages 43-44.

[2] Rainfall in inches. Spore frequency in average number of ascospores per cubic foot of orchard air filtered during periods indicated by arrows. Temperature in °F. Sunshine in hours of bright sunlight. Wind velocity in miles per hour. + = Sunshine recorded. --- = No sunshine recorded, or no rainfall.

Spore Germination Experiments

Viability and Longevity

Ascospores. Frequent tests made from year to year throughout the periods of natural discharge have shown that recently matured, freshly ejected ascospores possess a high degree of viability, their germination in sterile distilled water usually approximating 100 per cent. Under ordinary conditions, ascospores appear commonly to be discharged during the first sufficient rain period following their maturity, provided the ostiole is open. Consequently, if rain periods occur with ordinary frequency, most of the spores available for discharge will have been comparatively recently matured. Numerous inoculation experiments have confirmed germination tests in showing that naturally discharged ascospores constitute a very uniform and highly efficient inoculum.

The longevity of ascospores both in the asci and after discharge is of potential significance in relation to epidemiology. Its practical importance is minimized, however, by the following considerations:

(1) ascospores germinate readily when wet for a sufficient period at ordinary orchard temperatures, and (2) a new supply is ordinarily discharged during each infection period throughout the time of their production, thus furnishing a fresh inoculum. Some practical importance attaches to their ability (1) to withstand droughts while still enclosed in the asci, and (2) to survive periods of exposure after having lodged upon a susceptible host part in a moist period too short to permit infection.

Miss Curtis (1922) reports having observed appreciable discharge of ascospores from perithecia in leaves which had been exposed to an unbroken drought of three months' duration, but makes no statement regarding the viability of these spores. The present writers, however, have observed no case in which there was ejection of ascospores after a general loss of their viability.

On May 25, 1923, freshly collected air-dry apple leaves bearing perithecia which contained abundant mature ascospores were stored in a refrigerator at a temperature of 10° C. and a relative humidity of approximately 60 per cent. On November 16, a microscopic examination showed the perithecia to be in apparently healthy condition. When fragments of these leaves were moistened and placed above agar (2% agar dissolved in water) in Petri dishes, ascospores were discharged in abundance upon the surface of the medium. Approximately 90 per cent of these spores germinated, and cultures were readily isolated from this source.

On March 12, 1925, sub-samples of a single collection of air-dry apple leaves bearing abundant perithecia were placed (1) in diffuse light in a chamber in which the air temperature was held at 16° C. and the relative humidity at 78 per cent, (2) in a greenhouse held at approximately 16° C. without control of humidity, and (3) in a dark chamber (Altmann apparatus) in which the temperature was 16° C. and the humidity was not controlled. Tests of this material on March 12 showed that ascospores were discharged abundantly when representative leaf fragments were moistened, and that approximately 100 per cent of the ejected spores germinated vigorously in sterile distilled water. On May 21, similar tests of leaf samples from each of the three sources revealed abundant discharge of ascospores, which, however, germinated but sparsely in sterile distilled water. On May 22, in a parallel germination experiment, the spores were shot upon the surfaces of plates of agar (2% agar in water). After 24 hours microscopic examinations showed the following results, listed by places where the leaves had been stored: 16° C.—78 per cent relative humidity chamber, 95 per cent germinated, with an average germ tube length of 85 microns; 16° C. greenhouse, 80 per cent germinated, with an average tube length of 65 microns; 16° C. Altmann compartment, 90 per cent germinated, with an average tube length of 75 microns. Many tests and observations have shown that the development of ascospores is sharply checked when the moisture supply is reduced to the amounts employed in

this and the preceding experiment. It is evident, therefore, that most, if not all, of the ascospores which were discharged at the end of these experiments had remained viable in a mature condition in the asci during the periods covered by these tests.

In seven years of field experience in Wisconsin, the writers have not encountered a drought of sufficient duration to limit the viability of mature spores contained in the asci. Severe droughts in early spring, however, have been observed materially to retard and reduce the production of ascospores.

On July 6, 1920, ascospores from leaves which had been held for about six weeks in a refrigerator at 10° C. were discharged so as to fall upon clean slides which were then held dry in diffuse light at room temperature (commonly 20-27° C.) and humidity (variable). One slide from this series was at once covered with droplets of sterile distilled water and placed in a moist chamber at 20° C. for germination. Approximately 100 per cent of the spores germinated. On August 6, in a similar germination experiment, 10 per cent of the spores on another slide of this series germinated. Other similar tests showed that the longevity of ascospores after discharge varied considerably with their vitality at the time of discharge and the conditions to which they were subsequently exposed. The longevity of spores on glass under these experimental conditions is, of course, no index of their survival in the field. Further studies relating to the probabilities of the survival of discharged ascospores of *V. inaequalis* during intervals between rain periods are reported in other connections.

Conidia. Numerous germination experiments have shown that conidia freshly produced in nature possess a high degree of viability. In sterile distilled water on glass, however, the germination of conidia is frequently much less consistent than that of ascospores. On the surfaces of agar plates or on susceptible host organs conidia frequently germinate in greater percentage and with more vigor than in water on glass.

On July 1, 1920, severely scabbed apple leaves were collected from an unsprayed seedling tree and placed in a standard shelter for housing meteorological instruments. At intervals conidia from these leaves were placed for germination in drops of sterile distilled water on glass slides in moist chambers at temperatures near 20° C. The results, which are summarized in Table II, show that under these conditions a high percentage of the conidia survived the winter. It is noteworthy that the lowest temperature recorded during this period by the Madison Station of the United States Weather Bureau was —10° F. on Dec. 28. During this winter, temperatures below 0° F. occurred only four times. It should be noted that the conidia which survived the winter were protected from wetting. The writers have not succeeded in finding viable conidia of *V. inaequalis* which had survived the winter under natural conditions.

TABLE II.—RESULTS OF TESTS OF THE LONGEVITY OF CONIDIA OF V. INAEQUALIS, MADISON, WIS., 1920

Dates of tests	Spores germinated	Average length of germ tubes	Duration of tests
1920	%	Microns	Hours
July 2	99	91	36
July 13	35	78	24
July 23	60	78	24
Aug. 5	10	26	24
Aug. 18	25	10	24
Sept. 8	50	26	24
Oct. 6	70	130	24
Nov. 5	50	52	24
Dec. 3	70	104	24
1921			
Jan. 7	50	104	48
Feb. 21	80	104	24
Apr. 1	80	78	48

Relations of Moisture

The relations of moisture to spore germination are of primary importance to an adequate understanding of conditions which limit infection.

Continuous wetting. Data on the rapidity of germination of ascospores when continuously wet at various constant temperatures appear in Figure 7. A consideration of Figure 7 and Table III suggest that the minimal periods of continuous wetting necessary to permit leaf infection at the various temperatures studied permit the development of germ tubes of fairly constant average length in the hanging drop cultures.

Discontinuous wetting. Aderhold (1900, p. 562, 576-577) states that the spores of the apple and pear scab fungi germinate readily when exposed to brief periods of alternate wetting and drying and that brief periods of drying stimulate the formation of appressoria by bringing the germ tubes in close contact with a firm substratum. Results by Wiltshire (1915) and the present writers are in general conformity with those of Aderhold.

Exposure to water vapor. On the basis of observations and of limited experiments in highly humid atmospheres, Aderhold (1900, p. 561) opines that liquid water is necessary for the germination of the spores of the apple and pear scab Fusicladia. He states, however, that complete immersion in water is not necessary, since germination occurs readily when spores float on the surface of a drop. Wiltshire (1915, p. 337) states that the conidia of these fungi will not germinate in an atmosphere saturated with water vapor. The results of numerous observations and experiments by the present writers suggest that in nature the spores of these Fusicladia ordinarily germinate only when in contact with liquid water. The difficulties of controlling the conditions of germination tests at high humidities with such accuracy as to preclude the possibility of the presence of minute amounts of liquid water are so great, however, that fully convincing experiments on the

relation of high concentrations of water vapor to the germination of these spores are lacking. Further trials are in progress.

Relations of Temperature

Aderhold (1900, p. 557-559) states that conidia of the pear scab fungus germinate in open drops of rain water on glass slides through a range of temperatures extending from 2° to 30° C., the optimum appearing to be near 22°. From 11° to 22° C., the germination was vigorous, while above 22° the vigor waned rapidly. From similar experiments with ascospores of *V. inaequalis*, Frey (1924, p. 333) states that optimal germination and growth occurred at 10-18° C., and that vigor waned rapidly at higher temperatures.

Ascospores from perithecia in moistened apple leaves were ejected so as to fall into sterile redistilled water. Droplets of the spore suspension thus obtained were transferred by means of a platinum loop to clean cover glasses and suspended as unsealed hanging drops over glass rings in moist Petri dishes. The procedure was standardized with the aim of having the size of the drops and the number of spores in each as uniform as feasible. These cultures were placed in duplicate for germination at a series of constant temperatures (variable 1° C.). At stated intervals records were made from 50 spores taken at random in the cultures at each temperature, showing the per cent germinated and the length of the germ tubes. In earlier experiments the spores were placed in drops of water on glass slides in moist Petri dishes. In such cultures, however, there were two distinct types of germ tube development, depending upon whether or not the tubes were in close contact with the surface of the glass. If they attained such contact, they were usually comparatively short and thick: if not, they were long and slender. This variable detracted somewhat from the value of comparative data on the length of germ tubes in such cultures. Furthermore, spores near the edge of drops sometimes appeared to germinate more vigorously than those which rested on the surface of the slide near the middle of the drops. Since other experiments have indicated that spores of *V. inaequalis* have a comparatively high oxygen requirement for germination, particularly as their viability wanes, it seems probable that these variations were due to inqualities in oxygen tension in different parts of the drops. The hanging drop technique was used, therefore, to prevent the contact of the germ tubes with a hard surface and to provide as uniform aeration as feasible, the spores accumulating in the lower part of the drop. It was found desirable to use a comparatively small number of spores in each drop (about 100 to 200), since germination sometimes appeared to be inhibited if large numbers were closely aggregated at the bottom of the drop. The average length of germ tubes recorded in five series of tests appears in Figure 7. The data on the per cent of spores germinated are not given in detail, since they add little to the results shown by length of germ tube. Germination occurred at

temperatures ranging from less than 1° (after 24-36 hours at ½° C.) to 32° C., but not at 35° C. The optimal temperature for the elongation of term tubes under the conditions of these experiments appeared to be in the range of 16 to 22° C. The data of Figure 7 suggest a minor bi-modality within this optimal range. Germination and germ tube growth were quite vigorous from 11° to 22° C. Below 11° C.

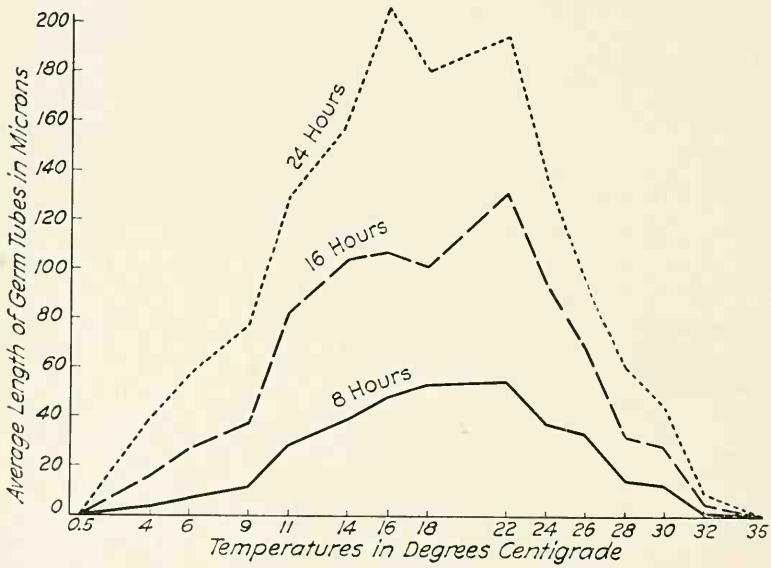

FIG. 7.—THE RELATION OF TEMPERATURE TO THE GERMINATION OF ASCOSPORES OF V. INAEQUALIS IN HANGING DROPS OF STERILE DISTILLED WATER

the development of germ tubes was normal but retarded. At 28° C. and above, the germ tubes were much retarded in development and frequently assumed aberrant forms.

Numerous germination tests of conidia of *V. inaequalis* in open drops of sterile redistilled water on glass slides in moist chambers have shown thermal relations very similar to those just reported for ascospores.

Relations of Light

No differences in development were observed when ascospores and conidia of *V. inaequalis* were germinated comparatively in diffuse light and darkness. Ascospores and conidia which had been placed on the upper surfaces of leaves of potted apple plants which were then exposed to full sunlight for periods up to six days germinated well. It is evident that these spores are able to tolerate considerable exposure to sunlight without losing their viability.

Infection Experiments

Throughout the course of this investigation, numerous infection experiments have been performed, both in the field and upon potted plants in the greenhouse or out of doors. In the field experiments, suitable moisture conditions were provided by the method described on page 5 or by one of the methods devised by the senior author (1917, p. 22; 1918, p. 547) for the study of other diseases. In the earlier experiments in the greenhouse the potted plants were inoculated in a moist chamber previously described by the senior author (1918, p. 548). Conidia from various sources or naturally discharged ascospores from overwintered apple leaves were used as the inoculum. As the work on epidemiology progressed, it seemed increasingly desirable to study the details of infection and of its variability, particularly in relation to the play of certain factors of the natural environment. The technique decribed below was developed for this purpose. This technique proved so much more reliable and satisfactory than any hitherto employed that the results of the earlier experiments will not be given in detail. They constitute a check on the results obtained by the later method, with which they conform in all important particulars.

Apparatus and Method for Experiments under Controlled Conditions

Through the kindness of Drs. J. G. Dickson and James Johnson,[1] their apparatus for the control of air temperature and humidity was made available for our work. This equipment was very useful for studies after the fungus was sufficiently well established in the host tissues to be independent of an external water supply. For studies during the initial stages of spore germination and infection, however, it was necessary to devise a chamber in which a saturated atmosphere could be maintained at a wide range of constant temperatures. This was accomplished in the apparatus shown in Figure 8. Potted apple plants of suitable size were placed in this apparatus, and inoculated by abundant natural discharge of ascospores of *V. inaequalis* from moist overwintered apple leaves which were suspended on moveable trays of wire netting in the upper part of the inner chamber. The abundance of the inoculum was observed by microscopic examination of plates exposed in representative situations in the chamber. Condensation water on the susceptible parts of the host constituted an adequate supply of moisture for spore germination and infection. This method provides an inoculum which is remarkably uniform and free from extraneous material, and which in an unusual degree parallels natural conditions.

The period during which an adequate supply of ascospores is available may be much protracted by suitable care of material. Ascospores may be forced to maturity by midwinter if the leaves in which they

[1]For an account of this apparatus see Dickson (1926) and Johnson (1921).

are developing are given suitable conditions of moisture and temperature, or their development may be much retarded by cold storage.

In certain experiments conidia or ejected ascospores, applied in suspension in sterile distilled water by means of atomizers, were used as the inoculum.

Two potted trees (two or three years old) were used for each test. The dates at which the lesions first showed olivaceous color were recorded. After an adequate incubation period records were made of the average number of infected leaves per twig, and the average number of lesions on the most severely infected leaf of each twig. Only the infection on the upper surfaces of leaves was considered. The total number of twigs on the two trees of each test averaged about eight. These records were found to constitute a satisfactory basis for comparison of results.

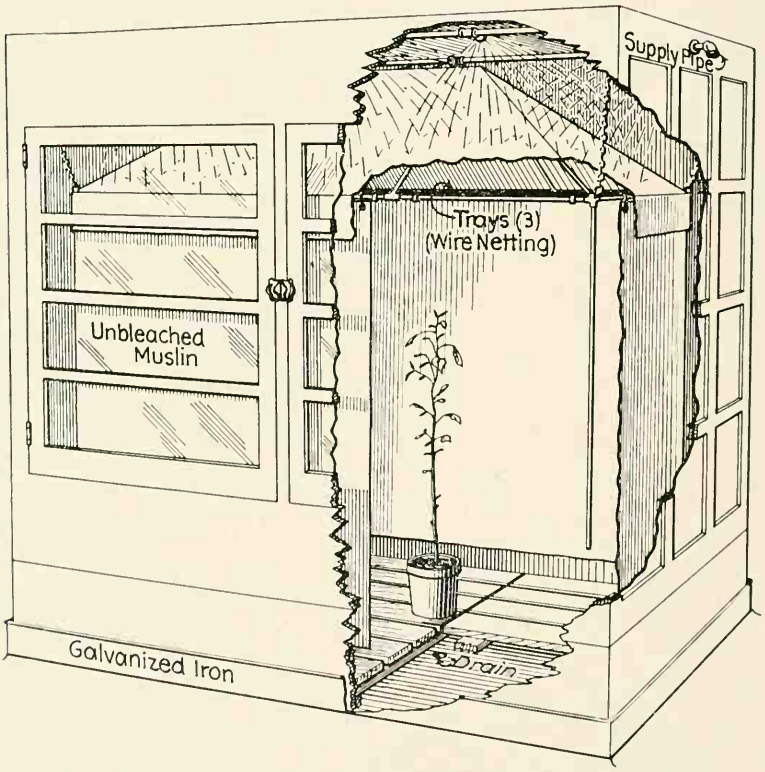

FIG. 8.—INOCULATION CHAMBER

The desired temperature and saturated humidity are maintained by spraying water of the appropriate temperature upon the inner chamber. Hot and cold water from supplies kept at suitable constant temperatures are mixed through metal valves to give the desired temperature. The cloth parts of the inner chamber are washed and sterilized at frequent intervals to preclude the possibility of injurious effects from the growth of microörganisms which might attack the cloth or give off gases which inhibit infection (see Brown, 1922).

Mode of Penetration of the Fungus

The mode of penetration of host tissues by *V. inaequalis* and certain closely related species has received considerable attention. Frank (1883), working with *Fusicladium tremulae* Frank, observed that when conidia germinated on the surfaces of healthy leaves of *Populus tremula* the apical parts of the germ tubes developed into more or less swollen structures, the flat bases of which were closely appressed to the cuticle. He states that the epidermal cells of the host are penetrated by hyphae which grow through germ pores in the basal walls of these structures. He ascribes to these bodies the function of preparation for infection, and suggests that they be called "Appressorien oder Haftorgane." Büsgen (1893, p. 61-62) describes the formation of appressoria by germinating conidia of *Fusicladium pyrinum*. He states that they are developed when the germ tubes come in contact with a firm substance, and that they are surrounded by a thin, colorless, gelatinous layer which serves to attach them to the substratum. Aderhold (1896, p. 895; 1900, p. 562) describes the formation of appressoria by *V. inaequalis* and *V. pyrina*, and states that infection hyphae from these structures penetrate the cuticle. Without giving any experimental evidence, Fischer (1909) advances the opinion that *V. inaequalis* is unable to infect the apple in the absence of wounds. Voges (1910, p. 389-391) confirms Aderhold's description of the formation of appressoria and the penetration of infection hyphae. Wallace (1913, p. 567) states that . . . "it seems that the germ tube bores directly through the cuticle and continues to grow between the cuticle and the epidermis." He did not observe the formation of appressoria in the material studied. Wiltshire (1915) confirmed and somewhat extended the work of Aderhold. He describes in detail the formation of appressoria by conidia of *V. inaequalis* and *V. pyrina* and the penetration of the cuticle by infection hyphae from these structures.

The mode of host penetration described by Aderhold (1896) and Wiltshire (1915), in which the formation of a well differentiated appressorium is followed by direct penetration of the cuticle by an infection hypha from the basal surface of this organ of attachment, was commonly observed by the present writers in their studies of infection of the leaves of potted apple trees by ascospores and conidia of *V. inaequalis*. Certain variations in the details of the process of infection appear, however, to merit further consideration.

The details of the development of anchorage of the germinating spores prior to infection are imperfectly understood. As has just been shown, the fact that appressoria adhere tenaciously to a firm substratum is well established. Voges (1910, p. 390), however, suggests but does not prove that the conidia and ascospores of *V. inaequalis* are surrounded by "Schleimhülle" which serve to attach them to their substrata after dissemination. Wiltshire (1915) states: "The comparative difficulty with which conidia about to germinate are washed from

the slide on which they have rested suggests a definite attacking envelope, but although indications of an outer gelatinous layer have been seen under the microscope they are in all probability optical illusions, for similar indications are found with the conidia of other fungi."

Drops of freshly prepared suspensions of ascospores and conidia of *V. inaequalis* in distilled water were placed upon glass slides for one minute, during which time most of them settled to the surface of the glass. Practically all of these spores were washed off when the slides were gently flooded with water from a pipette. Similar results were obtained when the drops were dried quickly before washing. If the spores were permitted to stand in the drops in contact with the glass at laboratory temperatures, however, they soon showed an increasing resistance to removal by washing. This increased adherence was easily perceptible within 15 minutes, and quite marked within 30 minutes. It was not uncommon for 50 per cent of ascospores which had stood in drops of water on a glass slide for 30 minutes to adhere when the slide was held for 20 seconds at an angle of 45 degrees under a stream of water approximately one centimeter in diameter falling 15 centimeters from a faucet upon which the water pressure was approximately 50 pounds to the square inch. At the end of an hour's wetting comparatively few of the ascospores were removed by such washing. Drying prior to washing somewhat increased the resistance of the spores to removal during the earlier phases of their acquisition of adherence. The conidia became adherent similarly, but slightly less rapidly. It was observed, particularly in the earlier stages of the acquisition of adherence, that the conidia which resisted removal by washing were very commonly attached to the slide at the apical end, which is ordinarily the region from which the germ tube emerges. The localization of this attachment was readily demonstrated under the microscope by inducing currents in a drop of water placed over the spores. The conidia oscillated freely about the places of attachment. No such localization of attachment was commonly observed in the case of ascospores.

In the germination of both ascospores and conidia the germ tubes were of two types: (1) comparatively short and thick, or (2) long and slender. The first type was firmly adherent to the substratum throughout its entire length, while the second was not so attached, as witnessed by its ready lateral motion in microscopic currents of water. After a brief period of desiccation followed immediately by washing, however, even these slender tubes adhered tenaciously to the surface of the slide. A similar demonstration was made with spores which had been germinated in hanging drops and permitted to dry on a glass surface. The shorter and thicker germ tubes commonly formed appressoria, while the slender type failed to do so unless it attained close contact with a firm surface. In less extensive observations, spores were found to adhere to the surfaces of apple leaves as readily as to glass slides. Observations of these spores, germ tubes, and

appressoria mounted in suitable dilutions of India ink or nigrosin failed to reveal the presence of a clearly recognizable layer or sheath about the fungal wall. The nature of the phenomenon of adherence manifested by these bodies is not sufficiently understood, and appears to merit further investigation.

Studies were made of infection of leaves of potted apple plants by conidia and ascospores of *V. inaequalis*. For examinations *in toto*, portions of inoculated leaves at the desired stages of infection were placed, successively, for suitable periods (a) in a solution composed of two parts by volume of absolute alcohol to one of glacial acetic acid, and (b) in a saturated aqueous solution of chloral hydrate (see Peace, 1910). They were then stained lightly in a very dilute aqueous solution of aniline blue, washed, and mounted in water. The fungus on the surface of the leaf stained blue, while the leaf tissues usually took very little stain. The sub-cuticular fungus was not readily stained by this method, but was discernible upon close observation, especially in the more advanced stages of infection. The process of clearing was expedited without disadvantage by warming the reagents. Paraffin sections were prepared, using several fixing agents and stains. Formal-acetic-alcohol and Haidenhain's haemotoxylin were usually employed.

The results with conidia were generally confirmatory of those reported by Aderhold (1896) and Wiltshire (1915). The development of a well differentiated appressorium commonly preceded infection. Not infrequently, however, comparatively short, thick germ tubes appeared to function as appressoria, without the formation of clearly differentiated hold-fasts.

Similar studies showed that infection was very frequently induced by ascospores without the development of a well differentiated germ tube. In such cases, the ascospore itself functioned as an appressorium, from which an infection hypha penetrated directly into the cuticle. This type of infection appears to be of significance in relation to epidemiology and control, because it shortens the time necessary for infection and reduces to a minimum the exposure of delicate-walled fungal hyphae to the action of fungicides.

It is projected that a more detailed account of this work and of experiments in progress will be reported by the senior author in a later paper.

Relations of Temperature

In studying the relations of temperature to infection, it has seemed desirable to differentiate between the initial stages of infection, up to the time when the parasite becomes independent of an external water supply, and the subsequent stages of disease development.

TABLE III.—RELATIONS OF TEMPERATURE AND MOISTURE TO THE INFECTION OF WEALTHY APPLE LEAVES BY ASCOSPORES OF V. INAEQUALIS

Section, number and date of Inoc.	Inoc. chamber		Incubation		Final results—averaged per twig	
	Temp.	Period in	Conditions[1]	Period	No. leave infected	Max. No. lesions on one leaf
1925	°C.	Hrs.		Days		
Sect. I.						
Apr. 2	6	24	20-25° greenhouse	11	2.5	11
Apr. 13	9	24	do	10	3.1	19
Apr. 10	15	24	do	9	3.2	27
Apr. 30	20	24	do	11	3.3	40
Apr. 20	24	24	do	14	2.8	22
Mar. 27	26	24	do	13	1	2.4
May 28	28	24	do	--	0	0
Sect. II.						
Apr. 2	6	13	do	--	0	0
Apr. 2	6	18	do	11	2.2	5
Apr. 2	6	24	do	11	2.5	11
Apr. 2	6	36	do	11	2.9	21
Apr. 2	6	44	do	11	3.1	26
Sect. III.						
Apr. 13	9	9	do	--	0	0
Apr. 13	9	11	do	11	2	7
Apr. 13	9	13	do	10	2.9	10
Apr. 13	9	24	do	10	3.1	19
Sect. IV.						
Apr. 10	15	8.5	do	9	2.2	19
Apr. 10	15	10	do	9	2.7	27
Apr. 10	15	12	do	9	3	21
Apr. 10	15	24	do	9	3.2	27
Sect. V.						
Apr. 30	20	4	do	--	0	0
Apr. 30	20	6	do	12	1.7	19
Apr. 30	20	8	do	12	2.6	34
Apr. 30	20	24	do	11	3.3	40
May 8	20	6	do	14	0.8	0.6
May 8	20	24	do	12	2.6	30
May 19	20	6	do	15	0.7	6
May 19	20	24	do	13	1.3	13
June 8	20	4	do	--	0	0
June 8	20	24	do	14	3.5	20
Sect. VI.						
Apr. 20	24	4	do	--	0	0
Apr. 20	24	6	do	14	2.3	10
Apr. 20	24	8	do	14	3.1	21
Apr. 20	24	24	do	14	2.8	22
Sect. VII.						
Mar. 27	26	6	do	--	0	0
Mar. 27	26	8	do	--	0	0
Mar. 27	26	10	do	13	0.9	2
Mar. 27	26	24	do	13	1	2.4
Sect. VIII.						
May 28	28	24	do	--	0	0
May 28	28	30	do	--	0	0
Sect. IX.						
Mar. 17	8	20	do	10	2.5	12
Mar. 17	8	20	8°-78% chamber	17	3	35
Mar. 17	8	20	28° greenhouse	--	0	0
Apr. 13	9	24	20-25° greenhouse	10	3.1	19
Apr. 13	9	24	8°-78% chamber	14	3.4	35
May 8	20	24	20-25° greenhouse	12	2.6	30
May 8	20	24	26°-80% chamber	--	0	0
May 15	20	24	20-25° greenhouse	13	0.7	19
May 15	20	24	26°-80% chamber	13	2.3	5
Apr. 20	24	24	20-25° greenhouse	14	2.8	22
Apr. 20	24	24	8°-78% chamber	17	3.8	19
Apr. 20	24	24	26°-80% chamber	--	0	0
Sect. X.						
Mar. 16	20	21	20°-90% chamber	8	3.4	33
Mar. 16	20	21	20°-80% chamber	8	3.2	32
Mar. 16	20	21	20°-50% chamber	10	2.4	24
Apr. 8	20	21	20-25° greenhouse	9	2.9	15
Apr. 8	20	21	20-90% chamber	9	3	18
Apr. 8	20	21	20°-80% chamber	9	2.9	16
Apr. 8	20	21	20°-50% chamber	9	3.1	15

[1]Temperatures in degrees C.: relative humidities in per cent. In the greenhouse, no effort was made to control humidity. The chambers used in the experiments reported in sections IX and X are discussed on page 25.

During initial stages in moist chamber. The experimental plants were inoculated and held in the moist chamber at various constant temperatures ranging from 6° to 28° C. (Table III). Infection resulted at temperatures ranging from 6° to 26° C. (Sect. I). It is probable that further studies will somewhat extend this range, especially toward the lower limit. The data on the number of leaves infected and the maximal number of lesions per leaf agree in suggesting that the optimal temperature for rapid development of the initial stages of leaf infection is near 20° C. The incubation periods appear to suggest a slightly lower optimum. It should be noted, however, that the factor of error in recording incubation periods is comparatively large, due to the difficulty of determining when lesions should be classed as macroscopic. A further criterion of the relation of temperature to the initial stages of infection is the time required for the fungus to establish itself in the host sufficiently to become independent of an external water supply. The results of experiments on this aspect of infection appear in sections II-VII of Table III. Infection occurred freely at 6° C., the lowest temperature tried, although the process was much retarded. The periods necessary for infection were progressively shortened at temperatures of 9°, 15° and 20° C. At 24°, as at 20° C., infection occurred when plants were subjected to a six-hour moist period, but not when the period was cut to four hours. At 26° C. the period was considerably lengthened. It is noteworthy that, in all of these tests, an increased amount of infection attended the longer periods in moist chamber. The data now available suggest that the optimal temperature for rapid development of the initial stages of leaf infection is near 20° C.

During incubation. From limited data (Table III. Sect. IX) it appears that the effect of temperature during the incubation period parallels its influence upon the initial stages of infection. At 8° C. the minimal period of incubation was prolonged to 17 days, as compared with periods commonly ranging from 8 to 12 days at temperatures of 20-25° C. When the temperature during incubation was raised to 26° C., the disease developed sparsely after 13 days in one trial and failed to develop in two. It is evident, however, that in nature the organism frequently tolerates periods of summer heat in which temperatures materially exceed 26° C. The effect of intermittent temperatures is, therefore, of interest. Four successive daily exposures of 8 hours each at 31° C., beginning the second day after inoculation, did not preclude the development of the disease, though they appeared to inhibit it slightly. A single exposure of 24 hours at 31-32°C. did not preclude scab development. Similar exposures of 48 hours or more, however, prevented macroscopic development of lesions. These exposures to high temperatures were made in the chambers discussed on p. 25 at relative humidities near 80 per cent. The experimental plants were incubated in 20-25°C. greenhouse.

To parasite and host. The experiments reported on page 24 show

that in sterile distilled water ascospores and conidia of *V. inaequalis* germinate through a range of temperatures beginning at less than 1° and extending to 32° C., with an optimal rate of germ tube elongation in the range of 16° to 22° C. Limited observations on the rate of growth of the fungus on various agar infusions suggest that the temperature relations for mycelial growth on these media closely parallel those for germination of spores and elongation of germ tubes. While no extensive experiments have been made relative to the thermal relations of the host plant, observations of its behavior in the inoculation experiments in which temperature was controlled, supplemented by field experience, indicate that it has a distinctly higher temperature range than has the scab fungus. It is worthy of note that, within the temperatures at which the disease will develop, its thermal relations agree very closely with those for germination and growth of the parasite.

Relations of Moisture

The results of experiments on the relations of moisture to infection appear in Tables III and IV.

The minimal periods of continuous wetting necessary for infection. The results thus far available (Table III, Sect. II-VII) suggest that the minimal periods of continuous wetting necessary for leaf infection by ascospores fall within the following limits for the temperatures listed: 6°, 13 to 18 hours; 9°, 9 to 11 hours; 15°, not more than 8.5 hours; 20°, 4 to 6 hours; 24°, 4 to 6 hours; 26° C., 8 to 10 hours. It it noteworthy that an increased amount of infection accompanied prolongation of the moist periods to the limits reached in these tests. The longer periods of wetting undoubtedly increase the chances for infection by the less vigorous and less favorably placed spores, as well as those which happen to put out their germ tubes in directions which do not lead to an early contact with the cuticle of the host. From these data and from field records, it is apparent that most natural infections require somewhat longer periods of wetting than the minimal periods indicated in Table III.

Discontinuous wetting in relation to infection. Aderhold (1900, p. 562, 576-577) showed that spores of the apple and pear scab fungi germinate well when exposed to brief periods of alternate wetting and drying, and opined that suitable discontinuous wetting favors infection by bringing germ tubes in contact with the cuticle and stimulating the formation of appressoria.

The results recorded in Table IV show that abundant infection resulted from inoculations made under conditions of discontinuous wetting. At 20° C. two successive wet periods of four hours each were sufficient to permit infection (Inoc. 3). In one experiment three successive wet periods of four hours each appeared to be as effective as

TABLE IV.—RELATIONS OF DISCONTINUOUS WETTING AND OF VARIATIONS IN LIGHT TO INFECTION OF WEALTHY APPLE LEAVES BY V. INAEQUALIS

Date and No. of Inoc.	Inoc. chamber		Subsequent treatment of plant[1]	Incubation period	Final results—averaged per twig	
	Temp.	Period in			No. leaves infected	Max. No. lesions on one leaf
1925	°C.	Hrs.		Days		
June 8						
1	20	24	20-25° Grh	14	3.5	20
2	20	4	do	--	0	0
3	20	4	4 Hrs 20-25° Grh, 1 Hrs 20° IC, 20-25° Grh	14	2.4	7
4	20	4	4 Hrs 20-25° Grh, 4 Hrs 20° IC, 12 Hrs 20-25° Grh, 4 Hrs 20° IC, 20-25° Grh	14	3.8	22
5	20	24	4 days dark cellar 20°, 20-25° Grh	14	3	14
6	20	24	5 days dark cellar 20°, 20-25° Grh	14	5	9
7	20	24	7 days dark cellar 20°, 20-25° Grh	14	5	22
May 8						
8	20	24	20-25° Grh	12	2.6	30
9	20	6	1 day in sun out of doors, 20-25° Grh	--	0.2	0.2
10	20	6	1 day in sun out of doors, 18 Hrs 20° IC, 20-25° Grh	14	2.2	23
11	20	6	1 day 20-25° Grh, 18 Hrs 20° IC, 20-25° Grh	13	2.2	23
May 19						
12	20	24	20-25° Grh	15	1.3	13
13	20	2	1 day 20-25° Grh, 22 Hrs 20° IC, 20-25° Grh	15	1.5	14
14	20	2	1 day in sun out of doors, 22 Hrs 20° IC, 20-25° Grh	15	3	26
15	20	6	20-25° Grh	15	0.7	6
16	20	6	1 day 20-25° Grh, 18 Hrs 20° IC, 20-25 Grh	17	0.3	0.8
17	20	6	1 day in sun out of doors, 18 Hrs 20° IC, 20-25 Grh	15	2	20
18	20	6	1 day in sun out of doors, 20-25° Grh	15	0.6	1.6

[1]Temperatures in °C. Grh=Greenhouse. IC=Inoculation chamber.

one 24-hour period (Inocs. 4 and 1). In 1924 infection occurred on the youngest leaves of a potted apple plant which had been exposed to ascospore infection for two hours in moist chamber at 22° C., incubated out of doors in the day and in the greenhouse at night for six days without being wet, and placed in the moist chamber at 22° C. for 24 hours.

Humidity in relation to infection. In considering the relations of humidity to infection, at least three aspects should be taken into account: (1) its effect upon the host plant prior to infection, particularly in relation to the development of the cuticle, (2) its possible influence upon spore germination and the initial stages of infection, and (3) its effect during the period of incubation. The data now available (Table III, Sect. X) apply only to the last of these phases. These limited experimental variations in humidity do not appear to have exerted any significant influence upon the development of the disease.

Relations of Light

In relation to the possibilities of natural infection during discontinuous periods of wetting, it is desirable to know whether exposure to full sunlight during the dry intervals will check infection. In the experiment recorded above it was shown that exposure of the experimental plant to full sunlight for six days, following a two-hour inoculation period in the moist chamber, did not preclude infection on those leaves which had not become resistant when the plant was returned to the moist chamber. Similar exposures of shorter duration (Table IV, Inocs. 8-18) appear to have exerted no inhibiting influence on infection.

Inasmuch as most of the experimental plants used in the inoculation work were grown and incubated under glass, which in the later spring and summer must be shaded by suitable sprays to avoid excessive temperatures, some simple experiments were made to test the effects of different degrees of shading during the incubation period. The results appear in Table IV, Inocs. 1, 5-18. Abundant infection occurred under the most severe shading tried (Inoc. 7), although the experimental plants were severely etiolated and the foliage was somewhat spotted. Under conditions of excessive shading (Inocs. 5-7, 1), the average number of infected leaves per shoot showed a marked increase. If this relation is constant in repetitions of the experiment, it is of possible significance in relation to the experimental modification and the nature of resistance acquired by leaves as they mature. In comparing the results from plants incubated at the different light intensities it is impossible to attribute all differences to variations in lighting, since other factors of the environment were not sufficiently controlled. These experiments and those conducted in previous years, however, have given no evidence of any significant variations in results incident to differences in exposure to light under ordinary greenhouse conditions and out of doors.

Relations of the Stage of Development of Host Organs

Various investigators (e. g., Aderhold, 1900, p. 577-579; Clinton, 1901, p. 114; Wiltshire, 1915, p. 339) have observed that organs of apple and pear are much more susceptible to scab infection when young than when mature. In the infection studies of the present writers, hundreds of inoculation experiments have shown a striking relation between the stage of development of apple leaves and their susceptibility to scab. The bulk of the detailed data precludes publishing the results of more than the few experiments included in Table V. This work has shown that young growing apple leaves of all the varieties studied pass through a stage of maximal susceptibility into a period of increasing resistance. It is of potential significance in relation to the nature of this resistance that it develops at different rates and in different degrees in certain parts of the leaf. The ventral surface of the lamina (usually uppermost when the leaf is mature) leads in the rapidity and degree of development of resistance. Even on this surface, however, the development of resistance is not uniform, the larger veins lagging behind the remainder of the lamina. Not infrequently the disease develops rather generally over the midrib and larger veins while the adjacent surface of the lamina remains clean. Similarly, lesions are often observed to extend farther along the larger veins than over the adjacent surface. The dorsal surface lags behind the ventral both in rate and degree of acquisition of resistance. On this surface, increased resistance is manifested through a much prolonged incubation period and through a sparse and often diffuse and inconspicuous development of the fungus. This is well illustrated in Table V, Inoc. 2, where the disease developed abundantly on the dorsal surface even of the oldest leaf, but only after an incubation period of between 39 and 55 days. The data in this table show a rather consistent increase in the period of incubation for dorsal surface infection with the age of the leaf.

While data from inoculation studies on fruit and twigs are fragmentary, it is apparent from field observations and from the inoculation results available that all susceptible parts of the apple plant increase their resistance to *V. inaequalis* with age. The fruit lesions which result from late-season infections are smaller than those which occur when the fruit is younger. Since the external environment is frequently very favorable for scab infection in the fall, these differences can scarcely be attributed wholly to external factors. Bagging experiments on Shields (crab) in 1919, which will not be reported in detail, showed that pedicels which were protected from infection until ten days after petal-fall resisted natural infection throughout the season, while those which were continuously exposed became abundantly diseased. Scab infection periods occurred after the bags were removed. In all of the inoculation studies by the writers, no twig infection has

TABLE V.—RELATION OF STAGE OF DEVELOPMENT OF APPLE LEAVES TO INFECTION BY CONDIA OF V. INAEQUALIS

Inoculation data[1]	Dates noted	Number of lesions observed on stated leaves[2]: 1 32x29		2 40x34		3 45x42		4 56x42		5 62x45		6 65x45		7 75x45		8 70x36		9 32x25		10 30x20		11 20x5		12 18x--		13 10x--	
		V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D
	March 22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0
	do. 26	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	3	20	6	12	0	1	0	0	0	0
Inoculation No. 2	do. 29	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	3	20	6	12	0	1	0	0	0	0
	do. 31	0	0	0	0	0	0	0	0	0	0	0	0	0	+	1	+	3	20	6	12	0	1	0	0	0	0
	April 2	0	0	0	0	0	0	0	0	0	0	0	0	1	+	7	+	3	28	6	18	0	1	0	0	0	0
Duchess	do. 5	0	0	0	0	0	0	0	0	0	0	0	2	1	+	7	+	3	28	6	18	0	1	0	0	0	0
	do. 7	0	0	0	0	0	0	0	0	0	0	0	2	1	+	7	+	3	28	6	18	0	1	0	0	0	0
	do. 12	0	0	0	0	0	0	0	1	0	6	0	6	1	+	7	+	3	28	6	18	0	1	0	0	0	0
March 9, 1920	do. 17	0	4	0	5	0	16	0	6	0	6	0	9	1	+	7	+	3	28	6	18	0	1	0	0	0	0
	do. 21	0	4	0	5	0	16	0	6	0	6	0	20	1	+	7	+	3	28	6	18	0	1	0	0	0	0
	do. 24	0	n	0	n	0	n	0	n	0	6	0	20	1	+	7	+	3	28	6	18	0	1	0	0	0	0
	May 3	0	+	0	+	0	+	0	+	0	+	0	+	1	+	7	+	3	28	6	18	0	1	0	0	0	0

Inoculation data[1]	Dates noted	1 25x20		2 34x30		3 45x30		4 60x40		5 70x45		6 80x50		7 85x52		8 80x45		9 65x35		10 40x20		11 26x12		12		13	
		V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D
	Mar. 22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	6	3	0	---	---	---	---
Inoculation No. 5	do. 24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	4	3	7	---	---	---	---
	do. 26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	+	19	3	14	---	---	---	---
	do. 29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	19	3	14	---	---	---	---
Wealthy	do. 31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	19	3	14	---	---	---	---
	April 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	n	0	+	+	19	3	16	---	---	---	---
	do. 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	+	+	19	3	16	---	---	---	---
March 9, 1920	do. 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	0	+	+	19	3	16	---	---	---	---
	do. 17	0	0	0	0	0	0	0	0	0	0	0	5	0	30	0	30	0	+	+	19	3	16	---	---	---	---
	do. 24	0	0	0	0	0	0	0	n	0	0	0	n	0	50	0	+	0	+	+	19	3	16	---	---	---	---
	do. 27	0	0	0	0	0	0	0	n	0	0	0	n	0	+	0	+	0	+	+	19	3	16	---	---	---	---
	May 3	0	0	0	0	0	0	0	n	0	n	0	n	0	+	0	+	0	+	+	19	3	16	---	---	---	---
	do. 26	0	0	0	0	0	n	0	n	0	n	0	+	0	+	0	+	0	+	+	19	3	16	---	---	---	---

Inoculation No. 40		1 15x16		2 30x25		6 67x40		7 77x48		8 80x50		9 88x60		10 87x65		11 95x60		12 99x65		13 85x50		14 70x35		15 50x18		16 25x__	
		V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D
	April 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	n	0	3
	do. 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	23	0	10
Wealthy	do. 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	12	23	4	18
	do. 19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	28	32	30	40	4	18
	do. 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	10	28	32	30	40	4	18
March 31, 1920	do. 24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	31	37	+	30	40	4	18
	do. 29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	+	37	+	30	40	4	18
	May 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	n	0	n	15	+	37	+	30	40	4	18
	do. 11	0	0	0	0	0	0	0	0	0	0	0	4	0	15	0	+	0	+	[3]28	+	37	+	30	40	4	18
	do. 26	0	0	0	0	0	0	0	n	0	n	0	+	0	+	0	+	[4]8	+	28	+	37	+	30	40	4	18

Inoculation No. 44		1 11x12		8 93x50		9 95x57		10 80x40		11 85x54		12 90x55		13 90x48		14 93x49		15 90x49		16 85x45		17 76x39		18 50x20		19 32x15	
		V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D
	April 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0
	do. 19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	21	0
Wealthy	do. 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	4	0	4	0	30	0	21	10
	do. 24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	n	10	n	6	+	30	50	21	15
	do. 27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	n	15	n	10	+	30	50	21	15
April 5, 1920	May 3	0	0	0	0	0	0	0	0	0	0	0	0	0	n	0	n	3	n	15	n	26	+	30	50	21	15
	do. 11	0	0	0	0	0	10	0	0	0	n	0	n	0	n	0	n	5	+	25	30	26	+	30	50	21	15
	do. 26	0	0	0	n	0	+	0	+	0	+	0	+	0	+	0	+	5	+	25	+	26	+	30	50	21	15

Inoculation No. 46		1		4		5		6		7		8		9 99x60		10 85x47		11 70x37		12 55x27		13 40x17		14 20x10		15 10x__	
		V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D	V	D
	April 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40	0	5	0	0	0
	do. 19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	20	12	0	1	0
Fameuse	do. 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	4	+	20	12	0	1	0
	do. 24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	20	+	50	16	10	3	10
	do. 27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	20	+	50	16	15	3	10
	May 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	29	+	50	16	25	3	10
April 5, 1920	do. 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	21	20	46	+	50	16	25	3	10
	do. 26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	50	26	50	+	50	16	25	3	10

[1]All inoculations were made by atomizing both surfaces of each leaf with a suspension of conidia (in sterile distilled water) from 12- to 15-day-old cultures of *V. inaequalis* on potato agar. The potted experimental plants were incubated for 24 hours in a moist chamber at approximately 20-22° C. and then removed to a greenhouse in which the temperature varied from 20 to 25° C.

[2]The leaves are numbered serially in the order of their development, beginning with the oldest. Immediately below each number are given the length and width of the leaf in millimeters at the time of inoculation.

The results from the ventral surfaces (upper when mature) appear in the columns headed "V", and those from the dorsal surfaces in columns headed "D".

+ = more than 50 lesions. n = lesions numerous, but not clearly enough defined to count. On heavily infected leaves, confluence and imperfect demarcation often occasioned a margin of error of 5 to 10% in counts.

[3]Many lesions on larger veins.

[4]Lesions occur only on larger veins.

TABLE VI.—RELATIONS OF CERTAIN FUNGICIDAL APPLICATIONS TO INFECTION OF LEAVES OF POTTED FAMEUSE APPLE TREES BY ASCOSPORES OF V. INAEQUALIS

Date and No. of Inoc.	Inoc. Chamber		Fungicidal treatment[1]		Incubation period	Final Results—averaged per twig	
	Temp.	Period in	Time applied	Material		No. leaves infected	Max. No. lesions on one leaf
1925	°C.	Hrs.			Days		
Apr. 16							
19	20	24		Untreated	12	2.3	6
20	20	24	24 Hrs. before Inoc. began	L-S 1-40	--	0	0
21	20	24	do	do.+AL 1-50	--	0	0
22	20	24	do	BM 4-4-50	12	2	5.4
23	20	24	do	S dust	--	0	0
24	20	24	do	S-Ars dust 90-10	--	0	0
Apr. 1							
25	6	33		Untreated	10	3.1	42
26	6	33	24 Hrs. before Inoc. began	L-S 1-40	--	0	0
27	6	33	do	do.+AL 1-50	--	0	0
28	6	33	do	BM 4-4-50	12	3.4	35
29	6	33	do	S-dust	--	0	0
30	6	33	do	S-Ars dust 90-10	--	0	0
31	6	33	33 Hrs. after Inoc. began	do	12	3	39
32	6	33	do	S dust	12	3	35
33	6	33	48 Hrs. after Inoc. began	L-S 1-40	12	2.7	13
34	6	33	do	do.+L-S 1-50	12	2.6	14
35	6	33	do	BM 4-4-50	12	2.9	27
36	6	33	do	S dust	12	2.7	25
37	6	33	do	S-Ars dust 90-10	12	3	33
38	6	33	81 Hrs. after Inoc. began	L-S 1-40	12	2.6	17
39	6	33	do	do.+AL 1-50	12	2.4	20
40	6	33	do	BM 4-4-50	12	3	35
41	6	33	do	S dust	12	2.9	20
42	6	33	do	S-Ars dust 90-10	12	3.1	35
May 22							
43	20	24		Untreated	12	2.4	8
44	20	24	24 Hrs. before Inoc. began	L-S 1-40	--	0	0
45	20	24	do	do.+AL 1-50	--	0	0
46	20	24	do	BM 4-4-50	14	1.5	14
47	20	24	do	S dust	--	0	0
48	20	24	do	S-Ars dust 90-10	--	0	0

[1]L-S 1-40=commercial liquid lime-sulphur (33°B.) at the rate of one part by volume to 39 parts of distilled water. AL 1-50=commercial powdered arsenate of lead at the rate of one pound in 50 gallons. BM=Bordeaux mixture. S dust=sulphur dust (flour). S-Ars dust=sulphur-arsenate dust, 90-10. All these preparations were from the same sources and prepared and applied in substantially the same manner as described on pages 58-60.

The experimental plants were incubated in the greenhouse at 20-25°C.

been observed. Only in rare instances has twig infection been observed in Wisconsin, except on certain unsprayed crab trees. It is evident that this type of infection is limited by a very narrow range of conditions.

These data offer strong indication, though not conclusive evidence, that the cuticle plays a very important part in determining the susceptibility or resistance of host organs to scab infection. It is projected that a further discussion of this phase of the subject will be given in a later paper.

Relations of Certain Fungicidal Treatments

The literature of spraying and dusting contains many apparently conflicting reports concerning the effectiveness of fungicides. The many factors which contribute to these discrepancies will not be discussed here. One potentially important consideration, however, is the influence of environmental factors, particularly moisture and temperature, upon the effectiveness of fungicidal treatments. While many field observations have been made concerning these relationships, little work has been done under conditions in which the fungicidal applications could be related at will to the experimental production of the disease under controlled conditions. Such studies, which were made possible by the apparatus and method described above, have been started on apple scab in the hope of gaining a clearer understanding of the efficiency and adaptations of fungicides in combating this disease under the range of conditions commonly met in practice. Only a small beginning has been made on this work. The results now available appear in Table VI.

The most striking feature of these results is the inefficiency of Bordeaux mixture, as compared with the sulphur fungicides, under the conditions of these tests. The Bordeaux sprayed plants, in all cases, developed essentially as severe scab infection as did the controls, whereas all the sulphur treatments tested commonly gave excellent control of the disease if applied prior to the beginning of the infection period. These results with Bordeaux are at variance with most reports of field experience, and with the commonly prevalent idea that it is a more effective fungicide than lime-sulphur for apple scab control. The results of spraying experiments reported herein confirm very extensive data of other investigators in showing that in practice Bordeaux mixture can effectively control scab on the fruit. The present writers' field results do not, however, bear out the idea that Bordeaux mixture is more effective than lime-sulphur for this purpose, except as each application of Bordeaux is effective throughout a longer period of adverse weather conditions than is a treatment with lime-sulphur. In certain programs, therefore, critical periods for scab control may come at a time when the lime-sulphur applications are more in need of renewal than the Bordeaux, and lead to

results indicative of superior efficacy of the latter spray. This superiority is very real under the conditions stated, but seems to disappear when the programs provide the appropriate time and number of applications of lime-sulphur. The following explanation of the occurrence of abundant scab infection on leaves recently sprayed with Bordeaux, as reported in Table VI, is offered tentatively, pending further study. As pointed out by Barker and Gimingham (1911-12), the more important possible modes of rendering the "insoluble" copper of Bordeaux mixture residues soluble, and therefore available for fungicidal activity, may be grouped under three headings: (1) the solvent action of inorganic compounds in the air or in meteoric water, notably carbon dioxide; (2) the solvent action of compounds emanating from the host plant; and (3) the solvent action of compounds emanating from the fungus. In the infection experiments under consideration (Table VI), it seems unlikely that the Bordeaux residues had weathered long enough for any considerable solvent action to have been exerted upon the copper compounds by inorganic chemical compounds from the air or the water which condensed upon the plants. It seems unlikely that any considerable solvent action had occurred from compounds from within the leaves, since the experimental plants had been grown in the greenhouse under conditions which would minimize wounding, and the work was confined to the upper (ventral) leaf surfaces, upon which the cuticle is highly resistant to exosmosis and few if any stomata occur. It would appear probable, therefore, that the solvent action responsible for fungicidal activity under these conditions resulted chiefly from materials which exosmosed from the germ tubes of the germinating spores in contact with or close proximity to particles of the Bordeaux residues (see Barker and Gimingham, 1911-12, p. 82-90, and Aderhold, 1899, p. 262-271). However, the detailed studies of penetration of the cuticle by the fungus under the conditions of these experiments, as reported above (p. 29), revealed the hitherto unobserved fact that the infection hyphae very commonly grew directly from the ascospores, which themselves functioned as appressoria, without the development of distinctly differentiated germ tubes. In this way infection was accomplished within six hours at 20° C. and with a minimal exposure of delicate-walled fungal hyphae to the toxic action of the fungicide. The extent of this type of infection in nature, and whether the infection hyphae can penetrate thin films of the Bordeaux mixture or must chance to strike unprotected leaf surface, are subjects for further investigation.

A point of special practical and theoretical interest is the apparent effectiveness of the various sulphur treatments, including sulphur dust used alone, at 6° C. These data are in accord with the results from field experiments reported herein and similar results of other workers, in which satisfactory control of scab was repeatedly effected by lime-sulphur and other sulphur fungicides at comparatively low temperatures in early spring. They are, therefore, at variance with

the view of those investigators who have maintained that finely divided sulphur is not effective as a fungicide, save at comparatively high temperatures (see Table VI, Inocs. 25-27, 29-30).

The experiments in which the fungicides were applied after infection occurred are too limited to be conclusive. In certain instances, they suggest some inhibition by the lime-sulphur preparations. In preliminary experiments in 1924, which will not be reported in detail, distinct inhibition of scab development on apple leaves attended spraying with lime-sulphur, 1-40, plus arsenate of lead, 1-50, 24 hours after the infection period began.

Studies of the relation of fungicides to infection under controlled conditions are being continued.

The Development of Epidemics

In the light of the results recorded in the foregoing sections, it is sought in the following pages to trace the development of epidemics and to define critical periods in their development and control.

Modes of Overwintering of the Fungus

It is now generally accepted that the most important mode of overwintering of the apple scab parasite is by the formation of the ascigerous stage in the dead leaves on the ground, as first scientifically demonstrated by Aderhold (1894, 1896) and later confirmed by many other investigators. In the studies of the present writers, the ascigerous stage has been found to occur in such abundance and to mature at such periods as to furnish a source of inoculum which has seemed sufficient to account for all the primary infection which they have observed.

It has long been known that the scab fungus may overwinter on infected apple twigs. The amount of twig infection varies greatly with varieties and with environmental conditions. The literature bearing on this subject has been reviewed by Wallace (1913, p. 577-578) and Morse and Darrow (1913). This type of overwintering appears to be of minor importance in relation to epidemiology, save on certain very susceptible varieties in environments which unusually favor twig infection. In Wisconsin, the observations of the present writers during a period of ten years have revealed no instance in which overwintering of the fungus in twig lesions was of practical significance.

It has been suggested that the scab fungus may overwinter in the conidial stage, or by means of appressoria or of superficial mycelial growth developed from spores which germinate upon the surfaces of buds, twigs, or other host parts (see discussion by Wallace 1913, p. 576-578). In extensive studies of field material, the present writers have

found no evidence to indicate that any of these types of overwintering play a significant rôle in the life history of the fungus under Wisconsin conditions.

The information now available appears to justify the conclusion that, under present conditions of commercial apple culture in Wisconsin, the scab fungus overwinters to a significant extent only through formation of the perithecial stage in dead leaves.

Production and Dissemination of Ascospores

Since ascospores appear to be by far the most important inoculum for primary infection, and the only primary inoculum of economic significance under Wisconsin condition, an adequate understanding of their production, dissemination, and rôle in epidemiology is of first importance in relation to control measures. Aderhold (1900, p. 583) observed that perithecia of the apple and pear scab fungi matured before the blossoms of their respective host plants opened, and states that the first application of spray would be too late if made after petal-fall. Clinton (1901, p. 121) notes that perithecia containing mature ascospores were found in Illinois in April and May. Lawrence (1904, p. 6-7), having worked in Washington, writes: "About the time the flower buds commence to open the spores of the winter stage are matured and set free." As a result of his observations in New York, Wallace (1913, p. 559-560) states: "In nature the ascospores usually begin to mature at or about the time when the apple blossoms are ready to open . . . and the ripening process may continue for about one month." The idea of a synchronism of the maturity of perithecia of the scab fungus and the opening of the apple blossoms has had wide acceptance, and was for many years a leading consideration in timing the first fungicidal application for scab control just before the blossom buds opened. Working under comparatively mild and moist climatic conditions at Hood River, Oregon, however, Childs (1917) found that ascospore discharge began in 1916 before the first apple buds opened and continued at intervals for more than three months. In 1917, in consequence of the unsatisfactory control of scab in the experiments reported above, Frey and Keitt (1925) began a study of spore dissemination of *V. inaequalis* in relation to the seasonal development of apple scab. By means of a filtration apparatus devised for the purpose, frequencies of ascospores in orchard air were determined throughout the season. The first natural discharge, which was much retarded by drought, was recorded on May 19 (when most of the blossoms had opened) and the last on July 18. The maximal concentration of ascospores, 71 per cubic foot of orchard air filtered, was registered on May 21. Significant discharges occurred only when the leaves were wet by rain. If abundant asci were in condition to eject their spores when wet, heavy discharge started soon after the rain began, and continued with continuous wetting for periods varying from

3 to 15 hours. Following a continuation and extension of these studies in 1919, the senior author of the present paper (1920, p. 58) records heavy discharges of ascospores at Madison and Sturgeon Bay, Wisconsin, well in advance of the apple blossoming period, and reports failure of the four-spray programs of lime-sulphur or Bordeaux mixture then in common use in many sections to control scab adequately except when an additional application was made soon after the blossom buds became exposed in the clusters. Further preliminary reports upon the continuation of this work by the same author (1921, 1922) and the present writers (1924 a and b) have recorded additional evidence of the occurrence and importance of early discharges of ascospores. Miss Curtis (1921, 1922), Bennett (1923), Krout (1923), Schneiderhan and Fromme (1924), Williams (1924), Adams (1925), Schneiderhan (1926), and others have contributed to the development of a valuable body of data concerning the time of ascospore discharges. This work, in general, points to the importance of early discharges, but indicates much variation with seasonal and regional climatic conditions.

Apparatus, Methods, and Records of Field Studies

The work of the present writers on the production and discharge of ascospores in nature covers a period of seven years. The simpler studies, which were made by a slight modification of the method used by Wallace (1913) and Childs (1917), are described on page 4 and

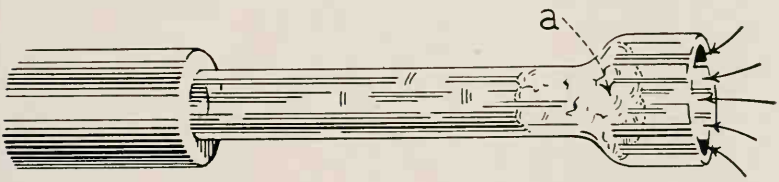

FIG. 9.—INTAKE OF THE FILTRATION DEVICE USED FOR DETERMINING THE FREQUENCIES OF SPORES OF V. INAEQUALIS IN ORCHARD AIR

reported graphically in Figures 1-6. The more intensive studies were made at Sturgeon Bay in 1924 through the use of the following apparatus and technique (Keitt and Jones, 1925 a).

A nitrocellulose filter was placed in a suitably fashioned glass tube (Fig. 9) which was connected by thick-walled rubber tubing to the intake of a motor-driven blower with suction capacity of approximately one cubic foot per minute (Pl. VI, A). A gas meter was connected in series between the filter and the blower to measure the volume of air filtered. At the end of each run, the nitrocellulose filter, bearing the spores caught, was placed in a straight-walled flat-bottomed

glass container of suitable size and dissolved in a liquid composed of three parts by volume of ether to one of absolute alcohol. This preparation was agitated in a manner designed to facilitate uniform distribution of settling spores, and allowed to evaporate to a thin layer of transparent gel, in which the spores were distributed at but little variance from an optical plane. Counts were then made of the number of ascospores of *V. inaequalis* in 50 microscopic fields. The use of a mechanical stage permitted random selection from representative parts of the film without duplication. The olivaceous color and characteristic form and size of the ascospores of the scab fungus, and the scarcity of other spores in the orchard air during rains, made identification easy. From these data the average number of ascospores per cubic foot of orchard air filtered in each run was computed. This method is a modification of one employed by Pasteur (1862) in his early investigations of the microbial content of air.

The resistance offered by the filter occasioned a factor of error because of the resulting slight attenuation of the air when it was measured. The difference in pressure between the air as it entered the meter and that outside was determined experimentally for ten filters through the use of a suitably connected U-tube of mercury. The results, which showed only minor variations, were averaged, and by the application of Boyle's law the factor of error was computed to be 8 per cent of the volume measured. Corrections were made accordingly, and care was exercised to make the filters as nearly uniform as feasible in size and resistance.

The apparatus just described was set up in the Dudley block of the Sackett orchard on the afternoon of May 6, 1924, and run as continuously as feasible until natural discharge of ascospores ceased. The Dudley block of this orchard was abundantly strewn with leaves bearing the perithecia of *V. inaequalis*. Although the orchard was disked shortly before the blooming period and at frequent intervals thereafter, no special effort was made to cultivate close to the bases of the trees or to accomplish a maximun of sanitation by turning under the old leaves. The result of these experiments, which are summarized in Table I, are discussed in the following paragraphs in relation to data from other sources.

Time of Earliest Maturity of Ascospores

Data on time of earliest maturity of ascospores in relation to the blooming period of the apple appear in Table VII. Representative illustrations of stages of advancement of the host plant in relation to these data appear in Plates I-III. From these records it is evident that under Wisconsin conditions ascospores of *V. inaequalis* are commonly available for discharge by the time of first exposure of susceptible host tissue to infection. However, there may be considerable

variation in the relative rates of development of host and parasite, since all environmental factors do not exert an equal influence on both.

Periods of Ascospore Discharge

The periods over which natural discharges of ascospores were observed to occur at intervals in the seasons covered by these studies, as shown in Figures 1-6 and Table I, varied from about five to nine weeks. The number and duration of the individual discharge periods and the quantity of ascospores ejected were influenced greatly by the amount and distribution of rainfall. In the event that there was sufficient rainfall in the early spring, the major discharges occurred well in advance of the blooming period, as at Sturgeon Bay in 1919 and 1924 (Figs. 1 and 6). If the early spring was very dry, however, as at Sturgeon Bay in 1920, 1921, and 1923 (Figs. 2, 3, and 5), discharge was delayed or in extreme cases much reduced.

Frequencies of Ascospores in Orchard Air

In the filtration experiments of 1924 (Table I), the maximal frequency of ascospores in orchard air was recorded on May 13, 17 days before the beginning of the blooming period of Wealthy. During a five-hour period ascospores were caught at the average rate of 289 per cubic foot of air filtered. Catches at the average rate of from 13 to 85 ascospores per cubic foot were made on May 10, 14, and 23, and June 6 and 9. A number of minor discharges were recorded. These frequencies are even higher than those reported by Frey and Keitt, in which the maximum was 71 per cubic foot.

TABLE VII.—TIME OF MATURITY OF ASCOSPORES OF V. INAEQUALIS IN RELATION TO THE BLOOMING PERIOD OF THE APPLE

Place	Year	Date of first observation of:		
		Ascospores mature in nature	Ascospore discharge from freshly collected leaves	Open blossoms on Wealthy
Wis.				
Madison	1916	April 26	------	------
do	1917	April 19	April 26	May 18
do	1919	April 17	April 17	May 15
do	1920	April 21	April 21	May 19
do	1921	April 14	April 14	May 3
do	1922	April 13	April 14	May 8
do	1923	April 23	------	May 15
do	1924	March 22	March 22	May 20
Sturgeon Bay	1919	May 3	May 3	May 28
do	1920	May 17[1]	May 17[1]	May 31
do	1921	May 12[1]	May 12[1]	May 16
do	1922	May 8[1]	May 8[1]	May 21
do	1923	May 5	------	May 26
do	1924	May 5[1]	May 5[1]	May 30

[1]The field laboratory was not opened in time to permit a record of the earliest maturity and discharge of ascospores.

Some Factors Which Affect Production of Perithecia and Discharge of Ascospores

An increasing appreciation of the importance of the time and amount ascospore infection in relation to epidemiology and control led the writers, with the assistance of Mr. E. E. Wilson, to begin a detailed study of major factors which affect the development of perithecia and the discharge of ascospores. In view of the fact that full responsibility for these phases of the work has now been undertaken by Mr. Wilson, the present discussion is limited to a brief preliminary report of the earlier results (see Keitt and Wilson, 1926).

A study of infected leaves which were removed from the tree at intervals from August to February and placed on the ground in the orchard under suitable conditions for the natural development of perithecia showed a marked relation between the time of leaf-fall and the time of maturity of ascocarps. In confirmation of the work of Killian (1917) and others, it was found that the fungus was commonly confined to its typical sub-cuticular position so long as the leaves remained alive on the trees, but soon after leaf-fall began to ramify the lamina with a vigorous mycelial development. Perithecia developed abundantly both in the older and the younger leaves, and in those which showed only a sparse diffuse infection on the under (dorsal) surface as well as those which bore large, well-defined lesions. Temperature and moisture appeared to be the cardinal environmental factors governing the rate of development of ascocarps. Leaves in which the most advanced perithecia were in an early stage of ascus formation were subjected to controlled conditions of temperature and moisture. In moist chambers in dark compartments (Altmann apparatus), the perithecia matured comparatively slowly at 4° and 7° C. The rapidity of development was successively increased at 12°, 16°, and 20° C. At 24° C., only occasional asci reached maturity. The optimal temperature for rapidity of development in the later maturation stages of the ascocarps appears, therefore, to be near 20° C. At 16° C. and relative humidity of 78 per cent, in one of the chambers used in the infection studies (p. 25), the maturation of perithecia was checked until the leaves were thoroughly wet. Discontinuous wetting led to more rapid maturation than continuous wetting. These results agree with field observations at Madison in 1917, 1919, and 1924, when the development of perithecia was definitely checked by dry weather in early spring.

As pointed out by Miss Curtis (1922) and Frey and Keitt (1925) and further confirmed by the data in Table I, an adequate supply of water is the chief requisite for the ejection of ascospores from asci which are in condition for discharge. In the present writers' experiments wetting by dew was never observed to be sufficient to occasion any discharge of significance. When abundant asci were in condition for discharge, heavy ejection commenced at the beginning of rain periods and continued with continuous moisture as long as the

supply of ripe asci lasted. The data in Table I indicate that on May 13 the heavy discharge lasted approximately five hours and on May 23 about eight or ten hours. These results accord well with those of Frey and Keitt (1925). In laboratory experiments the present writers have observed ascospore discharge to occur freely at temperatures ranging from ½ to 32° C. No trials were made at temperatures outside of this range.

The Occurrence of Primary Infection

Under Wisconsin conditions, where ascospores constitute the only important primary inoculum known, the occurrence and amount of primary infection are governed largely by (1) the abundance and timeliness of maturity of ascospores, (2) conditions of moisture and temperature in relation to spore dissemination and infection at critical periods in host development, and (3) the rapidity with which the host passes through its more critical periods for infection. It has already been shown that, under Wisconsin conditions, mature ascospores may commonly be found by the time the first susceptible host tissue is exposed in the spring, and that primary infection may occur at that time at ordinary orchard temperatures during rain periods of sufficient length (Pl. III). The relations of host development to primary infection and the extent to which the latter occurs merit further consideration.

The order in which the susceptible host parts are exposed bears an important relation to early infection. It is a matter of common knowledge that, in the case of the apple, the "fruit" or "cluster" buds, which contain the "blossom" buds, are the first to open, and that those borne on fruit spurs are generally more advanced in unfolding than those on terminal shoots (Pl. I, C, D). The apical portions of sepals and of leaves are the first susceptible parts exposed as the "fruit" buds open. The tips of sepals are found to be exposed to infection at the early stage of bud unfolding shown in Plate I, A, B. At Sturgeon Bay in 1924, abundant sepal infection occurred on buds at the stages shown in Plate III, A, B. The "leaf" buds are ordinarily some days behind the "fruit" buds in opening. In the early stages of leaf development the under (dorsal) surface is more exposed to wetting and, consequently, to infection, whereas, in the intermediate and later stages, the upper surface is more exposed. With the exception of the sepals, the "blossom" buds are very well protected from infection through a considerable period after the "fruit" buds begin to open, partly by the adjacent leaves and partly by hairs (Pls. I, II, A, B).

It has been pointed out (p. 35) that susceptible host parts pass through a period of maximal susceptibility when quite young into stages of increased resistance. The rapidity with which the more susceptible stages are passed may greatly affect the amount of primary infection. At Madison in 1922, in a season of comparatively rapid growth, the stages of bud development of Wealthy shown in Plates

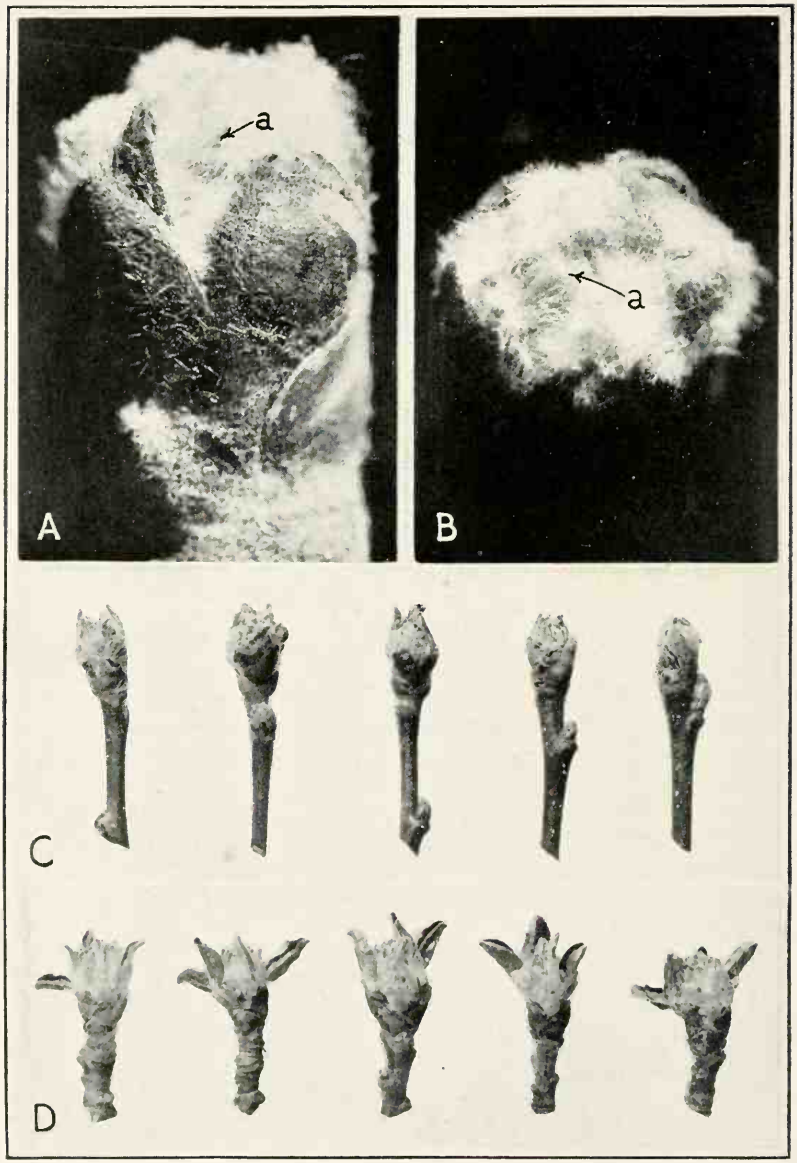

PLATE I.—STAGES IN UNFOLDING OF "FRUIT" BUDS OF WEALTHY APPLE

A.—Very early green tip stage, showing apical parts of sepals exposed at a (X 7). B.—Bud shown in A, viewed from above. C, D.—Later green tip stage, Madison, Wis., Apr. 28, 1922 (X 9/10): C, borne on terminal shoots; D, borne on fruit spurs. (See Pl. II for later stages.)

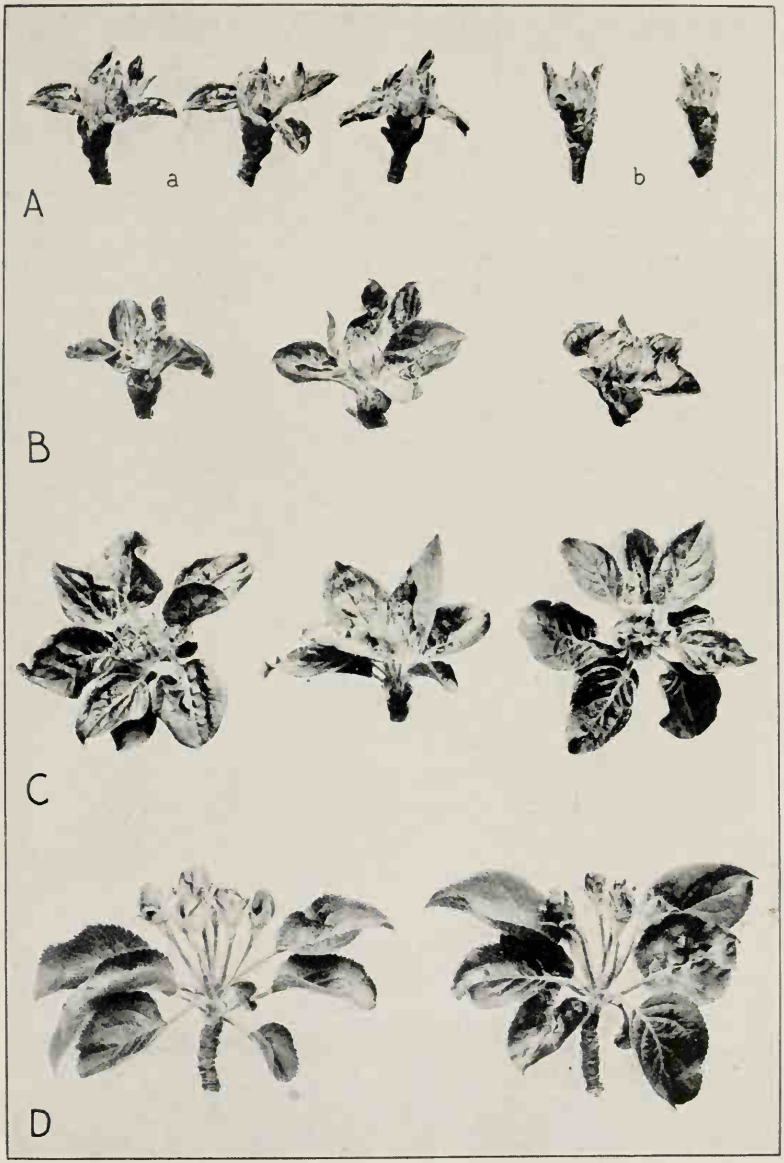

PLATE II.—STAGES IN UNFOLDING OF "FRUIT" BUDS OF APPLE, MADISON WIS., 1922

A.—Early closed cluster, Apr. 30: a, borne on fruit spurs; b, borne on terminal shoots. B.—Middle closed cluster, May 2. C.—Late closed cluster, May 4. D.—Open cluster, May 7. (All figures Wealthy X 6/10. Sec. Pl. I for earlier stage.)

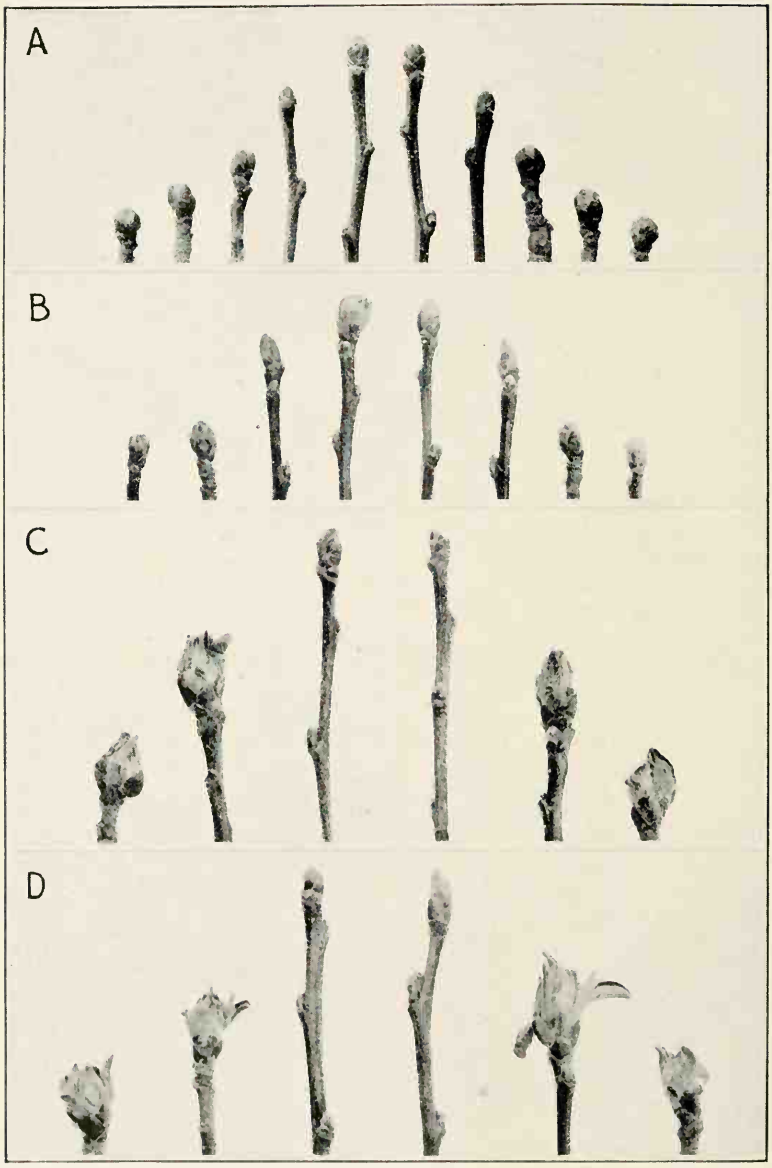

PLATE III.—THE DEVELOPMENT OF APPLE "FRUIT" BUDS IN RELATION TO SCAB INFECTION AND CONTROL, SACKETT ORCHARD, STURGEON BAY, WIS., 1924

A.—Wealthy, May 10. B.—Dudley, May 10. C.—Wealthy, May 15. D.—Dudley, May 15. Severe sepal infection occurred on both varieties prior to May 10.

I, C, D and II were passed in eleven days. In seasons of retarded growth a like development has been observed to require as much as four weeks.

The amount of primary infection varies greatly with the factors just considered. In seasons of rapid host development and unfavorable conditions for infection it may be sharply restricted (Figs. 2, 3, 5). In seasons of slow host development and suitable conditions for infection, particularly in the pre-blossom period, primary infection may be very abundant (Figs. 1, 4, 6). At Sturgeon Bay in 1924, a year of severe primary infection, counts made on unsprayed plots before secondary infection developed revealed sepal infection on 69 per cent of Wealthy blossoms and 87 per cent of Dudley. Not infrequently, several sepals on a single blossom were infected. Further data on the abundance of sepal infection appear in Tables XV and XVII. In considering the amount of primary infection on leaves shown in Figures 2-6, it should be remembered that the averages given there include the leaves which had become resistant as well as those which were susceptible. In the case of Lubsk's Queen, which was in a comparatively low state of vegetative vigor, the sparseness of foliage infection (Figs. 2-5) is largely attributable to the fact that leaf production was completed and resistance developed so early that opportunities for primary infection of foliage were minimized. To develop the greatest number of infections the individual leaf must be inoculated after it has expanded the maximal surface consistent with retention of the necessary degree of susceptibility. Only one or two leaves of a shoot ordinarily approximate this stage at a given time. The present writers have often found from 25 to 50 primary infections on individual leaves which were exposed to severe natural infection at about this stage.

The Special Significance of Early Infection of Sepals

The development of scab on sepals or other parts of "blossom" buds or blossoms has often been reported (Fairchild, 1894, p. 43; Beach *et al.*, 1899, p. 386; Scott and Quaintance, 1907, p. 21; Jackson, 1913, p. 238; Jackson and Winston, 1915, p. 13-14; Darnell-Smith and McKinnon, 1915, p. 26). The relations of early sepal infection to epidemiology and control appear, however, to merit further consideration.

The present studies of primary infection have shown that in years of severe epidemics abundant sepal infection occurred at a surprisingly early stage of bud development (Pl. III, A, B). In view of the fact that conidia of *V. inaequalis* are disseminated chiefly by meteoric water (p. 56), it was at once apparent that early sepal infection establishes the fungus in ideal position for secondary infection of the affected fruit during its period of greatest susceptibility. It seemed probable, therefore, that lesions on sepals might play a rôle in scab infection of apple fruit similar to that of twig

lesions in fruit infection of the peach by *Cladosporium carpophilum* (Keitt, 1917, p. 36-40). Consequently, the following studies were made.

In 1923, after abundant sepal infection had appeared but before secondary fruit infection was evident, 100 unsprayed Dudley fruits which showed sepal infection and 22 which did not were tagged for subsequent study. Records of the amount of infection were made on July 3, after the appearance of the first wave of fruit infection. The results, which appear in Table VIII, show that scab development on the sepal-infected fruit much exceeded that on the sepal-clean fruit, both in the percentage of fruit infected and the number of lesions per infected fruit. Furthermore, the percentage of drop was materially greater in the case of the sepal-infected fruits.

The data shown in Table IX were taken from the fruits (50 of each variety) which were used for seasonal development studies (p. 3-4). The records taken on June 24 show a strikingly greater development of scab on sepal-infected than on sepal-clean fruits. On July 30, after a further period of disease development, this difference was very little changed in the case of Lubsk's Queen, on which there was practically no leaf infection during the season (Fig. 5). On Dudley, however, upon which both leaf and fruit infection were very abundant, this difference was materially lessened, because of the abundant general inoculum from the leaves. Nevertheless, even under these conditions, the average number of lesions on the sepal-infected fruits exceeded that for the sepal-clean fruits by more than 75 per cent. Furthermore, though not shown by the tabulated data, the lesions on sepal-infected fruits were generally larger and more injurious than those on sepal-clean fruits, doubtless because of an earlier average date of infection. In the material studied, most of the badly scabbed, misshapen fruit developed from blossoms which sustained early sepal infection or else chanced very early to develop suitably situated lesions. The fact that a considerable percentage of sepal-infected fruits on the unsprayed trees did not develop secondary infection appears to be due primarily

TABLE VIII.—THE RELATION OF SEPAL INFECTION TO THE DEVELOPMENT OF SCAB ON YOUNG DUDLEY APPLES, STURGEON BAY, WIS., 1923

Classes of fruits observed	Results from fruits on tree, July 3			Fruits off, July 3
	Infected[1]		Clean[1]	Per cent
	Per cent	Ave. No. lesions	Per cent	
100 fruits which showed SEPAL INFECTION on June 10[2]	85	5.9	15	24
22 fruits which showed no infection on June 10	45	2.9	55	9

[1]Exclusive of sepal infection.

[2]No fruit infection other than that on the sepals was evident on June 10.

TABLE IX.—THE RELATION OF SEPAL INFECTION TO THE DEVELOPMENT OF SCAB ON APPLE FRUIT, STURGEON BAY, WIS., 1923

Variety and class of fruit[1]	Scabbed[2] fruits, June 24		Scabbed[2] fruits, July 30	
	Per cent	Ave. No. lesions	Per cent	Ave. No. lesions
Lubsk's Queen				
SEPAL-INFECTED on June 3 (48%)	54	8.2	59	8.4
CLEAN on June 3 (52%)	15.4	1	29	1.6
Dudley				
SEPAL-INFECTED on June 3 (56%)	54	7.3	100	34
CLEAN on June 3 (44%)	38	2.8	100	19
Wealthy				
SEPAL-INFECTED on June 3 (56%)	70	6.1	---	---
CLEAN on June 3 (44%)	43	1.4	---	---

[1]The numbers in parentheses refer to the percentage of fruits of each variety which showed sepal infection or were free from scab when observed on June 3 (see text).

[2]Exclusive of sepal infection.

to unfavorable exposure of the infected sepals and the adjacent surfaces of the fruits to wetting by meteoric water. This situation appears to be closely similar to that described by the senior writer (1917, p. 36-40, Fig. 1 of Pl. III) for peach scab. Typical stages of scab development following sepal infection appear in Plates IV and V. The relation of sepal infection to secondary infection is shown very emphatically in Plate IV, B, in which a sepal-infected fruit shows heavy secondary infection, while a sepal-clean fruit of the same cluster (a) remains free from infection.

Similar studies in 1924, another season in which sepal infection was abundant, gave results which confirmed those of the previous year. It is evident, therefore, that early sepal infection is of much importance in relation to epidemiology and control through establishing the fungus in a remarkably favorable situation for secondary infection of the affected fruit during the period of its greatest susceptibility and at a stage when thorough protection by fungicidal applications is difficult.

Production and Dissemination of Conidia

Under field conditions the production of conidia ordinarily begins before scab lesions become macroscopically visible, and on the individual lesion may continue throughout the season. Extensive observations have shown that sporulation occurs freely on scab lesions under ordinary field or greenhouse conditions without rain or artificial watering. The following record illustrates the rapidity and consistency of sporulation.

On a potted Wealthy plant in the 20-25° C. greenhouse, in which humidity was not controlled, a leaf bearing abundant lesions which had developed as the result of a single inoculation was atomized thoroughly with water by a standardized procedure. The water was recovered and examined for conidia, which were found in abundance.

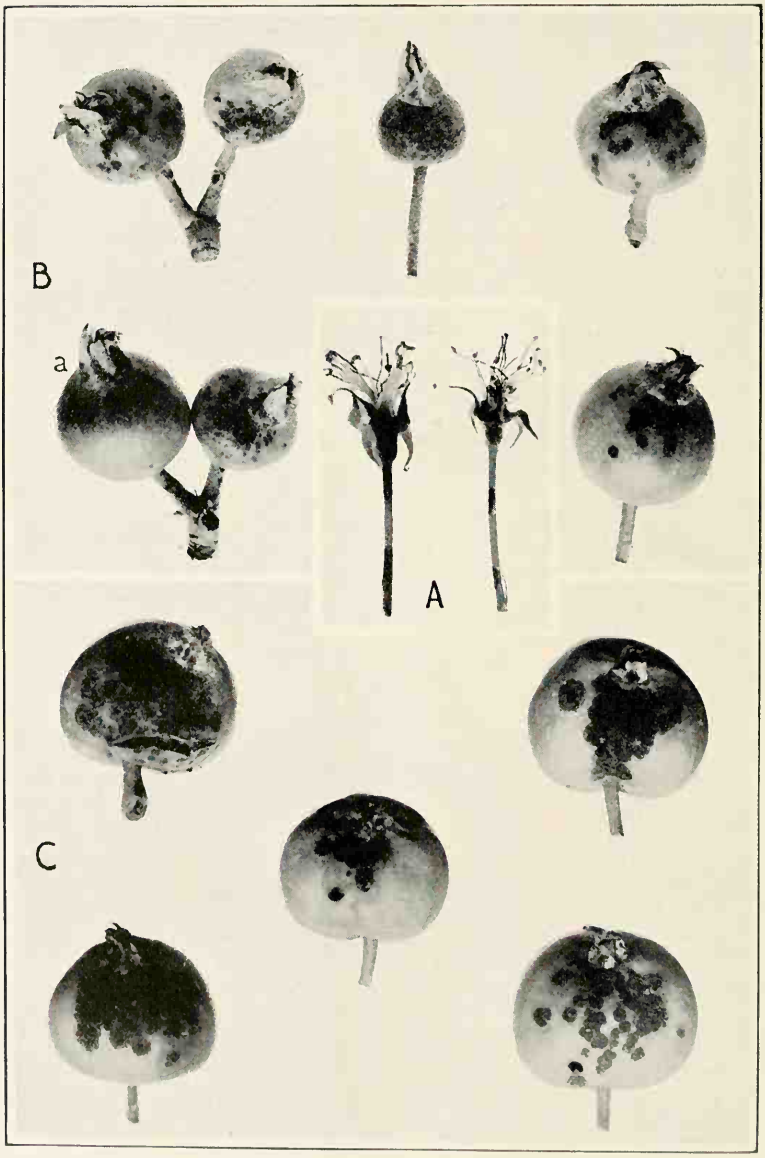

PLATE IV.—SEPAL INFECTION IN RELATION TO SECONDARY INFECTION

A.—Severe sepal infection on Virginia, June 6, 1924. B.—Sepal infection followed by abundant secondary infection, except a, which is sepal-clean and scab-free though in contact with a badly scabbed fruit. Summer Pear, June 1, 1921. C.—Secondary infection following sepal infection, Summer Pear, June 15, 1921. All material from Madison, Wis.

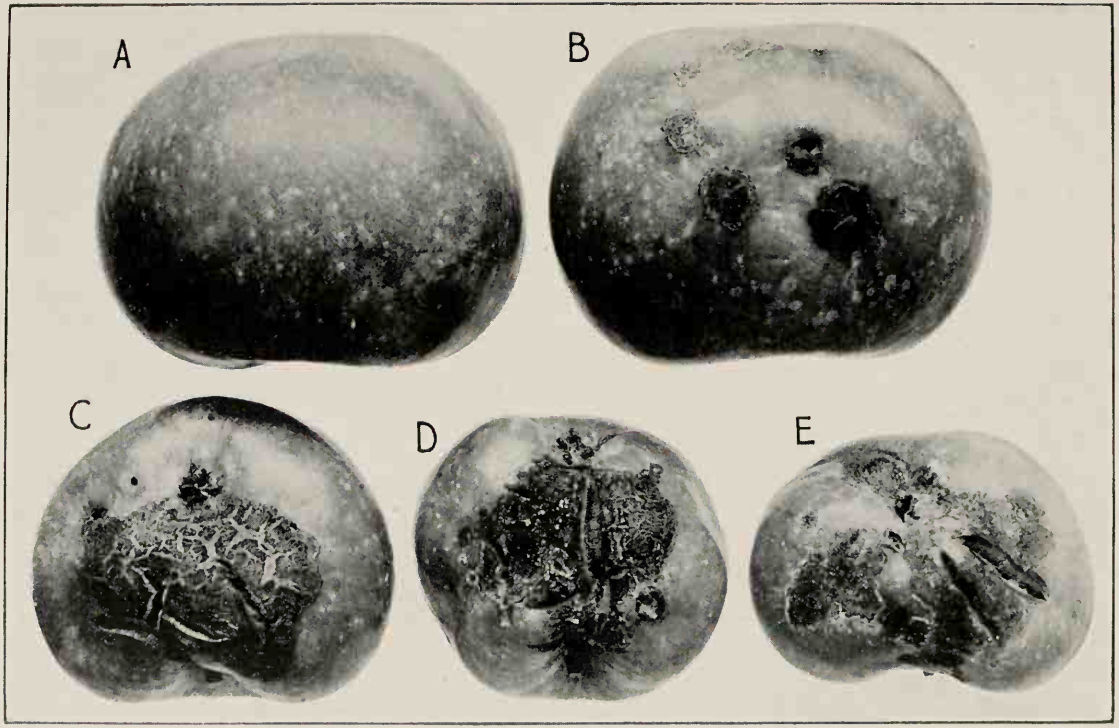

PLATE V.—SEPAL INFECTION IN RELATION TO SECONDARY INFECTION

A.—A sepal-clean fruit free from scab. B.—A sepal-clean fruit bearing several comparatively small lesions. C, D, E.—Sepal infected fruits, showing one-sided development and dwarfing due to early secondary infection. Summer Pear, Madison, Wis., July 30, 1921.

The leaf was then thoroughly washed with the aid of a camel's hair brush, after which examination of wash water showed only a trace of conidia. Repetitions of this test were made on the same leaf on the third, fourth, ninth, and thirteenth days following. In each atomizing test conidia were found in essentially undiminished numbers, while only traces of conidia were found after each thorough washing.

Field observations showed that an abundant source of secondary inoculum was never lacking on unsprayed trees after infection was once well established. The records of seasonal development of the disease (Figs. 2-6) may, therefore, be regarded as a rough index of production of conidia.

Frey and Keitt (1925, p. 537) showed that the conidia of *V. inaequalis* are very resistant to separation from their conidophores when dry, but promptly become detached in the presence of water. They state * * * "These results, in conjunction with those from the air filtration experiments, indicate that no important dissemination of conidia is to be expected in the absence of water, though undoubtedly some spores are dislodged by wind-whipping of leaves, fruit, or branches, by contact with wind-blown particles, and in other minor ways. It appears, therefore, that the important agency for dissemination of these conidia is meteoric water moving under the influence of wind and gravitation."

The present writers, using a slightly different technique, have repeated and somewhat extended the experiments of Frey and Keitt, with confirmatory results. Currents of air from an aspirator were driven against the surfaces of abundantly sporulating scab lesions on leaves and fruits in the laboratory. Glycerined slides were placed in a favorable position to catch samples of any conidia that might be dislodged. The catches of spores were *nil* or sparse, except in cases of excessive air velocity (estimated to be higher than velocities commonly attained in the orchards), when they were somewhat larger. Similar tests were conducted (a) in a saturated atmosphere in the moist chamber shown in Figure 8, using the same specimens after they had been held in a moist chamber for three hours, and (b) in the laboratory, using the same specimens after they had been atomized with water sufficiently to detach the conidia from their conidiophores, but not to scatter them widely or wash them off, and dried rapidly. Microscopic examination showed myriads of conidia in the droplets of water which stood on the lesions after atomizing. The results in the saturated atmosphere and after washing were not essentially different from those obtained from air-dry lesions in the laboratory.

In the filtration studies of the frequencies of ascospores in orchard air in 1924 (Table 1), watch was kept for conidia. They were found only four times, appearing in comparatively small numbers and only during rainy or windy periods.

The Occurrence of Secondary Infection

The occurrence of secondary infection depends chiefly upon the following factors, each of which has been discussed in other connections: (1) a sufficient inoculum, (2) sufficient moisture for spore dissemination and infection, (3) suitable temperatures, and (4) the presence of unprotected susceptible host parts. Records of secondary infection appear in Figures 2-6 and Tables VIII, IX, XV, XVII.

Critical Periods for the Development and Control of Epidemics

It is generally accepted that critical periods for scab infection and control occur in spring and fall. The foregoing studies seem to make possible a somewhat clearer understanding and definition to these periods.

Under Wisconsin conditions the most critical period for the development of epidemics extends from the time the apical parts of the sepals are first exposed in the opening "fruit" buds, (Pl. I, A, B) to an indefinite time some two to four weeks after petal-fall. The early part of this period is the more critical in relation to control for the following reasons:

1. Early infections provide an early secondary inoculum, which in the case of sepal infection is situated in a peculiarly favorable position for severe infection of fruit. An early and abundant secondary inoculum is of special importance in the case of apple scab, because (a) the fungus has a comparatively long incubation period, (b) the host passes rather rapidly through its period of maximal susceptibility, and (c) environmental conditions are more likely to be favorable for early than later secondary infection.

2. The rapid expansion of host parts in the pre-blossom period (Pls. I, II) makes it exceedingly difficult and expensive to keep them adequately covered by a suitable fungicide.

In cool summer climates, as at Sturgeon Bay, Wisconsin, if there has been sufficient primary infection to lead to the development of an abundant secondary inoculum, important infection periods may occur at any time prior to harvest. Hot dry weather, however, sharply checks scab development. Consequently, in warmer climates control during the summer is comparatively easy.

A second critical period for scab development occurs in the fall, when cooler weather and sufficient moisture may lead to important late infection of fruit and to the more abundant establishment of the fungus on the foliage, particularly on the younger leaves of late terminal growth and on the lower (dorsal) surfaces. If primary infection has been adequately prevented, however, the disease is easily controlled in its later stages.

SPRAYING AND DUSTING EXPERIMENTS

The spraying and dusting work, which was conducted in four commercial orchards at Sturgeon Bay during the six seasons, 1919-1924, included 249 plot experiments on the following problems:

1. The comparative effectiveness and desirability of liquid lime-sulphur, dry lime-sulphur and Bordeaux mixture.
2. The desirability of early applications of fungicides.
3. The effects of variations in the number and timing of applications.
4. The effectiveness and desirability of a mixed program of lime-sulphur and Bordeaux mixture in comparison with programs of lime-sulphur and Bordeaux, respectively.
5. The effects of certain variations in the lime-sulphur program in relation to fruit injury.
6. The comparative effectiveness of spraying with rod and gun.
7. The effectiveness of adding certain spreaders to sprays.
8. The effectiveness of certain dust programs.

In accumulating such a large amount of data, special efforts were made to systematize methods, with the aim of enhancing the comparative value of the experiments and facilitating the presentation of results. The following account of methods is, therefore, applicable to all the spraying and dusting work, except as otherwise noted.

Methods

Choice of Plots

The experimental plots were located with the aim of including: (1) varieties locally most subject to scab and those of leading commercial importance; (2) trees in good bearing and as uniform as feasible in size, condition, and surroundings; and (3) accessibility from the field laboratory. Care was taken to make plots of such size and location as to minimize any possible error occasioned by drifting of spray or dust. A brief description is given of the plots used in each experiment.

Spray Materials

Lime-sulphur (liquid). A commercial product obtained each year from the same company. Analyses have shown only slight variations from year to year. The specific gravity has varied little from 1.295 (33° B.).

Dry lime-sulphur. A proprietary product donated each year by the Sherwin-Williams Company, Cleveland, Ohio.

Copper sulphate. Technical copper sulphate crystals.

Lime. In 1919 and 1920, local commercial stone lime. In 1921, 1922, and 1923 hydrated lime. In 1924 a high grade stone lime which contained 98 per cent of calcium oxide.

Arsenate of lead. The commercial powdered acid arsenate of lead. Yearly analyses showed a satisfactory uniformity of product, the arsenic oxide content varying but slightly from 32.5 per cent by weight, and the arsenic in water soluble forms (expressed as metallic arsenic) never exceeding 0.5 per cent.

Glue. A very high grade finely ground glue obtained each year from the same company.

Gelatin. Gold label "WH No. 1866" obtained from the Arthur H. Thomas Company, Philadelphia, Pa.

Casein-lime. "Kayso," a proprietary product donated by The California Central Creameries, Inc., San Francisco, California.

Sulphur-arsenate dust. A commercial preparation (90% by weight of sulphur and 10% powdered lead arsenate) donated by The Niagara Sprayer Company, Middleport, N. Y.

Sulphur-arsenate dust A. This material was prepared by thoroughly mixing 10 parts by weight of powdered arsenate of lead with 90 parts of flowers of sulphur of which 99 per cent was of a fineness to pass through a 200 mesh sieve. This sulphur was 65 per cent soluble in carbon bisulphide.

Sulphur-dry lime-sulphur-arsenate dust. A mixture composed of 75 per cent by weight of finely ground dusting sulphur (flour), 15 per cent of finely ground dry lime-sulphur, and 10 per cent of powdered arsenate of lead (Giddings, 1921).

Copper-lime-arsenate dust. A mixture composed of 10 per cent by weight of finely ground anhydrous cupric sulphate, 80 per cent of hydrated lime, and 10 per cent of powdered arsenate of lead. In 1924, the following formula was substituted: 12 per cent of monohydrated cupric sulphate, 10 per cent of powdered arsenate of lead, and 78 per cent of hydrated lime.

Preparation of Sprays

Bordeaux mixture. Pound-to-gallon "stock solutions" of cupric sulphate and lime were prepared. About three-fourths of the desired volume of water was run into the spray tank. With the agitator and the tank filler running, the necessary amount of stock solution of copper sulphate was added, followed by the requisite amount of the stock preparation of lime, which was diluted with water as it passed through the strainer. The arsenate of lead was added in water suspension, and water was run in to make up the necessary volume. All materials were passed through a strainer. In 1919, 1920, and 1924 stone lime was used: in 1921, 1922, and 1923, hydrated lime. No correction was made for the decreased amount of calcium oxide in

the hydrated lime, as compared with the stone lime. Tests made with the materials used each season showed that a large, though varying, excess of calcium hydroxide occurred in all the Bordeaux mixtures used. The 1 to 1 ratio by weight of cupric sulphate to lime was used unless otherwise stated.

Lime-sulphur and arsenate of lead. The necessary amount of liquid lime-sulphur concentrate was added to about three-fourths of the required volume of water in the spray tank. The arsenate of lead was then added, as in the case of Bordeaux mixture, and water run in to make up the necessary volume. The agitator was run continuously during the mixing process.

Dry lime-sulphur and arsenate of lead. The necessary amount of dry lime-sulphur was vigorously stirred in a bucket of water and poured through a strainer into the partly filled spray tank, as in the case of liquid lime-sulphur. Arsenate of lead and water to make the required volume were added as in making Bordeaux mixture.

The Addition of Spreaders and Adhesives to Sprays

Gelatin. The gelatin was dissolved in a suitable volume of water by heating and stirring. This solution was added to the agitated spray mixture just before the latter was made up to volume.

Glue. Glue was added in same way as gelatin.

Casein-lime. The necessary amount of "Kayso" was placed in suspension in water and added to the spray as were gelatin and glue.

Technique of Application of Sprays

All sprays were applied with high grade power sprayers of 200-gallon tank capacity. When rods were used, each was equipped with two angled disc nozzles, with medium apertures, and pressures of from 225 to 275 pounds per square inch were maintained. The trees were thoroughly and evenly covered. Care was taken to avoid "drenching." One man sprayed from the top of the rig and another from the ground, covering one row at a time. When the spray gun was used, pressures of 250 to 300 pounds were maintained, and when feasible the sprayer was operated along rows running parallel to the wind direction. Each row was treated from two sides by a single gun operated from the top of the rig. In view of the fact that most of the apple trees in Door County are comparatively young, the spray gun tests were conducted on twelve-year-old trees.

Technique of Application of Dusts

All dust applications were made with a standard power duster equipped with a five horse-power engine. The duster was operated along rows as nearly parallel to the wind direction as feasible, and

each row was treated from two sides. Care was taken to make these treatments as thorough as possible without undue waste of materials.

Treatments

Unless otherwise stated, all spray treatments were made under conditions which permitted satisfactory application and thorough drying before rain fell. The conditions which attended each application of dust are noted in the appropriate connections.

Seasonal Development and Meteorological Records

Records relating to the seasonal development of host, parasite, and disease and meteorological data are reported above (p. 3-6). A special telegraphic weather forecasting service was furnished through the cooperation of the U. S. Weather Bureau.[1] This proved to be of much value in facilitating advantageous timing of applications.

Results on Fruit at Harvest

Count trees were chosen at random as regarded the condition of fruit. They were viewed from a distance and selected as representative of the plots in regard to the quantity of fruit borne. They were located as near the middle of the plots as was consistent with other requirements. Results were ordinarily taken from four trees in each plot. In certain minor experiments and in cases where the yields were unusually large, the count work was limited to the crop from two or three trees. The number of count trees used in each plot is recorded in the appropriate Table.

At harvest time, every fruit from each count tree, including fruits which lay upon the ground, was critically examined and its condition recorded according to the classification given below (see Pl. VI, B).

Scabbed fruit was divided into three classes, slight, commercial, and bad. It was aimed to class as slightly scabbed those fruits of which the market value was not seriously impaired except for their being thrown out of grade A (Wisconsin standard). The commercial scab class was designed to include fruits on which scab injury was greater than "slight," but not enough to preclude sale of fruit. The bad scab class was planned to contain those fruits which were so injured by scab that they were rendered nearly or wholly unsalable. While, of necessity, the judgment of the sorter played some part in the classification of fruit, the following standards were adhered to as closely as feasible.

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[1]Grateful acknowledgments are made to Prof. H. J. Cox and his associates of the Chicago Station of the U. S. Weather Bureau for their kind cooperation in furnishing this valuable service.

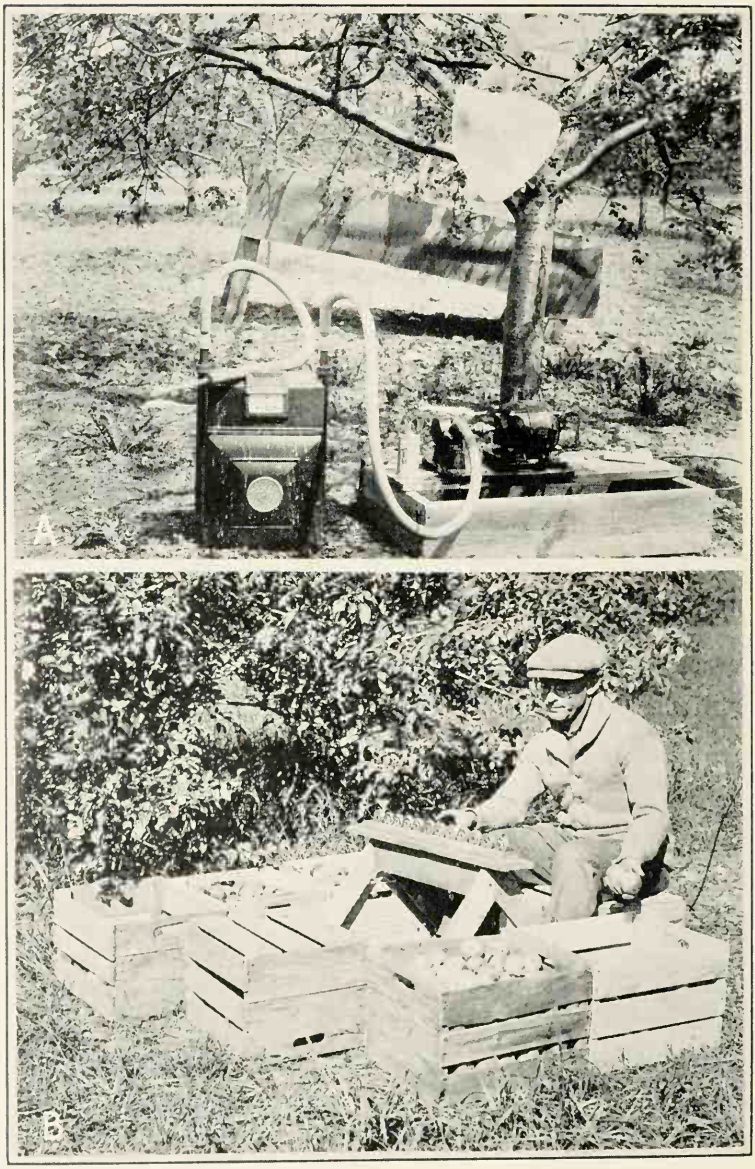

PLATE VI.—ILLUSTRATIONS OF APPARATUS AND METHODS

A.—Apparatus used for determining frequencies of spores in orchard air (see p. 43 11). B.—Taking results on fruit at harvest.

Slight scab. Maximum of scab per fruit: one spot 5 mm. in diameter and slightly cracked, two spots 5 mm. without cracking, three to four spots 2-4 mm. without cracking, or not more than five spots, 1-2 mm.

Commercial scab. Maximum scab per fruit: one spot 15 mm. in diameter without cracking, one spot 10 mm. with slight cracking, three to five spots 5 mm. without cracking, or 10-25 spots not larger than 2 mm.

Bad scab. Any scab injury more serious than "commercial," as just described.

Russeted fruit was classified as slight, commercial, or bad. The class of slight russet was designed to include those cases in which the grade of the fruit was not affected and its market value was not reduced except as might result from its inferior finish. Commercial russet was planned to include fruits sufficiently injured to throw them out of grade A (Wisconsin standard) and distinctly reduce their market value. The bad russet class was designed to include all more seriously russeted fruits, many of which were cracked and rendered unsalable. The judgment of the sorter was based as nearly as feasible on the following standards:

Slight russet. Maximum injury per fruit: light net russet covering 20 per cent of the surface of the fruit or solid russet of 1 square cm. without cracking.

Commercial russet. Maximum injury: heavy net russet without cracking, or solid russet covering 25 per cent of the surface without cracking.

Bad russet. All russet accompanied by cracking and all solid russet covering more than 25 per cent of the surface.

The time and labor required for taking results and the injury to fruit by handling were minimized by taking all data on each fruit from a single examination and recording the results upon a series of hand tally registers (Pl. VI, B). The data from each count tree were then computed on a percentage basis for each classification outlined above. The figures shown in the Tables X-XIV and XVI are the means of these tree percentages.

The standard deviation of the percentage of scab was determined by the formula, $S.D._{.} = \sqrt{\frac{\Sigma d^2}{n}}$, in which Σ represents summation; d, the individual tree difference from the mean; and n, the number of trees.

Experiments in 1919

Condition of Plots

Learned orchard. Well developed Lubsk's Queen trees about 30 years old. Orchard had been in sod for several years, and was not cultivated in 1919. Trees in rather low state of vegetative vigor.

Orchard on a western slope with excellent air and surface drainage. Soil sandy to light silt loam. Burned over about May 1. Many patches escaped burning, only about one-fourth of old leaves being destroyed. Scab had occurred in great severity in this orchard in previous years, and an abundant supply of ascospores escaped the fire.

Lawrence orchard. Well-grown, vigorous Fameuse and McIntosh trees, most of which were about nine years old. Orchard well elevated, with a slight slope to the east. Good air and surface drainage. Soil light to heavy silt loam. Orchard disked in early spring and cultivated until July 1, after which weeds were allowed to grow until they were mowed in early September.

Seasonal Conditions

Seasonal development and meteorological data appear in Figure 1.

No ascospore discharge from the leaves selected for the seasonal development experiment was observed until May 15, when the "blossom" buds were in the closed cluster stage. That ascospores were matured earlier, however, was shown by the fact that a sparse discharge was obtained on May 3 from freshly collected leaves which were moistened in the laboratory. Furthermore, sparse leaf infection was observed on May 24. This allows an incubation period of 18 days from the rain of May 6, which accords well with early-season incubation periods of other years. The more critical periods for scab control were May 19 to 21 and June 5 to 6. The rains recorded on May 15 and 17 were of short duration and apparently led to little if any infection. The season of 1919 was one of rather severe scab infection, the percentage of scabbed fruit on untreated plots varying from 69 to 97, of which a large part was severely infected.

Treatments and Results

A summary of treatments and of the results taken at harvest appears in Table X.

Liquid and dry lime-sulphur controlled scab as efficiently as did Bordeaux mixture, and occasioned much less russeting (plots 77, 71, 85, 87, 81, 94, 96, 90). There was little difference between the lime-sulphurs either in scab control or russeting. The mixed program of Bordeaux mixture and liquid lime-sulphur (plots 83-85, 87, 92-94, 96) gave about the same scab control as did the lime-sulphurs, but much more russeting. However, the mixed program occasioned much less russet than did the full program of Bordeaux mixture.

In no case was satisfactory scab control obtained on plots where application 0 was omitted, while very satisfactory control resulted on plots where it was applied in addition to the four-treatment program (plots 71, 72, 77, 78, 81, 82, 85-88, 90, 91, 94-97). The addition of this

TABLE X.—SUMMARY OF RESULTS OF SPRAYING EXPERIMENTS FOR THE CONTROL OF APPLE SCAB. STURGEON BAY, WIS., 1919

Plot No.	Variety, orchard, and treatment[1]	Count trees	Total fruits	Scabbed fruits					Russeted fruits			
				Slight	Com'l.	Bad	Total	S.D.[2]	Slight	Com'l.	Bad	Total
		No.	No.	%	%	%	%		%	%	%	%
	Lubsk's Queen (Learned)											
70	Unsprayed	4	13986	20.0	22.0	34.8	77	2.9	0.0	0.0	0.0	0
71	B.M., 0,1,2,3	4	2233	10.9	5.6	3.0	20	4.0	9.5	3.8	1.9	15
72	B.M., 1,2,3	4	7096	18.5	8.5	3.8	31	1.8	8.9	3.0	1.0	13
73	B.M., 1,3	4	6041	21.6	15.0	11.3	48	4.7	0.3	0.1	0.0	0
74	B.M., 1,2,3 (1, 3 days late)	4	2251	10.5	7.8	6.2	25	2.7	9.1	3.0	1.3	13
75	B.M., 1,2,3 (1, 3 days early)	4	8407	19.0	11.8	10.0	41	4.8	7.4	2.1	1.1	11
76	B.M., 1,2,3 (2 and 3, 7 days late)	4	3518	25.8	20.5	14.3	61	10.3	1.6	0.8	0.2	3
77	L-S., 0,1,2,3	4	8002	5.3	2.0	1.1	8	0.7	3.2	1.2	0.1	5
78	L-S., 1,2,3	4	4586	12.8	6.3	3.0	22	2.9	0.3	0.1	0.0	0
79	L-S., 1,2,3 (2 and 3, 7 days late)	4	2627	17.5	10.5	6.8	35	10.0	0.4	0.1	0.0	1
	McIntosh (Lawrence)											
80	Unsprayed	4	639	13.4	35.6	48.3	97	1.4	0.0	0.0	0.0	0
81	B.M., 0,1,2,3,4	4	922	2.2	3.4	3.7	9	2.5	14.9	9.0	9.2	33
82	B.M., 1,2,3,4	4	855	11.2	7.8	12.9	31	3.7	12.2	6.9	3.9	23
83	B.M., 0,1,4; L-S., 2,3	4	831	2.3	1.2	1.3	5	2.3	8.3	3.4	2.6	14
84	B.M., 1,4; L-S., 2,3	4	1108	9.1	5.4	6.0	21	4.2	3.7	2.4	1.0	7
85	L-S., 0,1,2,3,4	4	897	2.7	2.3	1.2	6	0.9	0.0	0.0	0.0	0
86	L-S., 1,2,3,4	4	1476	9.2	6.2	4.2	20	2.3	0.0	0.0	0.0	0
87	D.L-S., 0,1,2,3,4	4	943	2.2	1.8	1.4	5	2.7	0.0	0.0	0.0	0
88	D.L-S., 1,2,3,4	4	1003	12.5	8.8	8.8	30	6.6	0.0	0.0	0.0	0
	Fameuse (Lawrence)											
89	Unsprayed	1	239	25.0	20.8	23.0	69		0.4	0.4	0.8	2
90	B.M., 0,1,2,3,4	4	1413	2.2	1.9	1.8	6	2.1	19.0	15.0	32.6	67
91	B.M., 1,2,3,4	4	1892	9.7	6.8	11.6	28	0.5	20.3	11.1	28.0	59
92	B.M., 0,1,4; L-S., 2,3	4	1440	1.5	1.0	0.7	3	1.8	21.6	9.0	2.4	33
93	B.M., 1,4; L-S., 2,3	4	1914	13.3	4.4	4.1	22	2.3	10.1	7.2	6.6	24
94	L-S., 0,1,2,3,4	4	1675	1.4	1.0	1.2	4	1.5	3.2	1.5	1.2	6
95	L-S., 1,2,3,4	4	1451	9.0	4.3	3.2	17	2.3	6.9	3.4	1.7	12
96	D.L-S., 0,1,2,3,4	3	1493	2.9	0.5	0.3	4	0.3	2.1	0.3	0.1	3
97	D.L-S 1,2,3,4	4	743	11.3	6.1	4.7	22	2.2	1.0	0.7	0.2	2

[1]B.M.=Bordeaux mixture, 4-4-50. L-S.=Liquid lime-sulphur, sp. gr. 1.306, 1-40. D.L-S.=Dry lime-sulphur, 3-50. Arsenate of lead (powder) was added to each spray mixture at the rate of 1½ pounds to 50 gallons.

Dates of spray application at Learned orchard: 0, May 17; 1 (open cluster), May 27; 2, June 5; 3, June 19. At Lawrence orchard: 0, May 16; 1 (open cluster), May 26; 2, June 4; 3, June 18; 4, August 20.

For a general account of the methods used see pages 58-63.

[2]S.D.=Standard deviation (see p. 63.)

treatment to the four-spray program resulted in an increase of scab-free fruit amounting to from 11 to 25 per cent of the crop. The reason for these results is readily apparent upon referring to Figure 1, where it is shown that the most important ascospore discharges of the season occurred under favorable conditions for infection well in advance of the open cluster ("pink") spray.

Results from variations in the time and number of applications are valuable primarily as they are interpreted in relation to seasonal conditions. The decreased scab control when application 1 of Bordeaux was made three days early on Lubsk's Queen probably resulted chiefly from imperfect covering of the expanding "blossom" buds, which had not yet separated in the clusters. It is not surprising that an application three days later than usual gave increased efficiency in scab control, as no rain occurred between May 27 and May 30, and the later application had the advantage of covering a greater area of the unfolding parts (plots 75, 72, 74). Considerable increase in scab infection resulted when applications 2 and 3 were delayed seven days each (plots 76, 72, 79, 78). This appears to have been due primarily to lack of adequate protection during the important infection period of June 5-6. The increase in scab infection attendant upon the omission of treatment 2 is explained in the same way (plots 73, 72).

Experiments in 1920

Condition of Plots

Learned orchard. Same Lubsk's Queen trees as in 1919. Also, 48 nine-year-old Wealthy trees (plots 113-116) in same orchard. Wealthy block fairly well cultivated. Lubsk's Queen block, along with remainder of orchard, lightly disked in 1920, with the aim of bringing it gradually into clean culture.

Lawrence orchard. Same trees as in 1919, and same cultural methods. Increased attention, however, given to clean cultivation in advance of first ascospore discharge period.

Seasonal Conditions

Seasonal development and meteorological data appear in Figure 2.

The first discharge of ascospores from the leaves used in the seasonal development series was recorded on May 20, when the "blossom" buds were in the early closed cluster stage. The earliest observation at Sturgeon Bay was made on May 18, when abundant ascospores were found to be mature. The first infection of the season, which was rather sparse, appears to have occurred on May 17 and 18. The more critical periods for scab development occurred during the rains of June 7 to 11 and 15 to 16 and July 6 to 7. Less important infection periods occurred from May 20 to 23, July 17 to

18 and 22 to 23, and August 11 to 13. Pre-blossom infection was sharply limited by scarcity of rainfall of sufficient duration to cause infection. The season was one of moderately severe scab infection, the percentage of scabbed fruit on unsprayed plots varying from 72 to 91. The occurrence of abundant fruit infection on Lubsk's Queen in the absence of a significant amount of leaf infection is noteworthy (see p. 51).

Treatments and Results

A summary of treatments and of the results at harvest appears in Table XI.

Under the conditions of this season liquid lime-sulphur and Bordeaux showed little difference in effectiveness of scab control (plots 99, 101, 118, 122, 127, 131). Serious fruit russeting, however, made Bordeaux unsatisfactory commercially. The mixed program of Bordeaux and lime-sulphur controlled scab as well as did the full programs of Bordeaux or lime-sulphur. It occasioned less russeting than did Bordeaux, but more than lime-sulphur (plots 120, 118, 122, 129, 127, 131). In full programs which included treatment 0, dry lime-sulphur gave essentially as good results as did liquid lime-sulphur (plots 112, 111, 101, 124, 122, 133, 131). Where application 0 was omitted, however, the control of scab by dry lime-sulphur was slightly less efficient than by liquid lime-sulphur or Bordeaux (plots 125, 123, 119, 134, 132, 128).

On McIntosh and Fameuse, the disease was satisfactorily controlled without the addition of treatment 0, except in the case of dry lime-sulphur noted above. On Lubsk's Queen, however, the control was not fully satisfactory without this early treatment (plots 102, 101, 100, 99). The relatively small value of the pre-pink treatment this year is due to the dry period which followed its application.

Due to the dry weather, little difference in results attended the changes in timing of treatments 0, 1, and 2 (plots 106-108, 101).

The omission of treatments 1 and 2, respectively, let to a relatively small increase in the percentage of fruit scabbed (plots 105, 104, 101). It appears that either of these applications furnished sufficient protection to prevent severe infection during the period of June 7-16. No increase in scab development attended the omission of treatment 3 (plots 103, 101).

There was no evidence that the addition of gelatin to lime-sulphur was beneficial (plots 109, 101, 110, 103).

There was no significant difference in results from experiments in which the spray gun and rod were tested comparatively (plots 116, 114).

TABLE XI.—SUMMARY OF RESULTS OF SPRAYING EXPERIMENTS FOR THE CONTROL OF APPLE SCAB, STURGEON BAY, WIS., 1920

Plot No.	Variety, orchard, and treatment[1]	Count trees	Total fruits	Scabbed fruits					Russeted fruits			
				Slight	Com'l.	Bad	Total		Slight	Com'l.	Bad	Total
		No.	No.	%	%	%	%	S.D.[2]	%	%	%	%
	Lubsk's Queen (Learned)											
98	Unsprayed	2	9755	12.7	13.8	45.3	72	21.7	9.5	2.4	0.4	12
99	B. M., 0, 1, 2, 3	2	8627	3.2	2.5	3.4	9	1.8	39.8	24.7	4.3	69
100	B. M., 1, 2, 3	3	5723	2.5	5.5	6.9	15	3.4	48.8	22.8	5.9	78
101	L-S., 0, 1, 2, 3	2	6257	3.4	2.3	3.2	9	0.5	26.1	14.6	3.3	44
102	L-S., 1, 2, 3	2	4282	5.2	3.8	8.8	18	4.6	20.3	8.2	0.8	29
103	L-S., 0, 1, 2	2	1882	3.7	1.9	3.6	9	1.9	25.5	13.4	0.9	40
104	L-S., 0, 1, 3	2	2518	4.4	4.6	6.6	16	2.7	18.8	20.9	1.2	41
105	L-S., 0, 2, 3	2	3397	4.7	2.8	5.7	13	1.0	15.3	7.9	1.0	24
106	L-S., 0, 1, 2, 3 (0, 2 days early)	2	3689	2.5	1.5	2.5	7	1.7	13.5	10.7	1.6	26
107	L-S., 0, 1, 2, 3 (1, 3 days early)	2	3316	1.9	1.4	3.0	6	1.8	19.0	8.5	2.1	30
108	L-S., 0, 1, 2, 3 (2, 6 days early)	3	1844	1.4	0.9	2.3	5	2.7	19.9	7.7	0.7	28
109	L-S. +gelatin, 0, 1, 2, 3	4	2188	2.9	3.7	5.9	13	4.9	21.6	21.1	4.7	47
110	L-S. +gelatin, 0, 1, 2	4	2732	5.8	5.3	7.8	19	6.3	15.1	7.8	1.1	24
111	D. L.-S., 0, 1, 2, 3	2	7366	4.6	2.0	4.2	11	2.2	18.6	8.9	0.8	28
112	D. L-S. (4½-50), 0, 1, 2, 3	3	6364	1.4	2.4	3.7	8	2.4	16.1	14.3	1.3	32
	Wealthy (Learned)											
113	Unsprayed	3	2252	13.5	21.6	55.6	91	4.8	1.8	1.4	0.3	4
114	L-S., 0, 1, 2, 3, 4	4	3263	0.8	0.8	0.6	2	0.7	5.3	2.3	0.8	8
115	L-S., 1, 2, 3, 4	4	3643	1.6	1.0	0.8	3	0.3	6.5	2.3	0.7	10
116	L-S., 0, 1, 2, 3, 4 (gun)	4	3374	0.7	0.6	0.5	2	1.3	4.8	1.7	0.3	7
	McIntosh (Lawrence)											
117	Unsprayed	4	606	7.5	6.7	71.5	86	17.9	0.8	2.9	1.7	5
118	B. M., 0, 1, 2, 3, 4	4	1126	1.2	1.0	0.6	3	1.0	53.9	22.7	7.2	84
119	B. M., 1, 2, 3, 4	4	2018	0.3	0.1	0.3	1	0.2	61.7	21.9	3.9	88
120	B. M., 0, 1, 4; L-S., 2, 3	4	1450	1.7	0.3	0.2	2	1.1	52.3	12.8	3.0	68
121	B. M., 1, 4; L-S., 2, 3	4	2009	0.8	0.4	0.3	2	0.3	44.6	10.9	2.3	58
122	L-S., 0, 1, 2, 3, 4	5	1068	2.2	0.4	0.2	3	1.6	2.0	1.5	0.5	4
123	L-S., 1, 2, 3, 4	4	2225	1.8	0.4	0.3	3	0.8	3.7	0.9	0.3	5
124	D. L-S., 0, 1, 2, 3, 4	4	1383	3.6	1.4	1.2	6	2.1	5.6	3.2	0.5	9
125	D. L-S 1, 2, 3, 4	4	1743	8.6	2.9	1.6	13	5.6	3.9	1.1	0.5	6
	Fameuse (Lawrence)											
126	Unsprayed	4	935	18.1	19.5	43.3	81	7.7	1.7	1.8	0.1	4
127	B. M., 0, 1, 2, 3, 4	4	1502	0.7	0.7	0.4	2	0.6	57.2	21.6	3.4	82
128	B. M., 1, 2, 3, 4	4	1559	0.9	0.7	0.8	2	0.9	63.6	20.7	3.9	88
129	B. M., 0, 1, 4; L-S., 2, 3	4	1268	1.1	0.7	0.5	2	0.8	16.5	5.0	0.8	22
130	B. M., 1, 4; L-S., 2, 3	4	1209	1.0	1.1	0.1	2	0.7	14.1	3.5	0.6	18
131	L-S., 0, 1, 2, 3, 4	4	1710	0.9	0.7	0.2	2	0.8	4.7	0.4	0.0	5
132	L-S., 1, 2, 3, 4	4	1631	0.9	1.5	1.4	4	1.0	4.1	1.0	0.3	5
133	D. L-S., 0, 1, 2, 3, 4	4	1208	1.5	1.0	0.5	3	1.3	5.1	1.5	0.2	7
134	D. L-S., 1, 2, 3, 4	4	1759	3.7	4.1	2.5	10	2.8	5.9	1.3	0.1	7

[1]B.M. = Bordeaux mixture, 4-4-50. L.S. = Liquid lime-sulphur, sp. gr. 1.295, 1-40. D. L-S. = Dry Lime-sulphur, 3-50, unless otherwise noted. Arsenate of lead (powder) was added to each spray mixture at the rate of 1 pound to 50 gallons. Gelatin was used at the rate of ¼ pound to 50 gallons.

Dates of spray application at Learned Lubsk's Queen orchard: 0, May 24; 1 (open cluster), May 30; 2, June 12; 3, June 22. At Learned Wealthy orchard: 0, May 24; 1 (open cluster), May 29; 2, June 8; 3, June 23; 4, August 9. At Lawrence orchard: 0, May 24; 1 (open cluster), May 29; 2, June 9; 3, June 21; 4, August 6. For a general account of the methods used see pages 58-63.

[2]S. D. = Standard deviation (see p. 63).

Experiments in 1921

Condition of Plots

Learned orchard. Same Lubsk's Queen and Wealthy trees as in 1920. For dusting work an additional block of Wealthy and Lubsk's Queen used. These trees were part of same planting as Lubsk's Queen in previous experiments, and were of like vigor and general condition. Young Wealthy orchard (plots 153-156) continued in clean culture. Old orchard disked three times, but considerable blocks of sod were left about bases of trees.

Lawrence orchard. Same trees and cultural plan as in 1920. Special attention given to thorough cultivation prior to first period of ascospore discharge.

Goff orchard. Vigorous, well-grown, twelve-year-old Wealthy trees. Orchard well elevated, nearly level, with good air drainage. Soil light clay loam to silt loam. Well cultivated seven or eight times a year with disk harrow, beginning before buds opened.

Seasonal Conditions

Seasonal development and meteorological data appear in Figure 3.

The first ascospore discharge from the seasonal development experiment was recorded on May 13, when the "blossom" buds were in the open cluster stage. It should be noted, however, that no rain fell in the period between April 28 and May 13. The fact that sparse infection appeared on May 18 indicates that ascospores were mature in the rain period prior to and including April 28, when the initial infections evidently occurred. The dry weather throughout the spring and early summer so checked the development of the fungus that there was little scab, even on unsprayed trees.

Treatments and Results

A summary of treatments and of the results taken at harvest appears in Table XII. There was not sufficient scab infection to constitute a satisfactory test of the various programs used. Consequently, the detailed data on conditions at the time of dust applications and a detailed discussion of the results at harvest are unnecessary.

All the spray and dust materials used controlled the disease satisfactorily. As in previous years, however, Bordeaux mixture gave unsatisfactory results because of fruit russeting.

An unusual feature of the season's results was the occurrence on the lime-sulphur plots of a variable amount of fruit "burning" of a type which has not infrequently been reported by other investigators (Young and Walton, 1925). This injury was first observed on June

TABLE XII.—SUMMARY OF RESULTS OF SPRAYING AND DUSTING EXPERIMENTS FOR THE CONTROL OF APPLE SCAB, STURGEON BAY, WIS., 1921

Plot No.	Variety, orchard, and treatment[1]	Count trees	Total fruits	Scabbed fruits					Russeted fruits				"Burned"[3] fruits
				Slight	Com'l.	Bad	Total		Slight	Com'l.	Bad	Total	
		No.	No.	%	%	%	%	S.D.[2]	%	%	%	%	%
	Lubsk's Queen (Learned)												
135	Untreated	2	8205	8.8	6.0	6.4	21	1.9	1.1	0.6	0.1	2	0
136	B.M., 0,1,2,3	2	5457	0.4	0.1	0.1	1	0.0	16.8	7.6	5.0	29	0
137	B.M., 1,2,3	2	3427	2.1	1.3	1.0	4	0.7	18.6	7.0	2.6	28	0
138	L-S., 0,1,2,3	2	7375	0.3	0.1	0.1	1	0.4	1.5	0.4	0.1	2	5
139	L-S., 1,2,3	2	4544	0.6	0.2	0.1	1	0.0	1.2	0.4	0.1	2	4
140	L-S., 0,1,2	2	5680	0.6	0.2	0.1	1	0.1	1.7	0.5	0.1	2	0
141	L-S., 0,1,1A,2,3	2	2977	0.2	0.1	0.0	0	0.1	1.6	0.2	0.2	2	5
142	L-S., 0,1,2,3 (0, 2 days early)	2	2390	1.0	0.6	0.1	2	0.4	2.5	0.3	0.1	3	7
143	L-S., 0,1,2,3 (0, 3 days late)	2	2855	0.2	0.1	0.1	0	0.0	2.3	0.5	0.2	3	3
144	L-S. +gelatin, 0,1,2,3	2	3320	1.6	0.6	0.5	3	0.9	1.7	0.5	0.1	2	4
145	D.L-S., 0,1,2,3	2	4527	0.5	0.2	0.1	1	0.1	2.2	0.7	0.1	3	1
146	D.L-S., 1,2,3	2	2080	2.9	1.0	1.1	5	1.5	0.7	0.3	0.4	1	1
147	D.L-S. (3-50), 0,1,2,3	2	4262	1.0	0.3	0.2	2	0.4	0.8	0.4	0.1	1	2
148	C-L-Ars., **1,2,3,4,5,6**	2	2575	6.6	2.2	1.7	11	3.4	4.6	2.1	0.4	7	0
149	S-D.L-S-Ars., **1,2,3,4,5,6**	2	8960	3.8	1.0	0.7	6	0.1	1.9	0.7	0.1	3	0
150	S-Ars., **1,2,3,4,5,6**	2	5945	5.0	1.0	1.2	7	0.3	1.9	0.3	0.1	2	0
151	C-L-Ars., **1,2,3**; S-D.L-S-Ars., **4,5,6**	2	3671	3.6	1.7	1.1	6	2.5	5.3	1.9	0.5	8	0
152	S-Ars., **1,2,4,5,6**	2	4727	3.3	2.7	2.3	8	0.9	1.2	0.5	0.2	2	0
	Wealthy (Learned)												
153	Untreated	2	2217	10.7	7.3	3.5	22	4.6	0.9	0.5	0.1	2	0
154	L-S., 0,1,2,3,4	4	2116	2.9	0.6	0.3	4	1.5	1.6	0.5	0.0	2	6
155	L-S., 1,2,3,4	4	2860	3.3	1.6	0.5	5	2.1	1.4	0.8	0.0	2	3
156	L-S., 0,1,2,3,4 (gun)	4	1652	4.9	2.8	1.2	9	3.2	2.1	0.7	0.1	3	4
157	Untreated	2	1961	4.9	1.9	1.5	8	1.3	0.7	0.2	0.3	1	0
158	C-L-Ars., **1,2,3,4,5,6**	2	2468	2.9	1.2	0.6	5	0.1	4.2	1.1	0.2	6	0
159	S-D.L-S-Ars., **1,2,3,4,5,6**	2	2060	1.6	0.2	0.1	2	0.2	1.3	0.1	0.1	2	0
160	S-Ars., **1,2,3,4,5,6**	2	4223	1.8	0.2	0.1	2	0.3	0.9	0.3	0.2	1	0
	McIntosh (Lawrence)												
161	Untreated	2	1778	18.5	19.0	18.3	56	2.4	0.9	0.4	0.2	2	0
162	B.M., 0,1,2,3,4	3	1431	0.1	0.0	0.1	0	0.2	25.8	23.7	13.3	63	0
163	B.M., 1,2,3,4	3	2088	0.4	0.0	0.6	1	0.5	26.6	22.5	4.3	53	0
164	L-S., 0,1,2,3,4	4	1857	0.1	0.1	0.0	0	0.8	0.8	0.8	0.0	2	2
165	L-S., 1,2,3,4	3	1896	0.1	0.1	0.0	0	0.2	0.3	0.2	0.1	1	2
166	L-S., 0,1,2,3; B.M., 4 (3, delayed 7 days)	4	1928	0.3	0.0	0.1	0	0.4	0.5	0.3	0.0	1	7
167	L-S., 0,1,2; B.M., 3,4 (3, delayed 14 days)	3	2409	1.1	0.8	0.1	2	1.4	3.0	0.7	0.2	4	0
168	D.L-S., 0,1,2,3,4	4	2280	0.8	0.1	0.1	1	0.9	0.4	0.3	0.0	1	3
169	D.L-S., 1,2,3,4	3	1307	1.0	0.4	0.4	2	0.4	0.2	0.5	0.1	1	3

	Fameuse (Lawrence)												
170	Untreated	3	2207	3.1	6.2	3.0	12	2.7	1.1	0.4	0.1	2	0
171	B.M., 0,1,2,3,4	4	1000	0.2	0.0	0.0	0	0.3	34.9	29.1	2.3	66	0
172	B.M., 1,2,3,4	3	1819	0.6	0.3	0.1	1	0.5	29.3	25.0	2.7	57	0
173	L-S., 0,1,2,3,4	4	2582	0.1	0.1	0.0	0	0.1	0.9	0.4	0.0	1	9
174	L-S., 1,2,3,4	3	1884	0.1	0.1	0.1	0	0.1	0.4	0.2	0.0	1	10
175	L-S., 0,1,2,3; B.M., 4 (3, delayed 7 days)	3	1867	0.0	0.1	0.0	0	0.1	0.2	0.2	0.0	0	17
176	L-S.+lime, 0,1,2,3,4[4]	3	1630	0.1	0.1	0.0	0	0.1	0.6	0.1	0.0	1	4
177	D.L-S., 0,1,2,3,4	4	2695	0.1	0.2	0.1	0	0.3	0.6	0.3	0.0	1	2
178	D.L-S., 1,2,3,4	3	1687	0.2	0.3	0.1	1	0.5	0.7	0.3	0.0	1	5
	Wealthy (Goff)												
179	Untreated	4	2240	3.2	3.2	2.0	8	1.4	2.3	0.8	0.4	4	0
180	L-S., 0,1,2,3; B.M., 4	4	1635	0.4	0.1	0.1	1	0.2	5.1	2.5	0.5	8	0
181	L-S., 1; S-D. L-S-Ars., **1,4,5,6,7**	4	2395	0.9	0.9	0.2	2	0.4	2.2	1.2	0.2	4	0
182	L-S., 2; S-D.L-S-Ars., **1,2,3,5,6,7**	4	1573	0.4	0.4	0.0	1	0.1	5.1	2.3	0.3	8	0
183	L-S., 1,2; S-D.L-S-Ars., **1,5,6,7**	4	2131	0.8	0.6	0.3	2	0.6	5.2	1.5	0.3	7	0
184	C-L-Ars., **1,2,3,4,5,6,7**	4	2549	1.5	1.5	0.6	4	1.3	1.9	0.9	0.1	3	0
185	S-D.L-S-Ars., **1,2,3,4,5,6,7**	4	1679	1.6	0.8	0.3	3	0.6	3.5	0.7	0.1	4	0
186	S-Ars., **1,2,3,4,5,6,7**	4	2388	2.1	0.9	0.5	4	0.6	3.2	1.0	0.2	4	0
187	S-Ars., **1,2,4,5,6,7**	4	3845	1.6	0.5	0.3	2	0.6	2.4	0.8	0.1	3	0
188	S-Ars., **1,2,3,4,5,7**	4	1792	1.0	0.6	0.2	2	0.2	3.7	1.5	0.5	6	0

[1]B.M.=Bordeaux mixture, 4-4-50. L-S.=Liquid lime-sulphur, sp. gr. 1.283, 1-40. D.L-S.=Dry lime-sulphur, 4-50, unless otherwise noted. Arsenate of lead (powder) was added to each spray mixture at the rate of 1 pound to 50 gallons. S-Ars.=Ground sulphur-lead arsenate, 90-10. S-D.L-S-Ars.=Sulphur-dry lime-sulphur-lead arsenate, 75-15-10. C-L-Ars.=Copper-lime-lead arsenate, 10-80-10. Gelatin was used at the rate of ¼ pound in 50 gallons

Dates of spray application at Learned orchard: 0, May 6; 1 (open cluster), May 13; 1A, May 19; 2, May 28; 3, June 9-10; 4, July 30. At Lawrence orchard: 0, May 7; 1 (open cluster), May 13; 2, May 28; 3, June 8; 4, August 1. At Goff orchard: 0, May 8; 1 (open cluster), May 14; 2, May 30; 3, June 9; 4, August 2.

Dates of dust applications at Learned orchard: **1,** May 6; **2,** May 12; **3,** May 17; **4,** May 28; **5,** June 8; **6,** June 12. At Goff orchard: **1,** May 7; **2,** May 13; **3,** May 20; **4,** May 30; **5,** June 9; **6,** June 18; **7,** August 2.

For a general account of the methods used see pages 58-63.

[2]S.D.=Standard deviation (see p. 63).

[3]Fruits "burned" following the use of lime-sulphur in application 3.

[4]Hydrated lime added in each application at the rate of 5 pounds to 50 gallons.

18. It occurred mainly on fruit borne on the south side of trees and was confined to fruit parts which received direct sunlight during the hottest part of the day. The injury was greatest on fruit borne on the lower branches. It appeared as more or less circular, somewhat sunken, chocolate brown areas, often covering about one-half of the fruit. In cases of less severe injury, healing sometimes occurred and the dead tissue was later sloughed off. A study of Table XII will show that this injury was associated with the application of lime-sulphur in treatment 3 (plots 138, 140, 164, 167). The injury was slight on the experimental plots which received treatment 3 at the regular time (ten days after the petals fell), June 8-9. An increase in the amount of injury occurred on plots on which treatment 3 was delayed until June 15 (plots 175, 173, 166, 164). A similar relation of fruit burning to delay of treatment 3 was noted in various commercial orchards. The most severe injury noted occurred on a Dudley orchard which was sprayed on June 17. Seventy-five per cent of the fruit was badly injured. Reference to Figure 3 shows that, for the period of June 8-17, the maximum temperature was 93° F. on June 17, which was clear, hot, and calm. At no other time during this period was the temperature higher than 82°. June 18 was also a clear, hot day with a maximum temperature of 90°, followed by two days of cooler weather. Since no increase in the amount of injury was observed after June 20, it appears that the major portion of the injury occurred on June 17, with possibly some increase on June 18. The results of plots 173 and 176 indicate that the addition of hydrated lime to lime-sulphur at the rate of five pounds to 50 gallons reduced the amount of burning. These data, however, seem too limited to be conclusive.

Experiments in 1922

Condition of Plots

Learned orchard. Same trees as in 1921. Clean cultivation was practiced in the young Wealthy block (plots 203-212), while the old orchard was disked three times early in the season.

Goff orchard. Same trees and cultural practice as in 1921.

Sackett orchard. Well-grown, vigorous, Dudley, Wealthy, and Duchess trees mostly twelve years old. Orchard on a gentle western slope, well elevated, with good air and surface drainage. Well cultivated, beginning before first discharge of ascospores and continuing until the middle of July.

Seasonal Conditions

Seasonal development and meteorological data appear in Figure 4.

The first ascospore discharge from the seasonal development experiment was recorded on May 12, when the "blossom" buds were in the closed cluster stage. The first observations on ascospore maturity

at Sturgeon Bay were made on May 8. Abundant mature ascospores were found at this time. The first infection of the season was observed on sepals and leaves on May 22. This indicates that ascospores were mature at some time during the rain periods of May 3 to 9. A moderately severe infection period occurred May 18 to 19 and a lighter one May 30 to 31. The most severe infection period of the season occurred June 8 to 10. Abundant infection also occurred in the periods of June 15 to 17 and July 9 to 16.

The season was one of severe scab infection, the percentage of scabbed fruits on untreated plots varying from 85 to 100.

Treatments and Results

A summary of treatments and of the results taken at harvest appears in Table XIII. Supplementary data relative to dust applications follow:

Treatment 1, Learned's. Dusted from 8:00 to 10:00 A. M. Light rain prior to and during the first hour of treatment. Moderate breeze (about 5 miles per hour).

Treatment 2, Learned's. Dusted from 10:00 to 12:00 A. M. Light rain up to 8:30 A. M. Leaves fairly dry when dust was applied. Light breeze.

Treatment 3, Learned's. Dusted from 9:30 to 10:00 A. M. Light rain just before and during dusting. Light breeze.

Treatment 1, Goff's. Dusted from 4:00 to 5:00 P. M. Trees wet from rain just prior to dusting. Light breeze.

Treatment 2, Goff's. Dusted from 2:00 to 4:00 P. M. Trees dry. Moderate breeze.

Treatment 3, Goff's. Dusted from 9:00 to 10:30 A. M. Trees dry. Moderate breeze.

Treatment 4, Goff's. Dusted from 9:30 to 11:30 A. M. Trees dry. Strong breeze.

The control of scab was not consistently satisfactory with any fungicide used, due to the fact that abundant infection occurred before treatments 0 were made.

In general there was little difference in scab control between Bordeaux mixture and liquid lime-sulphur (plots 190, 192, 204, 209, 217, 219, 223, 225, 227, 229, 191, 193, 205, 211, 218, 220, 228, 230). As in previous years fruit russeting made the results from Bordeaux unsatisfactory. The results from dry and liquid lime-sulphur were not essentially different (plots 197, 192, 198, 193, 212, 210, 221, 219, 222, 220, 231, 229, 232, 230).

The most striking feature of the results from treatment 0 is their variability in different orchards and on different varieties. Unfortunately, much of the value of this treatment was lost because the spray

TABLE XIII.—SUMMARY OF RESULTS OF SPRAYING AND DUSTING EXPERIMENTS FOR THE CONTROL OF APPLE SCAB, STURGEON BAY, WIS., 1922

Plot No.	Variety, orchard, and treatment[1]	Count trees	Total fruits	Seabbed fruits					Russeted fruits			
				Slight	Com'l.	Bad	Total		Slight	Com'l.	Bad	Total
		No.	No.	%	%	%	%	S. D.[2]	%	%	%	%
	Lubsk's Queen (Learned)											
189	Untreated	2	10142	10.5	17.4	60.4	88	6.4	0.4	0.3	0.2	1
190	B. M., 0, 1, 2, 3	2	3403	4.0	3.1	1.2	8	5.1	18.6	8.1	2.6	29
191	B. M., 1, 2, 3	2	2550	7.7	5.7	2.4	16	5.6	10.0	3.3	1.8	15
192	L-S., 0, 1, 2, 3	3	2952	6.9	4.9	1.7	14	4.9	2.8	1.4	0.8	5
193	L-S., 1, 2, 3	2	1316	7.6	4.6	4.3	17	10.0	2.0	1.5	2.4	6
194	L-S. + gelatin, 0, 1, 2, 3	2	13958	12.4	16.0	18.1	47	1.1	3.2	1.5	0.6	5
195	L-S. + glue, 0, 1, 2, 3	3	9900	10.8	15.6	10.3	37	14.3	4.1	1.8	0.8	7
196	L-S. + Cas-lime, 0, 1, 2, 3	2	7445	2.6	3.3	3.2	9	4.0	4.6	2.1	1.2	8
197	D. L-S., 0, 1, 2, 3	2	6653	11.8	11.6	13.0	36	0.0	2.4	1.1	0.7	4
198	D. L-S., 1, 2, 3	2	4383	9.6	9.0	9.0	28	2.5	1.4	0.8	0.7	3
199	D. L-S (3-50), 0, 1, 2, 3	2	8455	8.2	8.9	9.0	26	10.8	1.5	0.9	1.6	4
200	C-L-Ars., **1, 2, 3**	2	5647	6.2	3.4	4.4	14	3.8	3.0	1.1	1.1	5
201	S-D. L-S-Ars., **1, 2, 3**	2	3851	4.1	3.0	5.3	12	4.4	5.2	1.8	1.7	9
202	S-Ars., **1, 2, 3**	3	2388	6.0	2.4	4.6	13	2.9	6.9	1.7	2.7	11
	Wealthy (Learned)											
203	Untreated	2	5984	23.1	29.0	33.1	85	10.9	2.3	0.1	0.1	3
204	B. M., 0, 1, 2, 3, 4 (gun)	4	6352	2.2	1.1	0.3	4	0.6	38.7	16.5	3.7	59
205	B. M., 1, 2, 3, 4 (gun)	2	3577	3.4	1.2	0.9	6	0.3	23.5	9.6	2.5	36
206	B. M. (4-10-50), 0, 1, 2, 3, 4 (gun)	2	3043	0.8	0.4	0.2	1	0.2	28.6	13.1	3.1	45
207	B. M., 0, 3, 4; L-S., 1, 2 (gun)	2	3096	1.1	1.0	0.9	3	1.3	1.7	0.5	0.1	2
208	B. M. + Cas-lime, 0, 1, 2, 3, 4 (gun)	2	3123	1.5	0.4	0.4	2	0.8	32.0	10.1	3.2	45
209	L-S., 0, 1, 2, 3, 4	2	2506	1.3	1.0	0.5	3	0.1	0.0	0.1	0.0	0
210	L-S., 0, 1, 2, 3, 4 (gun)	4	6129	0.4	0.3	0.3	1	0.5	0.1	0.1	0.0	0
211	L-S., 1, 2, 3, 4 (gun)	3	2474	1.3	0.4	0.7	2	1.8	0.2	0.1	0.1	0
212	D. L-S., 0, 1, 2, 3, 4 (gun)	4	6699	2.0	0.9	0.7	4	1.3	0.2	0.1	0.1	0
213	C-L-Ars., **1, 2, 3**	4	2392	6.3	2.5	2.0	11	2.2	3.9	1.5	0.3	6
214	S-D. L-S-Ars., **1, 2, 3**	3	2173	1.9	0.7	0.6	3	1.1	0.3	0.1	0.2	1
215	S-Ars., **1, 2, 3**	3	2625	2.1	0.3	0.1	3	1.2	0.1	0.1	0.1	0

	Wealthy (Sackett)											
216	Untreated	4	2625	0.9	12.4	86.4	100	0.2	0.1	0.0	0.0	0
217	B. M., 0, 1, 2, 3	4	4409	6.0	4.6	4.6	15	3.4	20.0	8.7	6.5	35
218	B. M., 1, 2, 3	3	1482	14.0	5.9	4.8	25	2.2	19.2	5.6	4.9	30
219	L-S., 0, 1, 2, 3	4	2900	10.0	4.3	3.3	18	8.9	1.2	0.5	0.1	2
220	L-S., 1, 2, 3	4	1890	20.2	12.0	8.3	41	4.1	0.7	0.4	0.1	1
221	D. L-S., 0, 1, 2, 3	4	3138	7.3	4.1	3.4	15	1.0	0.6	0.2	0.4	1
222	D. L-S., 1, 2, 3	4	4055	18.1	9.8	7.6	36	18.4	5.4	2.2	1.6	9
	Duchess (Sackett)											
223	B. M., 0, 1, 2, 3	3	1110	13.5	3.6	1.0	18	6.3	23.6	3.9	0.8	28
224	B. M., 1, 2, 3	4	695	16.6	4.9	1.9	23	15.6	19.6	6.5	1.4	28
225	L-S., 0, 1, 2, 3	4	1149	10.3	3.6	1.4	15	8.0	4.1	0.9	0.2	5
	Dudley (Sackett)											
226	Untreated	4	1110	0.0	0.6	99.2	100	0.3	0.0	0.0	0.1	0
227	B. M., 0, 1, 2, 3	3	2436	24.5	11.2	13.1	49	8.3	35.7	21.6	1.0	58
228	B. M., 1, 2, 3	4	1166	22.7	20.3	30.5	74	5.6	20.3	12.4	2.8	36
229	L-S., 0, 1, 2, 3	4	2809	18.5	13.8	10.3	43	6.4	3.5	1.2	0.2	5
230	L-S., 1, 2, 3	4	2167	25.0	29.2	34.0	88	8.2	2.0	0.7	0.1	3
231	D. L-S., 0, 1, 2, 3	4	3528	26.2	13.6	9.5	49	10.8	1.7	0.2	0.1	2
232	D. L-S., 1, 2, 3	4	3217	26.1	26.1	26.8	79	10.0	3.9	0.8	0.1	5
	Wealthy (Goff)											
233	Untreated	2	2470	2.8	18.3	78.7	100	0.4	0.0	0.0	0.0	0
234	L-S., 0, 1, 2, 3, 4	3	2031	1.1	0.8	0.4	2	0.5	1.4	0.3	0.1	2
235	L-S., 0, 1, 2, 4; B. M., 3	2	1337	0.8	0.6	1.0	2	0.9	0.2	0.1	0.0	0
236	L-S., 0, 1, 2, 4; B. M., 3A	2	1638	1.0	1.2	1.0	3	1.9	0.3	0.1	0.0	0
237	L-S., 0, 1, 2; S-D. L-S-Ars., **3, 4**	2	1788	1.8	0.5	0.5	3	1.5	0.1	0.1	0.1	0
238	L-S., 1, 2; C-L-Ars., **3, 4**	3	2363	7.4	4.9	1.8	14	3.3	0.5	0.3	0.0	1
239	C-L-Ars., **1, 2, 3, 4**	3	679	21.3	15.5	14.5	51	12.6	1.3	0.7	0.3	2
240	S-D. L-S-Ars., **1, 2, 3, 4**	2	1343	28.0	18.6	13.0	60	25.0	0.2	0.1	0.0	0
241	S-Ars., **1, 2, 3, 4**	3	2612	21.4	12.9	7.0	41	11.2	0.1	0.6	0.0	1

[1]B. M.=Bordeaux mixture, 4-4-50. L-S.=Liquid lime-sulphur, sp. gr. 1.306, 1-40. D. L-S.=Dry lime-sulphur, 4-50, except as otherwise noted. Arsenate of lead (powder) was added to each spray mixture at the rate of 1 pound to 50 gallons. Cas-lime=a proprietary casein-lime spreader, ½-50. S-Ars.=Ground sulphur-lead arsenate dust, 90-10. S-D. L-S-Ars.=Sulphur-dry lime-sulphur-lead arsenate, 75-15-10. C-L-Ars.=Copper-lime-lead arsenate, 10-80-10. Gelatin was used at the rate of ¼ pound to 50 gallons: glue, ½ pound to 50 gallons.

Dates of spray applications at Learned orchard: 0, May 12; 1 (open cluster), May 18; 2, June 1; 3, Lubsk's Queen June 9, Wealthy June 14; 4, August 5. At Sackett orchard: 0, May 13; 1 (open cluster), May 20; 2, May 29; 3, June 8. At Goff orchard: 0, May 13; 1 (open cluster), May 20; 2, May 31; 3, June 14; 3A, July 3; 4, August 11.

Dates of dust application at Learned orchard; **1,** May 12; **2,** May 31; **3,** June 9. At Goff orchard: **1,** May 19; **2,** May 31; **3,** June 10; **4,** August 7.

For a general account of the methods used see pages 58-63.

[2]S. D.=Standard deviation (see p. 63).

was not applied until well after the only important pre-blossom infection period of the season (May 3-9). In spite of this fact it greatly reduced the percentage of scabbed fruit on the more severely affected plots (maximum reduction of 45%, plots 229, 230). On the less severely scabbed plots the effectiveness of this treatment was relatively slight. The following factors were probably important in relation to these variations:

1. The greater abundance of ascospore material on the more severely scabbed plots (Sackett orchard), leading to more severe sepal infection. Counts made on May 22 on 50 fruits selected at random on each variety showed sepal infection on the following percentages of fruits: Dudley (Sackett's), 66; Wealthy (Sackett's), 36; and Lubsk's Queen (Learned's), 10.

2. Variations in the stages of advancement of the opening "fruit" buds at the time of the pre-blossom infection. This may in part explain the greater severity of the early outbreak of scab on Dudley than on Wealthy in the Sackett orchard, since yearly observations have shown that the Dudley buds open somewhat in advance of those of the Wealthy.

3. Variations in the timing of applications. Treatment 0 was applied one day later on the Sackett than on the Learned orchard, and slight differences occurred in the timing of the later treatments (Table XIII).

The addition of gelatin and glue, respectively, to liquid lime-sulphur appeared to detract from its effectiveness in scab control (plots 194, 195, 192). The apparent slight increase in the effectiveness of this fungicide by the addition of casein-lime is probably not significant (plots 196, 192).

There was no significant difference in results from experiments in which the spray rod and gun were tested comparatively (plots 209, 210).

Bordeaux mixture, 4-10-50, gave essentially the same scab control as Bordeaux, 4-4-50. The apparent decrease in russet which attended the use of the 4-10-50 formula is of doubtful significance (plots 206, 204). Both Bordeaux mixtures, however, occasioned too much russet to be satisfactory commercially. The substitution of lime-sulphur in treatments 1 and 2 of the full Bordeaux program (plots 207, 204) resulted in no significant difference in scab control and obviated most of the fruit russeting. The results from this program, however, were less satisfactory than from the full lime-sulphur program (plot 210), because of inferior finish of the fruit.

The substitution of Bordeaux mixture for lime-sulphur in the third treatment of the full lime-sulphur program was planned in relation to fruit burning. However, no fruit burning developed on any of the experimental plots in 1922. This substitution did not significantly affect scab control or russet. However, the fruit which received this program was somewhat inferior in appearance to that from the full

lime-sulphur program (plots 235, 234). The treatment of plot 236 was parallel to that of plot 235, except that treatment 3 (Bordeaux) was delayed 19 days. This was done to make it come at what appeared to be the most advantageous time for codling moth control. Under the conditions of this season this delay had no significant effect on scab control or fruit russet.

In the Learned orchard each of the three dust programs used controlled scab with approximately the same effectiveness as did the full program of liquid lime-sulphur (plots 200, 201, 202, 192, 213, 214, 215, 209). No significant fruit or foliage injury was caused by the dust treatments in either the Learned or the Goff orchard. In the Goff orchard, however, the dust treatments did not control scab satisfactorily (plots 239, 240, 241, 234). These sharp variations in the effectiveness of dust programs appear to be due primarily to the differences in the timing of treatments and in conditions under which the various applications were made. In the Learned orchard each dust application was made when the trees were wet, and, with the exception of the first treatment, during or immediately preceding a critical infection period. In the Goff orchard, all applications except the first were made on dry foliage, and usually in stronger wind than in the Learned orchard. Furthermore, the first application in the Goff orchard was made a week later than in the Learned orchard. No infection period, however, occurred during this interval. In the Goff orchard a mixed program consisting of liquid lime-sulphur spray in treatments 0, 1, and 2 and sulphur-dry lime-sulphur-arsenate dust in 3 and 4 gave essentially as good scab control as did the full lime-sulphur program (plots 237, 234).

Experiments in 1923

Condition of Plots

Learned orchard. Experiments confined to Lubsk's Queen trees used in previous years. Orchard disked four times early in season. However, sod remained practically unbroken in radius of about five feet from base of each tree.

Sackett orchard. Same orchard of Dudley, Wealthy and Duchess trees as in previous season. Disked four times prior to June 20, first cultivation being made May 8. Thorough cultivation continued until July 15.

Seasonal Conditions

Seasonal development and meteorological data appear in Figure 5.

The first ascospore discharge from the seasonal development experiment was recorded on May 20, when the "blossom" buds were in the late closed cluster stage. It is probable that ascospores were mature considerably earlier, since an examination of field material on

May 19 revealed many perithecia in which 50 per cent of the asci were mature. The rain periods of May 9 and 15 to 16 appear to have been of insufficient duration to permit infection. The first infection of the year appears to have occurred May 19 to 20. The second infection period, which was the most severe of the season, occurred during the rains of June 3 to 7. Other infection periods occurred July 6 to 7 and 23 to 24 and August 6 to 8.

The season was one of moderate scab infection, the percentage of scabbed fruits on unsprayed trees varying from 31 on Lubsk's Queen to 96 on Dudley. The scarcity of rain periods of sufficient duration to permit infection during the critical period of spring and early summer accounts for the lack of a more severe development of the disease.

Treatments and Results

A summary of treatments and of the results taken at harvest appears in Table XIV. Data regarding early infection on fruit are found in Table XV. Supplementary records pertaining to dust applications follow:

Treatment 1. Dusted from 9:00 to 10:00 A. M. A light rain fell prior to and during dusting and continued until 4:30 P. M. Light breeze.

Treatment 2. Dusted from 6:00 to 7:00 A. M. Very heavy dew. Trees wet. Very light breeze.

Treatment 3. Dusted from 8:00 to 9:00 A. M. Trees very wet from a light mist which continued throughout the day. Very light breeze.

Treatment 4. Dusted from 10:30 to 11:30 A. M. Trees moist from a fog which dissipated at about 10:00 o'clock. Wind velocity, 8 miles per hour.

Plots 251 and 252 were dusted on the dates of spray applications. All these treatments were made on dry trees in light to moderate breeze. In each case, however, the trees in plot 251 were wet just prior to dusting by being thoroughly sprayed with clean water. This was accomplished by connecting the spray rig between the tractor and the duster, and heavily spraying the trees by means of rods, using the same technique described above for applying sprays. The trees were thoroughly dusted from two sides.

Lime-sulphur and Bordeaux showed little difference in scab control (plots 244, 243, 261, 259, 274, 272) when the full program was used. While Bordeaux russet was less severe than usual, it was sufficient to render the results from this spray unsatisfactory commercially. On Lubsk's Queen, which was not severely scabbed, dry lime-sulphur gave about the same degree of scab control as Bordeaux and lime-sulphur (plots 248, 247, 243, 244). On Dudley and Wealthy, which were more severely affected, dry lime-sulphur gave a somewhat less satisfactory control (plots 263, 259, 261, 276, 272, 274). An unusual amount of russet occurred on Wealthy. A part of this was apparently oc-

casioned by injuries from spring frosts. Because of the irregularity of its occurrence, little significance can be attached to this russet in relation to spray injury.

In every instance, the addition of application 0 to the program materially increased its effectiveness in scab control (plots 244, 245, 259, 260, 261, 262, 263, 264, 272-277). The data contained in Table XV account for the effectiveness of this treatment. All programs of Bordeaux mixture or liquid lime-sulphur which included this early application controlled sepal infection adequately, whereas abundant sepal infection developed on all plots which did not receive a pre-pink treatment. On June 28, when the data of Table XV were taken, comparatively little scab had developed on fruits which were not infected on the sepals. A high percentage of the sepal-infected fruits, however, had developed secondary infection in spite of protection by the later fungicidal treatments.

Certain variations and substitutions were made in treatment 0 of the full lime-sulphur program. Where 90-10 flowers of sulphur-lead arsenate dust was substituted in this application, slightly less scab developed (plots 250, 244). No consistent difference in scab control attended the substitution of Bordeaux with or without casein-lime (plots 266, 265, 261, 279, 278, 274). In treatment 0 of the lime-sulphur program, no significant difference in control attended early application, increased concentration, or the addition of casein-lime (plots 270, 268, 269, 261, 282, 280, 281, 274).

Slightly less scab developed where casein-lime was added to lime-sulphur in the full program than where lime-sulphur was used without a spreader (plots 244, 246). This difference appears to be insignificant.

The substitution of Bordeaux mixture for treatment 3 of the full lime-sulphur program led to no important change in effectiveness of scab control or in the amount of russeting (plots 249, 244, 267, 261), but occasioned considerable foliage injury. This substitution was planned in relation to its possible bearing on fruit burning on lime-sulphur sprayed trees. There was not enough of this type of injury on the experimental plots to give a satisfactory test in this relationship.

Excellent control was obtained from each of the five dusting materials used. These tests, however, were not fully satisfactory in view of the fact that all the dusting work was done of Lubsk's Queen, on which the development of scab was light (31% of fruit scabbed on unsprayed plot). The various dust materials controlled scab on this variety with approximately the same effectiveness as did Bordeaux mixture and lime-sulphur (plots 253-257, 243, 244). A considerable amount of russeting attended the use of copper carbonate and the sulphur-dry lime-sulphur-arsenate dusts, and, to a less degree, the copper-lime-arsenate preparation. Where flowers of sulphur-arsenate dust was used comparatively on wet and dry trees (plots 251, 252) there was no significant difference in results. The control in this experiment was not significantly

TABLE XIV.—SUMMARY OF RESULTS OF SPRAYING AND DUSTING EXPERIMENTS FOR THE CONTROL OF APPLE SCAB. STURGEON BAY, WIS., 1923

Plot No.	Variety, orchard and treatment[1]	Count trees	Total fruits	Scabbed fruits					Russeted fruits			
				Slight	Com'l.	Bad	Total		Slight	Com'l.	Bad	Total
		No.	No.	%	%	%	%	S. D.[2]	%	%	%	%
	Lubsk's Queen (Learned)											
242	Untreated	2	7966	15.5	7.0	8.8	31	3.3	0.5	0.2	0.1	1
243	B. M., 0, 1, 2, 3	2	8977	0.3	0.4	0.3	1	0.9	10.2	4.4	5.5	20
244	L-S., 0, 1, 2, 3	2	8971	3.8	2.2	1.8	8	0.8	1.1	0.1	0.0	1
245	L-S., 1, 2, 3	2	6033	13.0	5.0	5.3	23	4.2	1.0	0.1	0.0	1
246	L-S. + Cas-lime, 0, 1, 2, 3	2	7089	0.8	1.1	1.4	3	0.7	0.7	0.2	0.1	1
247	D. L-S. (3-50), 0, 1, 2, 3	2	8237	2.4	2.8	3.7	9	1.6	0.5	0.1	0.1	1
248	D. L-S., 0, 1, 2, 3	2	7989	1.2	2.2	2.0	5	0.8	0.6	0.1	0.1	1
249	L-S., 0, 1, 2; B. M., 3	2	4829	5.4	2.9	3.6	12	2.5	0.5	0.2	0.0	1
250	S-Ars. A., 0; L-S., 1, 2, 3	2	10475	0.8	0.7	0.8	2	1.1	0.5	0.2	0.1	1
251	S-Ars. A., 0, 1, 2, 3[3]	2	11884	1.5	0.5	1.1	3	1.2	0.6	0.1	0.0	1
252	S-Ars. A., 0, 1, 2, 3[4]	2	6934	0.9	0.8	0.9	3	0.5	0.7	0.2	0.1	1
253	S-Ars. A., **1, 2, 3, 4**	2	9119	0.1	0.1	0.3	1	0.2	2.7	0.6	0.7	4
254	C-L-Ars., **1, 2, 3, 4,**	2	10402	0.7	0.6	0.8	2	0.9	9.2	1.2	0.9	11
255	S-D. L-S-Ars., **1, 2, 3, 4**	2	5960	0.1	0.1	0.0	0	0.2	23.6	8.2	12.7	45
256	S-Ars., **1, 2, 3, 4**	2	3248	0.5	0.7	0.6	2	0.9	2.2	0.3	0.1	3
257	Cop. Carb., **1, 2, 3, 4**	2	9937	0.2	0.2	0.1	1	0.1	23.7	8.5	11.6	44
	Dudley (Sackett)											
258	Untreated	4	1871	10.4	16.0	70.0	96	2.9	0.2	0.0	0.0	0
259	B. M., 0, 1, 2, 3	4	3239	2.5	2.1	0.2	5	0.2	5.3	1.0	0.1	6
260	B. M., 1, 2, 3	3	2735	12.8	9.0	4.2	26	8.8	5.3	1.3	0.1	7
261	L-S., 0, 1, 2, 3	4	5058	2.8	2.1	0.6	6	1.0	0.0	0.1	0.0	0
262	L-S., 1, 2, 3	4	3110	6.8	6.0	2.0	15	3.7	0.1	0.0	0.0	0
263	D. L-S., 0, 1, 2, 3	4	2502	11.3	3.4	0.7	15	2.1	0.3	0.1	0.0	0
264	D. L-S., 1, 2, 3	4	2907	14.5	9.4	4.0	28	4.3	1.3	0.5	0.0	2
265	B. M., 0; L-S., 1, 2, 3	4	3365	3.0	1.7	0.3	5	2.4	1.4	0.3	0.1	2
266	B. M. + Cas-lime, 0; L-S., 1, 2, 3	4	3493	1.9	0.5	0.1	3	1.1	1.3	0.4	0.1	2
267	L-S., 0, 1, 2; B. M., 3	3	1941	2.6	2.9	0.8	6	2.1	0.2	0.1	0.0	0
268	L-S., 0, 1, 2, 3, (0, 1-25, 8 days early)	4	4310	3.7	2.1	0.8	7	2.0	0.3	0.3	0.1	1
269	L-S., 0, 1, 2, 3 (0, 1-25 + Cas-lime 8 days early)	3	2841	4.3	3.4	1.6	9	2.3	0.7	0.2	0.1	1
270	L-S., 0, 1, 2, 3 (0, 8 days early)	4	3351	2.7	3.3	0.7	7	2.9	0.4	0.1	0.0	1

	Wealthy (Sackett)											
271	Untreated	1	852	30.6	15.3	14.3	60		6.7	7.1	2.6	16
272	B. M., 0, 1, 2, 3, 4	4	3409	1.5	1.6	0.2	3	1.8	29.0	22.8	6.6	58
273	B. M., 1, 2, 3, 4	2	997	6.9	5.0	2.6	15	1.9	23.2	23.8	30.6	78
274	L-S., 0, 1, 2, 3, 4	2	3057	1.2	1.8	0.1	3	0.2	6.3	2.8	0.5	10
275	L-S., 1, 2, 3, 4	2	1418	8.0	5.6	2.0	16	2.4	9.3	15.6	1.2	16
276	D. L-S., 0, 1, 2, 3, 4	2	2646	6.6	7.2	3.1	17	0.3	21.3	11.3	3.3	36
277	D. L-S., 1, 2, 3, 4	2	1887	11.4	11.0	5.6	28	5.3	19.0	15.0	1.0	25
278	B. M., 0; L-S., 1, 2, 3, 4	2	1526	2.0	3.2	1.0	6	1.2	8.4	5.0	4.4	18
279	B. M. + Cas-lime, 0; L-S., 1, 2, 3, 4	2	1983	1.3	2.3	1.2	5	1.7	22.2	19.2	8.6	50
280	L-S., 0, 1, 2, 3, 4 (0, 1-25, 8 days early)	2	1735	2.7	3.7	1.6	8	1.6	18.2	17.9	7.2	43
281	L-S., 0, 1, 2, 3, 4 (0, 1-25 + Cas-lime, 8 days early)	2	547	4.0	5.5	2.3	12	7.5	17.0	12.6	4.3	34
282	L-S., 0, 1, 2, 3, 4 (0, 8 days early)	2	1404	6.2	4.7	1.4	12	1.6	2.3	1.2	1.1	5

[1]B. M.=Bordeaux mixture, 4-4-50. L-S.=Liquid lime-sulphur, sp. gr. 1.295, 1-40. D. L-S.=Dry lime-sulphur, 4-50, unless otherwise noted. Cas-lime=a proprietary casein-lime spreader, ½-50. Arsenate of lead (powder) was added to each spray mixture at the rate of 1 pound to 50 gallons. S-Ars. A.=Flowers of sulphur-lead arsenate dust, 90-10. S-Ars.=Ground sulphur-lead arsenate dust, 90-10. S-D. L-S-Ars.=Sulphur-dry lime-sulphur-lead arsenate dust, 75- 15-10. C-L-Ars.=Copper-lime-lead arsenate dust, 10-80-10. Cop. Carb.=Copper carbonate dust.

Dates of spray application at Learned orchard: 0, May 16; 1 (open cluster), May 24; 2, June 8; 3, June 25. At Sackett orchard: 0, early, May 10; 0, May 18; 1 (open cluster), May 25; 2, June 8; 3, June 22; 4, August 9.

Dates of dust applications: **1,** May 15; **2,** May 25; **3,** June 7; **4,** July 7.

For a general account of the methods used see pages 58-63.

[2]S. D.=Standard deviation (see p. 63).

[3]Trees sprayed with clean water before dusting.

[4]Trees dry when dusted.

TABLE XV.—SUMMARY OF DATA ON THE RELATION OF SEPAL INFECTION BY V. INAEQUALIS TO THE EARLY DEVELOPMENT AND CONTROL OF APPLE SCAB, STURGEON BAY, WIS., JUNE 28, 1923[1]

Plot No.	Variety, orchard, and treatment[2]	Sepal-infected fruits	Sepal-infected fruits which developed scab on check	Sepal-clean fruits which developed scab on check	Total fruits infected
		%	%	%	%
	Dudley (Sackett)				
258	Untreated	54	70	35	70
259	B.M., 0,1,2,3	4	0	0	4
260	B.M., 1,2,3	36	67	9	42
261	L-S., 0,1,2,3	4	100	0	4
262	L-S., 1,2,3	42	33	10	48
263	D.L-S., 0,1,2,3	3	50	13	14
264	D.L-S., 1,2,3	54	45	11	58
265	B.M., 0; L-S., 1,2,3	2	0	4	6
266	B.M. + Cas-lime, 0; L-S., 1,2,3	4	0	0	4
268	L-S., 0,1,2,3 (0, 1-25, 8 days early)	4	50	6	10
269	L-S., 0,1,2,3 (0, 1-25+Cas-lime, 8 days early)	8	75	2	10
270	L-S., 0, 1,2,3, (0, 8 days early)	6	100	8	14
	Lubsk's Queen (Learned)				
242	Untreated	50	52	16	58
243	B.M., 0,1,2,3	4	50	0	4
244	L-S., 0,1,2,3	10	0	0	10
245	L-S., 1,2,3	38	21	3	40
247	D.L-S. (3-50), 0,1,2,3	12	50	0	12
248	D.L-S., 0,1,2,3	16	12	2	18
246	L-S. + Cas-lime, 0,1,2,3	4	50	0	4
250	S-Ars.A., 0; L-S., 1,2,3	2	100	2	4
251	S-Ars.A., 0,1,2,3 (Trees wet)	0	0	0	0
252	S-Ars., 0,1,2,3 (Trees dry)	8	0	0	8
253	S-Ars.A., **1,2,3,4**	6	0	0	6
254	C-L-Ars., **1,2,3,4**	4	0	0	4
255	S-D.L-S-Ars., **1,2,3,4**	0	0	0	0
256	S-Ars , **1,2,3,4**	2	0	0	2
257	Cop. Carb., **1,2,3 4**	0	0	0	0

[1]For each plot, results obtained from 50 fruits chosen at random from representative parts of three trees.

[2]For details of treatment see footnotes of Table XIV.

different from that of the same material applied according to the regular dusting program (plot 253). It is worthy of note, however, that few infection periods occurred in the critical stage of host development in spring and early summer, and that the spraying and dusting programs were very fortunately timed in relation to these periods. Under the conditions of this season, the spray applications, which were timed largely according to host development, and the dust treatments, which were timed primarily according to infection periods, were applied very nearly parallel throughout the most critical time for scab infection (Fig. 5).

Experiments in 1924

Condition of Plots

Learned orchard. Same Lubsk's Queen trees as in previous seasons. Orchard disked twice, beginning just before blossoms opened. Sod remained practically unbroken in radius of about five feet from base of each tree. "Set" of fruit was light.

Sackett orchard. Same Dudley and Wealthy trees as in previous years. First cultivation made with disk harrow May 16. Cultivations made at frequent intervals with spring-tooth harrow until July 5, when Hubam clover was sown.

Seasonal Conditions

Seasonal development and meteorological data appear in Figure 6 and Table 1.[1]

The first ascospore discharge from the seasonal development experiment was recorded on May 6, when the "blossom" buds were in the early green tip stage (see Pl. III). The first observations on ascospore development were made on May 5. The abundance of mature asci found indicates that ascospores had been mature for some days. The first infection period of the season occurred May 5 to 10. A second occurred May 12 to 15. Other infection periods occurred June 15 to 17 and 28, to 29; July 7 to 9, 15, 29 to 30; and August 5 to 6 and 15 to 16.

The season of 1924 was one of unusually severe scab infection, the percentage of scabbed fruits on unsprayed plots varying from 92 on Lubsk's Queen to 100 on Wealthy and Dudley. This severity of occurrence is attributed in large measure to the abundant early establishment of the fungus on the sepals. The foliage of Dudley was heavily infected, while that of Wealthy and Lubsk's Queen showed very little infection.

[1]In making cross-references it should be recalled that in Table I days extend from midnight to midnight, whereas in Figures 1-6 they are taken as 24 hours preceding 8 A. M. of the dates listed.

Treatment and Results

A summary of treatments and of the results taken at harvest appears in Table XVI. Data regarding early infection on fruit are found in Table XVII. Supplementary records pertaining to dust applications follow:

Treatment 1. Dusted from 9:00 to 10:15 A. M. Wind velocity 4-5 miles per hour. Trees were wet from fog and a light drizzle which fell during the operation and continued at intervals until next afternoon.

Treatment 2. Dusted from 7:15 to 8:30 A. M. Wind velocity 5 miles per hour. Trees were partly wet, following a trace of rain at 7:00 A. M.

Treatment 3. Dusted from 9:30 to 10:30 A. M. (plots 294, 293), and 2:00 P. M. (plot 292). Treatment interrupted to repair duster. Wind velocity 10 to 12 miles per hour. The trees were wet from rain which fell intermittently during the day and steadily through most of the night.

Plots 290 and 291 were dusted on the dates of spray applications. All these treatments were made on dry trees in light to moderate breeze. In each case, the trees in plot 290 were sprayed with clean water and dusted by the method described on pages 60-61.

On Lubsk's Queen, it would appear at first glance that Bordeaux mixture had given somewhat better scab control than liquid lime-sulphur (plots 284, 285). A consideration of the standard deviations, however, indicates that this difference is of doubtful significance. On Wealthy there was practically no difference in control between these fungicides (plots 308, 310). On Dudley, however, Bordeaux gave a strikingly more effective control (plots 296, 298). Much of the infection on the lime-sulphur plot appeared in the latter part of the season (see Table XVII, plots 296, 298). It appears probable, therefore, that the difference in effectiveness of these programs is to be attributed largely to a longer duration of effectiveness of Bordeaux in the final application under the very severe conditions of scab infection on Dudley. Bordeaux mixture russeted the fruit severely and occasioned considerable defoliation prior to harvest. Dry lime-sulphur appeared to give somewhat less satisfactory results than the liquid product, except on Dudley, where both programs were relatively ineffective (plots 288, 287, 285, 300, 298, 312, 310). Dry lime-sulphur, 3-50 and 4-50, used comparatively showed no significant difference in the control of the disease (plots 287, 288).

Application 0 showed considerable variations in effectiveness on the different varieties and with the different fungicides. On Lubsk's Queen in the lime-sulphur program (plots 285, 286), it showed no significant increase in effectiveness of scab control. On Wealthy, whereas treatment 0 in the Bordeaux program (plots 308, 309) gave

TABLE XVI.—SUMMARY OF RESULTS OF SPRAYING AND DUSTING EXPERIMENTS FOR THE CONTROL OF APPLE SCAB, STURGEON BAY, WIS., 1924

Plot No.	Variety, orchard, and treatment[1]	Count trees	Total fruits	Scabbed fruits					Russeted fruits			
				Slight	Com'l.	Bad	Total		Slight	Com'l.	Bad	Total
		No.	No.	%	%	%	%	S. D.[2]	%	%	%	%
	Lubsk's Queen (Learned)											
283	Untreated	2	9351	14.9	14.9	61.8	92	7.5	0.2	0.0	0.0	0
284	B. M., 0, 1, 2, 3	2	6420	2.0	1.0	1.2	4	2.0	39.3	29.7	21.2	90
285	L-S., 0, 1, 2, 3	2	9312	5.8	3.0	2.0	11	4.8	3.5	0.7	0.1	4
286	L-S., 1, 2, 3	2	3783	8.5	5.0	3.6	17	8.6	13.6	2.5	0.2	16
287	D. L-S. (3-50), 0, 1, 2, 3	2	8703	8.8	7.2	4.9	21	9.4	5.6	0.7	0.1	6
288	D. L-S. (4-50), 0, 1, 2, 3	2	12885	6.7	6.5	3.9	17	3.2	2.2	0.2	0.0	2
289	S-Ars., 0; L-S., 1, 2, 3	2	4018	1.9	1.3	0.9	4	1.0	19.7	5.2	1.1	26
290	S-Ars., 0, 1, 2, 3[3]	2	6178	6.4	7.0	3.6	17	12.1	24.7	4.9	0.3	30
291	S-Ars., 0, 1, 2, 3[4]	2	5239	7.5	6.4	3.3	17	3.0	15.4	2.8	0.3	19
292	S-Ars., **1, 2, 3**	2	3121	10.9	7.7	4.1	23	0.1	5.3	0.7	0.1	6
293	S-Ars. A., **1, 2, 3**	2	9531	16.5	17.7	10.6	45	22.2	1.1	0.1	0.1	1
294	C-L-Ars., **1, 2, 3**	2	5553	21.3	21.6	18.8	62	0.6	1.3	0.2	0.0	2
	Dudley (Sackett)											
295	Untreated	3	367	0.7	5.6	93.7	100	0.0	0.0	0.0	0.0	0
296	B. M., 0, 1, 2, 3	1	3045	10.2	12.7	6.8	30	3.6	45.5	24.7	0.2	70
297	B. M., 1, 2, 3	3	948	18.2	22.9	18.3	59	7.3	36.3	18.4	0.5	55
298	L-S., 0, 1, 2, 3	8	6641	35.9	20.8	8.9	66	7.3	0.5	0.1	0.0	1
299	L-S., 1, 2, 3	3	1167	31.9	24.2	18.6	75	9.8	1.5	0.1	0.0	2
300	D. L-S., 0, 1, 2, 3	4	2664	34.6	22.7	11.5	69	12.5	3.9	0.3	0.0	4
301	D. L-S., 1, 2, 3	3	1727	30.3	35.5	26.5	92	1.7	5.1	0.9	0.0	6
302	B. M., 0; L-S., 1, 2, 3	3	2229	24.6	24.3	17.7	67	5.7	6.2	0.7	0.0	7
303	L-S., 0, 1, 2, 3 (0, 1-25)	3	1672	22.8	13.8	7.0	44	11.2	0.8	0.3	0.0	1
304	L-S., 0, 1, 2, 3 (0, 4 days late)	3	2169	30.6	19.8	12.2	63	1.9	1.2	0.4	0.0	2
305	L-S. + Cas-lime, 0, 1, 2, 3	3	1438	19.7	12.7	4.5	40	17.6	3.8	0.4	0.0	4
306	L-S. + lime (5-50), 0, 1, 2, 3	3	2200	41.7	23.0	5.3	70	10.4	2.7	0.2	0.0	3
	Wealthy (Sackett)											
307	Untreated	3	1270	0.9	6.5	92.6	100	0.0	0.0	0.0	0.0	0
308	B. M., 0, 1, 2, 3, 4	3	595	2.9	5.6	0.0	9	1.2	25.9	63.7	9.4	99
309	B. M., 1, 2, 3, 4	3	902	3.8	3.5	2.5	10	0.7	40.8	49.9	7.5	98
310	L-S., 0, 1, 2, 3, 4	3	1106	3.3	3.7	1.4	8	0.6	0.7	0.3	0.0	1
311	L-S., 1, 2, 3, 4	3	949	10.6	10.2	3.4	24	1.4	0.6	0.1	0.0	1
312	D. L-S., 0, 1, 2, 3, 4	3	1163	11.1	11.2	7.5	30	7.7	0.8	0.4	0.0	1
313	D. L-S., 1, 2, 3, 4	3	995	19.5	16.1	8.8	44	3.7	0.6	0.8	0.0	1
314	B. M., 0; L-S., 1, 2, 3, 4	3	1043	6.4	8.6	2.0	17	1.7	1.4	0.1	0.0	2
315	L-S., 0, 1, 2, 3, 4 (0, 1-25)	3	935	6.5	5.8	3.2	16	4.8	1.1	0.2	0.0	1
316	L-S., 0, 1, 2, 3, 4 (0, 4 days late)	3	904	7.4	9.0	2.6	19	7.2	1.7	0.4	0.0	2
317	L-S. + Cas-lime, 0, 1, 2, 3, 4	3	1013	7.8	6.3	4.5	19	8.9	0.0	0.0	0.0	0
318	L-S. + lime (5-50), 0, 1, 2, 3, 4	3	593	11.2	6.9	2.3	20	5.3	0.1	0.0	0.0	0

[1]B. M.=Bordeaux mixture, 4-4-50. L-S.=Liquid lime-sulphur, sp. gr. 1.295, 1-40. D. L.-S.=Dry lime-sulphur, 4-50, unless otherwise noted. Arsenate of lead (powder) was added to each spray mixture at the rate of 1 pound to 50 gallons. S-Ars.=Ground sulphur-lead arsenate, 90-10. S-Ars. A.=Flowers of sulphur-lead arsenate, 90-10. C-L-Ars.=Monohydrated copper sulphate-arsenate of lead-hydrated lime, 12-10-78. Cas-lime=a proprietary casein-lime spreader, 1½-50.

Dates of spray applications at Learned orchard: 0, May 14; 1 (open cluster), May 29; 2, June 17; 3, July 1.

At Sackett orchard: 0, May 15; 1 (open cluster), May 29; 2, June 18; 3, June 30; 4, August 11.

Dates of dust applications; **1**, May 13; **2**, June 1; **3**, June 28.

For a general account of the methods used see pages 58-63.

[2]S. D.=Standard deviation (see p. 63).

[3]Trees sprayed with clean water before dusting.

[4]Trees dry when dusted.

TABLE XVII.—SUMMARY OF DATA ON THE RELATION OF SEPAL INFECTION BY V. INAQUALIS TO THE EARLY DEVELOPMENT AND CONTROL OF APPLE SCAB, STURGEON BAY, WIS., JULY 10, 1924 [1]

Plot No.	Variety, orchard, and treatment[2]	Sepal-infected fruits	Sepal-infected fruits which developed scab on cheek		Sepal-clean fruits which developed scab on cheek	
		Per cent	Per cent	Ave. No. lesions	Per cent	Ave. No. lesions
	Lubsk's Queen (Learned)					
283	Untreated	69	76	6.0	11.0	2.2
285	L-S., 0,1,2,3	28	12	2.0	1.0	1.0
286	L-S., 1,2,3	53	27	3.4	1.0	1.4
284	B.M., 0,1,2,3	37	4	1.0	0.0	0.0
288	D.L-S (4-50), 0,1,2,3	44	18	3.1	3.5	1.3
287	D.L-S. (3-50), 0,1,2,3	35	25	2.6	1.0	1.0
289	S-Ars., 0; L-S., 1,2,3	16	4	1.0	0.0	0.0
292	S-Ars., **1,2,3**	19	17	2.0	1.6	1.0
293	S-Ars.A., **1,2,3**	27	40	2.0	2.0	1.5
294	C-L-Ars., **1,2,3**	33	47	5.4	3.0	1.0
290	S-Ars., 0,1,2,3[2]	31	9	2.2	0.0	0.0
291	S-Ars., 0,1,2,3[2]	27	12	1.4	0.9	1.0
	Dudley (Sackett)					
295	Untreated	87	92	8.9	63.0	5.2
296	B.M., 0,1,2,3	47	41	4.9	2.5	1.5
297	B.M., 1,2,3	77	37	4.9	3.0	1.0
298	L-S., 0,1,2,3	49	37	3.9	5.0	2.0
299	L-S., 1,2,3	59	50	3.1	3.0	1.5
300	D.L-S., 0,1,2,3	61	43	5.0	7.0	1.8
301	D.L-S., 1,2,3	73	49	4.4	3.0	1.0
302	B.M., 0; L-S., 1,2,3	76	52	4.0	3.0	1.0
306	L-S. +lime (5-50), 0,1,2,3	51	34	4.2	1.0	4.0
305	L-S. +Cas-lime, 0,1,2,3	40	23	3.8	0.0	0.0
303	L-S., 0,1,2,3 (0, 1-25)	67	47	4.8	6.0	1.3
304	L-S., 0,1,2,3 (0,4 days late)	63	33	4.2	4.0	1.0

[1]For each plot, results obtained from 150 fruits chosen at random from representative parts of three trees

[2]For details of treatment see footnotes of Table XVI.

no increase in scab control, in the liquid and dry lime-sulphur programs (plots 310-313) a similar application improved the control. On Dudley this application in the Bordeaux (plots 296, 297) and dry lime-sulphur (plots 300, 301) programs gave increased control, but showed little effectiveness in the case of liquid lime-sulphur (plots 298, 299). The effectiveness of these early treatments was much marred by the fact that important infection periods occurred before they were applied (see Fig. 6 and Pl. III). The variations in effectiveness appear to have been influenced largely by differences in the severity of these early infections on different varieties, particularly on the sepals. On June 2 (at the beginning of the blooming period) counts of "blossom" buds showed the following percentages to be sepal-infected: Lubsk's Queen, 12; Wealthy, 37; Dudley, 74. These variations in the amount of early infection appear to have been due chiefly to differences in the amount of the ascigerous stage of the scab fungus developed in the fallen leaves of these varieties. On Lubsk's Queen this development was very sparse, due to the scarcity of foliage infection in the previous year (Fig. 5). On Wealthy the ascigerous development was intermediate in amount, while on Dudley it was extremely abundant. The importance of early sepal infection in relation to control is further shown in Table XVII. Even in this season of severe epidemic development and inadequate control, secondary infection prior to July 10 was readily held in check on the sepal-clean fruit by all the programs tried. On the sepal-infected fruits, however, the protection given by the strongest programs used was inadequate, and abundant secondary infection developed.

Certain variations and substitutions were again made in treatment 0 of the full lime-sulphur program. The value of these experiments was greatly lessened because of the infection which occurred prior to the earliest treatment. Consequently, the results appear to be of doubtful significance (plots 284-289, 296-304, 308-316).

In view of the high standard deviations, the results from lime-sulphur with and without the addition of lime (plots 306, 298, 318, 310) do not appear to be significant.

Where sulphur-arsenate dust, 90-10, was applied in comparison with spray applications on the spraying dates the results relating to scab control appear at first glance to be slightly in favor of the spray (plots 290, 291, 285). A consideration of standard deviations, however, suggests that this difference is probably not significant. No significant difference in control resulted when this dust program was applied comparatively on wet and dry trees (plots 290, 291). In the dusting experiments in which it was aimed to make an application in each important infection period before the fungus had time to establish itself in the host plant, sulphur-arsenate, 90-10, gave approximately as effective scab control as in the program in which the dates of spray application were followed (plots 292, 291). Flowers of sulphur-arsenate, 90-10, appears to have given less effective scab control

than the ground product (plots 293, 292). The large standard deviation, however, indicates that these results are of doubtful significance. The copper-lime-arsenate dust in this program gave distinctly less scab control than the sulphur dusts (plots 294, 293, 292).

Discussion of Results

In attempting to interpret the results of orchard spraying and dusting experiments, one should clearly bear in mind the limitations of the methods employed. In view of the many variables encountered in such work, the results from a single well-conducted set of experiments in a given year and locality are usually suprisingly consistent. Averages from a number of tests in different seasons or localities, however, may be very misleading. One important reason for this is the fact that field trials frequently do not reveal the margins of safety of the various programs used. A weak program may give as good results as a much stronger one when disease control is easy, yet give much inferior results under conditions which require greater efficiency. In such cases an average of the results would scarcely be a satisfactory criterion of the comparative efficiency of the two programs. Further limitations to the value of such averages are found in the variability in methods, environments, and materials dealt with in the several experiments.

The significance of these limitations may perhaps become more apparent when one contemplates the fact that the thousands of orchard tests that have been conducted have left us with a very unsatisfactory understanding of the relative efficiency and adaptations of the several fungicides in most common use for apple scab control. Notwithstanding these limitations, it is the view of the present writers that critically conducted field experiments are essential to further progress in apple scab control, and their hope that appropriate supplementary studies may make possible more accurate interpretations of results and more effective modifications of methods.

In view of the great number of contributions to the subject of apple scab control, and of the limitations which attend the interpretation of much of the data concerned, it has not seemed feasible or desirable to relate the following discussion to the literature, except in a limited measure in especially pertinent connections.

Comparison of sprays. During six seasons of comparative tests liquid lime-sulphur and Bordeaux mixture, in the full program of treatments (including two pre-blossom applications) have shown no significant differences in scab control, with the exception of one experiment in 1924, in which the control by Bordeaux was distinctly superior (discussed on p. 84). With the exception of the tests conducted in 1922 and 1924, when the first treatments were applied too late to control early infections, and of certain unexplained minor irregularities on Lubsk's Queen in 1919 and 1923, each of these fungicides gave con-

sistent and satisfactory scab control. The results from Bordeaux mixture were unsatisfactory commercially because of russeting, inferior finish of fruit, and foliage injury. Some foliage injury and fruit "burning" have attended the use of lime-sulphur. This, however, has not been of serious economic importance. In most of the comparative tests, dry lime-sulphur, 3-50 and 4-50, in the full program of applications, gave about the same degree of scab control as liquid lime-sulphur. In certain instances, however, it appeared to be less effective. Similarly, in the comparative tests of the 3-50 and 4-50 concentrations of dry lime-sulphur, no important differences in control resulted. Though inconclusive, these data suggest that liquid lime-sulphur (33° B.), 1-40, has a somewhat greater margin of safety for apple scab control than has dry lime-sulphur at the concentrations used.

The desirability of early applications of fungicides. Much confusion has existed regarding the importance of scab infection and of fungicidal applications in the earlier pre-blossom stages of host development. In the early years of spraying, a dormant treatment for apple scab was commonly recommended. With an increasing understanding of the life history of the fungus, this treatment has fallen into disuse, except as it may be applied in cases where twig infection occurs to a serious extent (Morse and Darrow, 1913, p. 269). Following the pioneer work of Goff and Taft (Clinton, 1901, p. 112-113) and a great many orchard trials by other workers, the use of a single pre-blossom treatment came to be widely accepted. The timing of this application in relation to host development varied considerably, and in many cases was not clearly defined. In the earlier years it was commonly recommended to be applied at some time between the first appearance of pink color in the expanding "blossom" buds and the opening of the blossoms. In later years the tendency has been to make this application just before the blossoms open, preferably when the buds are separated in the clusters and more readily covered by the fungicide (Wallace, 1913, Pl. XI).

Wallace (1913) appears to have been the first investigator to attempt to relate detailed studies of the life-history of the scab fungus to the development of the spraying program. He summarizes his results as follows (p. 571): "From the above data it appears that the leaves and buds of the apple are susceptible to infection as soon as they are exposed, but that infection does not occur until the ascospores have matured or until the first appearance of weather conditions favorable for infection following the maturity of ascospores. According to observations during the past three years, the spores do not reach maturity until the blossoms are either opening or just ready to open. It seems, therefore, that there is little danger of abundant infection earlier than about the time when the blossom buds show pink." These results accorded so nearly with prevailing practice that they became widely accepted as having general application, despite the fact that

numerous conflicting observations and suggestions are to be found in the earlier literature. Fairchild (1894, p. 44), for example, having observed the occurrence of the disease on blossoms, emphasizes the importance of early treatments, and makes the following tentative recommendation: "The first treatment should be made as the fruit buds are unfolding in the spring and the scales upon opening reveal the clusters of young unopened flowers. The second spraying should be done after the young flower clusters are expanded, but before the individual blossoms have opened." Lowe and Parrott (1902, p. 405-407), working with lime-sulphur-salt wash for the control of San José scale, noted satisfactory control of scab on Baldwin following a single application. Regarding the time of treatment, they state: * * * "In many cases the buds had already burst and in some cases the leaves were well out, while in others only the tips of the young leaves were beginning to appear." Scott and Quaintance (1907, p. 21) state: * * * "The fungus may attack and destroy the blossoms and even the unopened buds . . . The scab first appears early in the spring on the young buds and unfolding leaves, and new infections may continue to take place throughout the season." Nevertheless, they recommend that the first scab treatment be applied . . . "after the cluster buds have opened, but prior to blooming." Cordley (1908) calls attention to the value in scab control shown by delayed dormant applications of lime-sulphur for scale, and states that it was such observations that led him to try lime-sulphur as a summer spray.

Beginning in 1913, Blair and his colleagues conducted an extensive series of spraying experiments in which programs with two pre-blossom scab treatments were compared with others which carried only one. In 1913 the first pre-blossom application, which was made "when the leaf buds were unfolded slightly," played an important part in scab control (Robinson, 1914). Discussing the work of 1916, Blair 1917, p. 136) states: * * * "The results would seem to show that one spray [before bloom] may be sufficient to give reasonably good scab control. The results were very similar to those obtained in 1914 and 1915. However, as pointed out last season, should the leaves expand early as in 1913, due to warm weather, but be delayed in their development by cool weather, the period [pre-blossom] should be covered by two applications of spray mixture rather than one."

Jackson and Winston (1915?) noted that in 1914 very beneficial results in scab control followed the application of a delayed dormant lime-sulphur spray in certain commercial orchards. Winston and Childs (1916?) report that in their spraying experiments of 1915 a delayed dormant application . . . "in practically every case produced decidedly beneficial results." Childs (1917) made a valuable study of the periods of ascospore ejection at Hood River, Oregon, in 1916. He reviews previous literature and states (p. 11): "The study of ascospore discharge demonstrates beyond a doubt that the delayed-dormant application given while the leaves are small and undeveloped cannot be

safely dispensed with in the Hood River and Willamette Valleys, at least, since the ejection of ascospores begins before the foliage has even started. This would probably also hold true in all sections of Western Oregon and Washington . . . It is not at all unlikely that other sections could also be included except for our present limited knowledge."

Morse (1916 a, p. 90-92 and 1916 b, p. 180-182) reports no significant advantage in scab control following a delayed dormant application of lime-sulphur in his experimental orchard (see also Morse and Darrow, 1913). The same writer (1918, p. 116) reports unsatisfactory scab control from all programs used in 1917, and states: * * * "Undoubtedly in practical work an additional, earlier application of a fungicidal spray when the leaves are about one-fourth inch in diameter . . . would be very effective in Maine under such conditions as these [1917]."

Whetzel (1918) calls attention to the importance of preventing primary infection, and reports that in 1917 growers who applied the delayed dormant spray got best results. He states: "As a matter of insurance, this delayed dormant spray may well be made."

In 1919, therefore, the commonly accepted recommendations for apple scab control throughout the north-central and north-eastern apple belt of the United States called for the first treatment shortly before the blossoms opened, and the need appeared to exist for a more detailed study of pre-blossom infection in relation to the later development and the control of the disease. In a preliminary publication on such studies conducted in 1919, the senior writer (1920) reported that the heaviest discharges of ascospores at Sturgeon Bay, Wis., occurred prior to the open cluster spray, and that on certain badly scabbing varieties programs beginning with the usual open cluster application failed to control the disease satisfactorily, whereas excellent control resulted when an earlier pre-blossom treatment was added (for further preliminary reports on this work, see: Keitt, 1921, 1922, 1923, and Keitt and Jones, 1924 a, 1925 b). The present writers (1924 b) reported on the special significance of sepal infection in relation to the epidemiology and control of apple scab.

Since 1920, a valuable body of data has been accumulated in the north-central and north-eastern apple belt of the United States, the general tendency of which is to attach increasing importance to the occurrence and the prevention of scab infection prior to the open cluster stage of bud development (e. g., Manns, 1921; Whetzel, 1921, 1924; Dutton and Johnston, 1922; Bennett, 1923; Krout, 1923 1924; Cullinan and Baker, 1924; Doran and Osmun, 1924; Thurston, *et al.*, 1924; Walton, 1924; Williams, 1924; Butler, 1925; Morse and Folsom, 1925). Working farther south, Schneiderhan (1923, 1926) and Schneiderhan and Fromme (1924) have reported less valuable results from the delayed dormant treatment. These results show sufficient variation with season and section, however, to indicate the desirability of studies under local conditions as a basis for practice.

Important gains in effectiveness of scab control attended the addi-

tion of an early pre-blossom application to the regular spray programs in four of the six years covered by the experiments herein reported. In the other two seasons, the pre-pink application was of little or no value. These results are readily understood in the light of the data presented in Figures 1-6, and discussed in other connections. In 1919, 1922, 1923, and 1924, years in which the disease was not satisfactorily controlled on the more severely attacked varieties without the use of a pre-pink treatment, early spring rains of sufficient number and duration led to serious scab infection prior to the application of the open cluster spray. In 1920 and 1921, although ascospores were matured well in advance of the open cluster stage, the early rains were of such distribution and duration that little or no infection occurred prior to the open cluster spray. From these data and from the results discussed elsewhere, it is apparent that the importance of green tip or closed cluster treatments is conditioned primarily upon the abundance and time of maturity of ascospores and the occurrence of seasonal conditions favorable for infection and disease development during the critical period preceding the open cluster stage. The experience of 1922-1924 shows that serious sepal infection may occur at a very early stage in the unfolding of "fruit" buds, and that subsequent control of the disease on fruits which sustain early sepal infection is much more difficult than on those which escape it (Pls. III-V, pp. 50, 54, 55, Tables XV, XVII). Under the epidemic conditions of 1924, two pre-blossom treatments failed to control early-season infection on the more severely scabbed varieties. While this situation was foreseen, it was decided to adhere to the original program on the experimental plots and test its effectiveness, rather than to introduce an additional application. In certain commercial orchards, however, a third pre-blossom treatment timed between those at the green tip and open cluster stages gave good control. A program carrying three pre-blossom applications is being included in further experiments.

The effects of variations in the pre-pink treatment are so greatly influenced by local conditions that conclusions are not justified from the data available. It is apparent, however, that timeliness of application was more important than the material used. No significant advantage appears to have attended the substitution of Bordeaux for lime-sulphur in this treatment, or the modification of lime-sulphur by increased concentration or addition of casein-lime. In two trials sulphur-arsenate dust, 90-10, substituted for the pre-pink application of the lime-sulphur program gave as good results as the spray. Dust appears to have important potentialities for use in this critical period.

It should be observed that even a program with three pre-blossom applications is aimed primarily at control of the disease on the fruit, and may not safe-guard against infection of leaves which unfold between fungicidal treatments (see Pls. I, II). In the presence of an abundant source of inoculum, the control of scab on the foliage of the more susceptible varieties is very similar in its difficulty to control of

apple rust by fungicides. Often, however, scab is well controlled on the fruit, notwithstanding a considerable amount of leaf infection. The seriousness of such foliage infection in relation to epidemiology and control is discussed in other connections.

The number and timing of spray applications. It is now coming to be increasingly accepted that no single fungicidal program for apple scab is applicable to all conditions. The immediately preceding discussion has indicated the necessity of adequate protection for the fruit, at least, during the pre-blossom period, and has shown that under severe epidemic conditions in commercial orchards three applications may be required for this purpose. Subject to minor changes to meet local needs these treatments would naturally be timed (a) in the green tip stage (from the stage shown in Pl. I, A, B to that shown in Pl. I, C, D), (b) in the earlier part of the closed cluster stage (Pl. II), and (c) in the open cluster stage (Pl. II, D). Under less severe conditions, fewer applications are necessary. If only two pre-blossom treatments are to be given, the most critical periods for application appear to be in the green-tip stage and just before blooming, preferably in the open cluster stage. In such cases, if good weather forecasting service is available, the timing of the first application may be modified to advantage.

If the disease is adequately controlled in the earlier part of the season, its later control is comparatively easy. Supplementing the treatments just outlined, the following program of applications, which has long been widely used, has given satisfactory results: (d) after about three-fourths of the petals are off and before the calyx lobes close, (e) about ten days later, and (f) in summer at a time chosen for efficiency in codling moth control (ordinarily Aug. 1-20 at Sturgeon Bay). On early varieties, the final treatment of the program just outlined should be omitted.

It should be observed that this program of applications is arranged primarily for scab control. In the Sturgeon Bay district, where infestations of codling moth are comparatively light, it has not seemed necessary to accommodate the scab program to the insecticidal program, save in the final application. On the advice of the Department of Economic Entomology, arsenate of lead powder, 1-50, has been added to each application of spray. Further information relating to the control of insects may be obtained from the Department of Economic Entomology.

The desirability of a mixed program of Bordeaux and lime-sulphur. The mixed programs of Bordeaux and lime-sulphur gave no significant differences in scab control from the full Bordeaux and lime-sulphur programs. While the use of lime-sulphur in treatments 2 and 3 materially lessened russeting, as compared with the full Bordeaux program, the fruit sprayed with this mixed program was ordinarily distinctly inferior in finish and amount of russet to that from the full lime-sulphur program.

Variations in the lime-sulphur program in relation to fruit injury. During the six years of the experiments at Sturgeon Bay, "burning" of lime-sulphur sprayed fruit occurred in a significant amount only in 1921. In that year it appeared only on plots which received lime-sulphur in application 3, and was of minor importance when this treatment was timed according to schedule (ten days after petal-fall). It is evident that there is danger of this type of injury in the event that hot, clear weather occurs during or soon after a lime-sulphur application (Young and Walton, 1925). The substitution of Bordeaux mixture in treatments 3 or 4 of the lime-sulphur program has occasioned no significant difference in scab control. The fruit from such mixed programs, however, has ordinarily been less satisfactory in appearance than that from the full lime-sulphur program, and in certain instances considerable foliage injury has occurred. In 1922 a program in which there was a similar substitution of sulphur-dry lime-sulphur-arsenate dust gave as good results as did the full lime-sulphur program. The results from experiments designed to test the effect of the addition of lime to lime-sulphur in relation to fruit burning are inconclusive. Caution should be exercised in the use of lime-sulphur on apples in hot weather.

Effectiveness of spraying with rod and gun. The results of three years' comparative tests with spray rod and gun on trees of moderate size showed no significant differences.

The effectiveness of adding certain spreaders to sprays. The addition of gelatin and glue, respectively, to lime-sulphur appears to have decreased, rather than increased, its effectiveness in scab control. No significant difference in scab control appears to have attended the addition of casein-lime to lime-sulphur and Bordeaux mixture, respectively.

The effectiveness of certain dust programs. The dusting experiments were planned primarily with the aim of testing the effectiveness of the different dust schedules and materials under varied conditions.

The results of 1921 are not significant because of the scarcity of scab in that season. In 1922 and 1923, fair to good control of scab was obtained from a dusting program in which applications were made during major infection periods when the trees were wet. In these tests there was no significant difference in scab control by ground sulphur-arsenate, copper-lime-arsenate, sulphur-dry lime-sulphur-arsenate, copper carbonate (in 1923), and flowers of sulphur-arsenate (in 1923). On Lubsk's Queen in 1923, considerable russet followed the use of sulphur-dry lime-sulphur-arsenate and copper carbonate in this program. In 1924 this program gave less satisfactory control than in the previous years. These results suggest that in emergencies considerable benefit might be derived from dust applications made during the infection periods before the fungus became established.

In 1922 in the Goff orchard, a program closely paralleling that mentioned above, but applied on dry foliage under less favorable wind conditions and without immediate timeliness in relation to major infection periods, failed to control scab.

In 1923 and 1924, a dust program in which the applications were made on the same dates as the treatments in the full spray program gave essentially as satisfactory scab control as liquid lime-sulphur. Where applications of sulphur-arsenate dust in this program were made comparatively on wet and dry foliage, there was no significant difference in results. Experiments with sulphur and sulphur-arsenate dusts are being continued with an increased number of applications, particularly in the pre-blossom period.

OTHER STUDIES BEARING ON CONTROL MEASURES

The studies of epidemiology which have been reported in other connections have shown:

1. That, under Wisconsin conditions, and probably wherever twig infection does not occur to a significant extent, an abundant supply of ascospores is essential to the development of difficultly controllable epidemics of apple scab.

2. That the most critical time for the control of scab by the type of fungicidal program now in general use is in the period of rapid expansion of susceptible host parts early in the season.

3. That the fruit phase of the disease is more readily controlled by fungicides than the leaf phase.

4. That the fungicidal programs now in general use are adapted primarily for the control of scab on the fruit, and may permit sufficient foliage infection to lead to the development of a serious ascosporic inoculum for the following year.

These facts make it increasingly apparent that great promise for the future improvement of scab control lies in preventing the development and timely discharge of an abundant supply of ascospores. On general grounds, measures directed toward this end have been recommended ever since the establishment of the genetic relations of the Venturia and Fusicladium stages of the scab fungus. Even before that time, destruction of fallen leaves was recommended. Yet, scab epidemics continue to take their toll, and comparatively little progress seems to have been made towards checking them in their inception by other means that the direct protection of susceptible host parts by fungicides. Special consideration is therefore being given to methods for limiting the production and timely discharge of ascospores. The results of this work will appear in a later paper.

SUMMARY AND CONCLUSIONS

In trials made in Wisconsin in 1916 and 1917, the programs then in most common use for apple scab control in the North-Central and Northeastern United States, failed to give satisfactory results. Consequently, work was initiated along two general lines: (1) field studies of the disease and its control in relation to the natural environment, and (2) laboratory and greenhouse studies of the development and prevention of the disease under conditions in which certain factors of the environment were controlled.

For the years 1919 to 1924, inclusive, records of the seasonal development of the host, parasite, and disease are presented, in correlation with meteorological data taken in the experimental orchards, and records of control experiments. In general agreement with many previous observations by other investigators, these data show that the moisture and temperature factors of the natural environment play a leading part in determining the severity of occurrence of the disease and the difficulty of its control. These records are further discussed in connection with the topics to which they are most pertinent. They have been especially useful in defining critical periods in epidemiology and control and in contributing to a more effective orientation of control programs to these critical periods.

Studies of the viability and longevity of ascospores and conidia of *V. inaequalis* and of relations of moisture, temperature, and light to their germination are reported.

Infection experiments were conducted under conditions in which certain factors of the environment were controlled.

Ascospores and conidia which were allowed to lie in drops of water in contact with hard surfaces rapidly acquired a high degree of adherence to the substratum. Germ tubes which developed in close contact with a firm substratum were firmly adherent throughout their entire length. Direct penetration of the cuticle by infection hyphae from well differentiated appressoria, as described by Aderhold and others, was very commonly observed. Not infrequently, however, comparatively short, thick germ tubes appeared to function as appressoria, without the development of clearly differentiated hold-fasts. Infection hyphae frequently developed directly from ascospores, which functioned as appressoria without the formation of clearly differentiated germ tubes. Relations of this type of penetration to the rapidity of infection and to the action fo fungicides are discussed.

Infection occurred at temperatures ranging from 6° to 26° C., with evidence that the temperature most favorable for rapid development of the disease is near 20° C. Germination of ascospores and conidia and growth of the fungus were observed to occur from ½° to 32° C. The optimal temperature for spore germination and growth of the fungus in water or in certain culture media agreed closely with that for development of the disease. The temperature range for development of host plant appears to be somewhat higher than that of the parasite or the disease.

The results thus far available suggest that the minimal periods of continuous wetting necessary for infection of apple leaves by ascospores of *V. inaequalis* fall within the following limits for the temperatures listed: 6°, 13 to 18 hours; 9°, 9 to 11 hours; 15°, not more than 8.5 hours; 20°, 4 to 6 hours; 24°, 4 to 6 hours; and 26° C., 8 to 10 hours.

Abundant infection occurred following suitable periods of intermittent wetting.

Variations of the relative humidity from 50 to 90 per cent at 20° C. during the period of incubation exerted no perceptible influence upon the development of the disease.

No significant difference in disease development was observed when the plants were incubated comparatively out of doors and in the greenhouse. The disease developed on plants which were held for 7 days in a dark cellar during the incubation period. Under these conditions the development of resistance on the upper surfaces of the leaves appeared to be retarded.

The reports of other investigators that apple leaves and fruits are more susceptible to scab in their earlier stages of development were confirmed. It was observed that different parts of leaves acquire resistance at different rates and in different degrees. These facts are of possible significance in relation to the nature of this resistance.

In a limited number of trials at controlled temperatures, lime-sulphur and sulphur dust, respectively, each in the concentration commonly used in practice, controlled the disease perfectly on the leaves of potted apple plants at 6° C., the lowest temperature tried. Under the conditions of these experiments, the sulphur fungicides controlled the disease much more effectively than did Bordeaux mixture. The relative inefficiency of the latter fungicide is tentatively attributed in part to the penetration of infection hyphae directly from ascospores, as described herein, without the development of germ tubes, thereby greatly reducing the chances of the parasite to aid in dissolving its lethal dose of copper from the Bordeaux residues by exosmosis of solvent substances from thin-walled hyphae in contact or intimate association with these residues.

The results of the field and laboratory studies are discussed in relation to the development of epidemics.

Under Wisconsin conditions, the apple scab fungus was found to overwinter to a significant extent only through the formation of the perithecial stage in dead leaves. Mature ascospores were ordinarily observed by the time susceptible host tissue was exposed in the unfolding "fruit" buds. The periods during which ascospore discharges occurred in each season varied from about five to nine weeks. Discharges occurred only when the leaves bearing the ascocarps were wet. Severe drought in early spring delayed the discharge and reduced the quantity of ascospores produced. The frequencies of ascospores in orchard air

throughout the season of their discharge in 1924 were determined by means of a specially devised filtration apparatus. The maximal concentration, 289 ascospores per cubic foot of air, was recorded during a five-hour period on May 13, when the "fruit" buds were in the green tip stage. Factors affecting the production and discharge of ascospores are discussed.

In the six seasons studied primary infection commonly occurred during the first sufficient rain period following the exposure of susceptible host tissue in the unfolding "fruit" buds. The apical portions of sepals and of leaves were observed to be the first susceptible host parts exposed, and sepals the most exposed parts of the "blossom" buds during the early stages of their expansion. Early sepal infection places the fungus in a peculiarly favorable position for local infection of the adjacent surface of the blossom or young fruit by water-borne conidia. The special significance of sepal infection in relation to epidemiology and control is discussed.

The production of conidia ordinarily begins before the scab lesion becomes macroscopically visible, and may continue under favorable conditions until the end of the season. Field observations showed that an abundant source of secondary inoculum was never lacking on unsprayed trees after infection was once well established. In confirmation of the work of Frey and Keitt it is shown that conidia are very resistant to detachment from the conidiophores when dry, but quickly become detached in water. Their chief mode of dissemination in nature is in meteoric water moving under the influence of wind and gravitation. The occurrence of secondary infection is discussed.

Under Wisconsin conditions the most critical period for the development of apple scab epidemics extends from the time the apical parts of the sepals are first exposed in the unfolding "fruit" buds until an indefinite time some two to four weeks after petal-fall. The most critical part of this period is that prior to the open cluster stage of bud development. Other important infection periods may develop at any time during the summer. A second critical period occurs in the fall, when cooler weather and sufficient moisture may permit serious late infection of fruit and abundant establishment of the fungus on the foliage. If primary infection is adequately controlled, however, the disease is easily held in check later in the season.

Control experiments conducted during the years 1919-1924, inclusive, are reported.

Bordeaux mixture, liquid lime-sulphur, dry lime-sulphur, and various mixed programs of Bordeaux and liquid lime-sulphur were tested comparatively. Arsenate of lead was added to each spray mixture. Bordeaux or liquid lime-sulphur, in appropriately timed programs, ordinarily controlled the disease satisfactorily. Bordeaux mixture, however, was unsatisfactory commercially because of injury to fruit and foliage. Liquid lime-sulphur, 1-40, appeared to be the most satisfactory spray tried. Dry lime-sulphur, 3-50 and 4-50, in the full program of

treatments, ordinarily controlled the disease in about the same degree as the liquid lime-sulphur, 1-40. The results of certain experiments, however, suggest that the dry product at these concentrations has a somewhat less margin of safety than liquid lime-sulphur, 1-40. Mixed programs of Bordeaux and lime-sulphur showed no significant difference from the lime-sulphur program in controlling the disease, but were less satisfactory than lime-sulphur from the standpoint of appearance of fruit and injury to foliage.

The time and number of applications of spray should be arranged to meet the requirements of individual situations. The necessity of providing adequate protection of at least the blossom parts during the critical pre-blossom period is discussed. For use under severe epidemic conditions in Wisconsin, the following program of lime-sulphur applications is tentatively recommended, subject to modification in relation to seasonal or local conditions: (a) in the green tip stage; (b) in the early closed cluster stage; (c) just before the blossoms open, preferably in the open cluster stage; (d) after about three-fourths of the petals are off and before the calyx lobes close; (e) about ten days later; and (f) in the summer at a time chosen for efficiency in codling moth control. Modifications of this program to meet varied conditions are discussed.

During the six years of experimentation, "burning" of fruit which had been sprayed with lime-sulphur occurred in a significant amount only in 1921, and then was of minor importance on plots sprayed according to program. Considerable injury occurred, however, in cases where the treatment scheduled for ten days after petal-fall was delayed a week or two. It is evident that there is danger of this type of injury in the event that hot, clear weather occurs during or soon after an application of lime-sulphur.

The results of three years' comparative tests with spray rod and gun on trees of moderate size showed no significant differences.

No significant increase in effectiveness of Bordeaux mixture or lime-sulphur attended the addition of glue, gelatin, or casein-lime.

Dusting experiments were conducted with the aim of testing the efficiency of several materials under varied conditions of application. In 1922 and 1923 all the materials used gave fair to good control of scab when the applications were made during major infection periods when the trees were wet. In 1924 this timing of applications gave less favorable results. These results suggest that in emergencies considerable benefit may be derived from dust applications during infection periods before the fungus becomes well established in the host. In 1923 and 1924, sulphur-arsenate dust applied on the same dates as the treatments in the spray program gave essentially as satisfactory results as liquid lime-sulphur. The data on dusting reported in this paper are not considered conclusive. Experiments with sulphur and sulphur-arsenate dusts are being continued.

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The Classification of Plant Viruses

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Contents

The Classification of Plant Viruses[1]

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THE CAUSAL AGENCY of mosaic and related plant diseases, though unknown, is generally regarded as a filterable virus, thereby suggesting an invisible and infectious entity. The results of certain investigators indicate that only one virus is concerned with the disease on a number of widely different hosts, while the results of others suggest that a virus is plastic, the symptoms, host range, and properties being modified by existing conditions. On the other hand, the work of several investigators has shown that a single host may be affected by a number of different virus diseases and that a number of plant species are affected with apparently specific viruses. The evidence to date on the specificity of the viruses affecting a single host is based largely on symptoms and on differential hosts. In the case of the viruses affecting tobacco this evidence can, in most cases, be supplemented by differential properties of the viruses as will be shown in this bulletin.

While it is admitted that a single virus may affect a wide variety of unrelated hosts, and that a virus may be changed in virulence or have its properties measurably influenced by conditions, the fact seems to remain that specific viruses exist in nature with approximately the same constancy as is, for instance, exhibited by the bacteria.

The failure to recognize the specificity of certain of these virus diseases has resulted in much confusion in the literature. It seems to be important, therefore, to describe adequately and to classify the virus diseases of plants as far as our knowledge of each group will permit.

A system of nomenclature for plant viruses is greatly needed. The present system of applying names on the basis of host attacked, or symptoms exhibited, is quite inadequate for present needs. The number of different viruses attacking a single host species limits at once the usefulness of host names. Nowhere in the realm of plant pathology are symptoms of less value in description than in the plant virus diseases, because of the remarkable influence of environmental factors (Pl. IA.), and the possible co-existence of two or more viruses in a single plant. Symptoms have, however, some valuable diagnostic features when properly interpreted in comparative studies (Pl. I, B, C.). We have found it very difficult to apply a different descriptive name to all the viruses occurring in one host, and this seems to be a good reason for the use of an arbitrary system of nomenclature. In the development of such a system the established

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[1]Cooperative experiments with the office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture.

term already applied to any virus disease should, however, be left for what it is; i. e., a name for the disease, but not for the specific causal agency. An important objection may be raised to the proposal to name the causal agents in the absence of any known or visible entity. It seems most likely, however, that no visible causal agency will be found to be associated with these diseases and that the workers with viruses must be content to follow the example of other branches of science where names have long been applied to definite but unseen entities.

Material and Methods

This bulletin will deal only with certain viruses of which tobacco and other solanaceous plants are hosts. The first of these viruses is the causal agent of ordinary tobacco mosaic. The writer has previously described two viruses affecting tobacco which were secured from apparently healthy potatoes, namely those of spot-necrosis and ring-spot (4). Recently (5) we have shown by means of differential hosts that four other virus diseases affect tobacco, namely cucumber mosaic, "speckled" tobacco mosaic, "mild" tobacco mosaic, and petunia mosaic. This bulletin will add to the list four more diseases which will be referred to as yellow tobacco mosaic, medium tobacco mosaic, bleaching tobacco mosaic, and tomato stem-necrosis, although it must be recognized that these terms are descriptive of the diseases in question only under special conditions. Petunia mosaic, previously described (5) was not available for property studies.

The separation and classification of the viruses described is based on the symptoms, if any, produced on ten or more different species of hosts, their longevity *in vitro*, thermal death-points, lethal effect of chemicals, and such minor differences as relative infectivity and length of incubation period.

The inoculations to tobacco and other hosts have been made by introducing the extracted juice from mosaic plants into healthy plants by punctures and scratches with a needle, wrapped near the point with a small quantity of absorbent cotton to carry the extract more readily. Five plants were used in each series of inoculations. These plants were grown in four-inch pots, in very fertile soil under warm greenhouse conditions (27-32°C.) The inoculations were made on the plants when very young. The inoculation of old plants or young stunted plants will usually lead to symptoms with some viruses but not with others, so that such results would be of questionable value.

The longevity *in vitro* was determined by merely aging the extracted viruses from the tobacco plants in stoppered test tubes without preservatives for the desired length of time. The thermal death-points were determined by immersing five cubic centimeters of the newly extracted virus from tobacco plants in thin-walled test-tubes into an automatically regulated and strongly agitated water bath. The time of exposure was ten minutes in all cases, including the time between actual immersion and removal from the bath. Thermometer readings showed that the temperature of the

extract jumped rapidly to within five degrees of the desired temperature but that it usually required two to three minutes from the time of immersion before it reached the desired temperature. Determinations were usually made only at five degree intervals between 55°C and 95°C. Certain circumstances will vary the thermal death-points appreciably and they cannot ordinarily be regarded as significant for classification purposes unless differing by more than 5°C, even when the material is carefully chosen.

The effect of chemicals on the virus was determined by exposing the virus, diluted only one-half, to certain concentrations of the chemicals used for a definite length of time, usually one hour and one day. This promising field for the separation of viruses has been barely opened up in our investigations, and only the tests with alcohol and nitric acid for this purpose will be reported. As a result of Allard's work (1) and our own with other chemicals we are convinced that the lethal action of various chemicals on the different viruses will form a valuable means of classification and separation of viruses.

The incubation period for any one virus naturally varies greatly, depending upon the the condition of the host and other environmental factors. The first symptoms of tobacco mosaic may be evident under exceptionally favorable conditions three days after inoculation, whereas, under unfavorable conditions, ten or more days may elapse before symptoms develop. In our experiments symptoms were usually pronounced after five or six days. When the different viruses are compared under similar conditions on the same host, symptoms characteristically follow in a fairly definite chronological order; for example, cucumber mosaic usually requires considerably longer to develop symptoms on tobacco than does tobacco mosaic on tobacco.

The percentage of infection secured with the different viruses varies considerably, particularly on some hosts. With some viruses, one hundred per cent infection is the rule, whereas, with other viruses, only forty or sixty per cent infection is characteristic. The conditions influencing this relation are as yet inadequately understood. We are convinced, however, that at least two factors are concerned, namely, the relative susceptibility of the host, and the source of the inoculum. *N. tabacum* is, for example, much more susceptible to cucumber mosaic than is *N. glauca*, when the same inoculum is used on both species. On the other hand, cucumber mosaic from tobacco is a very good source of inoculum, whereas, cucumber mosaic from pokeweed, potatoes, and certain other plants, is a very poor source of inoculum. Comparing good and poor sources of inoculum in one group of experiments with cucumber mosaic, the records show 94 per cent infection secured in the former case as compared with only 7 per cent in the latter case. Results of this nature led us to doubt at one time that we were securing systemic infection with a virus disease even though marked symptoms occurred, since the virus could not be recovered from the inoculated hosts (5). We have since come to the conclusion that when symptoms are definitely secured, the species should be regarded as a host,

even though the virus cannot be again secured therefrom. Good examples of this sort are tobacco mosaic on *N. glutinosa* and several of the viruses occurring on tobacco when transmitted to the potato. It is evident that while a host may be poor source of inoculum for one virus disease, it may be a good source for another. In cross inoculation work one should, therefore, make certain that a suitable source of inoculum is being used in cases of negative results.

Experimental Results

Seven of the viruses with which we have been concerned have been previously described as to their source and behavior on differential hosts in particular (4, 5). Since these publications, four other viruses have been collected and studied along with the others in comparative trials. Two of these four viruses (yellow tobacco mosaic and medium tobacco mosaic) were secured from tobacco fields on farms near Madison, the source of the others (bleaching mosaic and tomato stem necrosis), is not definitely known, except that they arose during efforts in the experimental work to segregate the various viruses with which we were working. These viruses are described in comparison with the others in the tables and in a later chapter. It cannot be argued that some of these viruses are not closely related, and in view of recent results in which we have secured decided attenuation of some of these viruses by exposure to heat (6), it is not inconceivable that they are modified by other conditions as well. Such a behavior is characteristic of many organisms and until more is known about changes in virulence, mutations, etc., this phase of the subject will remain obscure. This does not, however, obviate the needs of a system of classification, but rather adds to the importance of it. For present purposes, it seems advisable to give all forms of viruses equal rank in classification, although the need for grouping them into related classes seems already evident.

The separation of the viruses with which we have worked is based upon the results secured in the inoculation of several thousand plants. The data in its entirety would to too voluminous and confusing to present in detail. It is, therefore, only summarized in Table I. with relation to the differential hosts. The larger number of inoculations to tobacco is significant only for the purpose of showing the number of times the viruses have been transferred without losing their identity. The results only are presented in the cases of aging *in vitro*, and thermal death-points (Table II.). In the case of the lethal influence of chemicals, the result of only one series of tests is given as an illustration (Table III.).

The symptoms given in Table I, as typical on the different hosts, are in a sense only relative. The natural variations and modifications due to environment and other circumstances are so great as to render them in many cases of little value, except in repeated comparative studies. Certain of the hosts, however, give remarkably specific symptoms for a specific virus, and these are the ones which should be selected for purpose of determina-

tions, and are consequently indicated in the description of the viruses as being particularly significant. The proper comparative host studies are sufficient in most cases to determine the viruses described in this paper, and in some cases prove the only available method. In other cases, determinations of the longevity of the virus *in vitro*, the thermal death-point, and the influence of chemicals may be necessary or will lend valuable additional information as to the certainty of the determination.

The hosts shown in Table 1 represent those most commonly used in the experiments. The results of the previous season's work (5) are added to those secured as a basis for the present report. *Solanum nigrum* and *Solanum melongena* are, however, dropped from the list of differential hosts since they are not well suited for this purpose and henbane and potato (Bliss Triumph) substituted. Symptoms with one or more of these viruses have been secured, however, upon a number of other hosts in the course of the work. Most of the solanaceous plants seem to be susceptible to one or more of the viruses described. Belladonna, *S. dulcamara* and Lycium seem to be the most "resistant" to virus diseases of all solanaceous plants tried in the experiments. We have secured symptoms on Belladonna with one virus, the classification of which is, however, uncertain. *Physalis alkekengi* and *Physalis franchetti* have given fairly good symptoms of tobacco mosaic in some cases and these species cannot be regarded as symptomless carriers (2, 7). *Solanum atropurpureum* has been infected with all the viruses except ring-spot, the latter inoculation not being tried. *Solanum lacinatum* gave symptoms with tobacco mosaic, cucumber mosaic, and spot necrosis. Tobacco mosaic has also given symptoms on *Solanum miniatum*, *S. rostratum*, *S. sisymbrifolium*, and *Nicandra physaloides*.

In addition to the names which we have applied to the specific virus diseases, we are tentatively applying a name to the specific causal agency of each disease, with the expectation that it may be more useful in future classifications than in the present one. The suggestion is simply to name a virus from the host on which it is first discovered, together with a number to indicate the specific virus in question. The well known tobacco mosaic virus under this scheme is to be technically known as *Tobacco virus 1*, and the other viruses described in this paper, originally noted on tobacco become simply *Tobacco virus 2, 3, 4, 5, 6, 7, 8*, and *9*.[1] The cucumber mosaic virus, though it occurs on tobacco, should remain *Cucumber virus 1*, since it was orginally found and described on cucumber. Question of priority in the naming of or numbering of viruses, will naturally arise, and it has been suggested that this should be regulated by some committee selected for this purpose. It is not believed, however, that simultaneous descriptions and questions of priority will greatly interfere with the practical application of such a system of nomenclature. Should this suggestion meet with any approval among the workers with plant viruses, the details of its application should be more adequately presented than can be done in this paper.

[1] In naming the virus, it may be preferable in some or all cases to use the Latin generic name or binomial in place of the common name of the host plant.

TABLE I. SUMMARIZED RESULTS OF INOCULATIONS OF VARIOUS VIRUS DISEASES ON DIFFERENTIAL HOSTS[1] WITH MOST DISTINCTIVE SYMPTOMS MANIFESTED[2]

DISEASE	VIRUS	HOSTS				
		Tobacco (Nicotiana)				Pepper
		N. tabacum	*N. glauca*	*N. glutinosa*	*N. rustica*	*C. annuum*
Tobacco mosaic	*Tobacco virus* 1	M,MF,S 435 429	s 25 24	S,N 25 ±17	m,N,mf,s 20 20	C,n,s 28 28
Cucumber mosaic	*Cucumber virus* 1	m,mf,c 265 147	mf,m 10 3	M,s 25 10	c,mf 15 7	mf,c,s 18 14
Speckled tobacco mosaic	*Tobacco virus* 2	m,s 200 187	m 10 10	20 0	mf,s,n 5 4	15 0
Mild tobacco mosaic	*Tobacco virus* 3	m 150 118	20 0	c,s,n 65 25	m,mf 35 19	m 15 6
Spot-necrosis	*Tobacco virus* 4	m,N 525 411	m 10 10	m,n 10 10	m,n 5 5	m,n 15 12
Ring-spot	*Tobacco virus* 5	m,N 85 70	5 0	n 5 5		n 8 5
Yellow tobacco mosaic	*Tobacco virus* 6	M,s,c 105 103	m,C 20 13	s,N 5 ±5	n 10 10	c,m,s 10 8
Medium tobacco mosaic	*Tobacco virus* 7	M,s 120 114	m 10 3	s,n 5 2	n 10 10	c,m,s 15 15
Bleaching mosaic	*Tobacco virus* 8	m,c,s 166 157	m,c 15 3	M,mf 20 7	mf,s,n 5 4	10 8?
Tomato stem necrosis	*Tobacco virus* 9	m 525 411	30 0	N 55 ±28	n 10 9	m 10 14
Petunia mosaic	*Petunia virus* 1	m,s 65 40	m 10 10	m,mf 20 6	mf,s,n 10 9	10 0

TABLE I. (Continued)

DISEASE	VIRUS	HOSTS					
		Tomato *S. esculentum*	Physalis *P. pubescens*	Petunia *P. violacea*	Henbane *H. niger*	Potato (Triumph) *S. tuberosum*	Pokeweed *P. decandra*
Tobacco mosaic	*Tobacco virus* 1	m,s 30 30	m,s 25 25	m,s 20 20	M 10 10	N 10 ±10	10 0
Cucumber mosaic	*Cucumber virus* 1	mf,s 28 13	m,mf 20 8	m,s,n 26 17	m 10 5	N,c,m 10 ±7	M 35 25
Speckled tobacco mosaic	*Tobacco virus* 2	m 25 16	m 30 7	mf,s,n 20 20	c,mf 10 10	c,n 5 ±5	10 0
Mild tobacco mosaic	*Tobacco virus* 3	m 70 33	s,c 30 17	m 15 2	c,n 5 2	m,n 10 ±6	15 0
Spot-necrosis	*Tobacco virus* 4	m,n 5 5	m,n 15 11	m,n 5 2	m,n 10 8	m,n 330 265	5 0
Ring-spot	*Tobacco virus* 5	n 10 10	n 10 8	n 5 1?	n 10 7	25 0	5 0
Yellow tobacco mosaic	*Tobacco virus* 6	m 35 35	m,s 15 15	m,c 15 15	M,c 5 5	N,c 15 ±15	5 0
Medium tobacco mosaic	*Tobacco virus* 7	m 15 11	m,s 15 15	m 5 4	M 5 5	N,c 10 ±10	5 0
Bleaching mosaic	*Tobacco virus* 8	m 25 18	N,m,c 20 12	m 10 7	c,s 10 9	m,n 15 ±13	M,n 5 5
Tomato stem necrosis	*Tobacco virus* 9	N 90 82	m 40 29	m 25 18	m 10 10	m,n 20 ±20	10 0
Petunia mosaic	*Petunia virus* 1	m 10 4	m,mf 20 4	m 15 7			m,n 10 2

[1]Upper figure is number of plants inoculated; lower figure number of plants infected.
[2]M—Mottling; MF—Malformation; S—Stunting; C—Chlorosis; N—Necrosis.

Small type indicates mild forms of symptom indicated.
±Virus not readily if at all obtainable from infected plants.

TABLE II.—RESISTANCE TO AGING, *IN VITRO*, AND THERMAL DEATH-POINTS OF VARIOUS VIRUSES ON TOBACCO

Virus disease	Resistance to aging *in vitro*	Thermal death-point ° C 10 min.
Tobacco mosaic	2 years +	90
Cucumber mosaic	3 days −	60
Speckled mosaic	3 months +	90
Mild mosaic	6 days −	60
Spot necrosis	14 days −	70
Ring spot	14 days −	70
Yellow tobacco mosaic	3 months +	90
Medium tobacco mosaic	3 months +	90
Bleaching mosaic	3 days −	75
Tomato stem necrosis	3 months +	90

TABLE III.—RESISTANCE OF VIRUSES TO THE LETHAL ACTION OF ALCOHOL AND NITRIC ACID. (FIGURES INDICATE NUMBER OF PLANTS INFECTED OUT OF FIVE INOCULATED.)

Virus disease	Alcohol, 50 per cent		Nitric acid, 1 to 200	
	Treated		Treated	
	1 hour	1 day	1 hour	1 day
Tobacco mosaic	5	5	5	5
Cucumber mosaic	0	0	0	0
Speckled tobacco mosaic	5	5	0	0
Mild mosaic	0	0	0	0
Spot necrosis	0	0	0	0
Ring spot	0	0	0	0
Yellow tobacco mosaic	5	5	5	5
Medium tobacco mosaic	5	5	5	5
Bleaching mosaic	5	5	1	0
Tomato stem necrosis	5	3	5	0

Description of the Viruses

The following abbreviated descriptions of the viruses with which this bulletin is concerned are presented below, with the hope that they may serve as a tentative basis for classification. It is to be expected that future investigations will necessitate additional details and modifications of the present descriptions. Other valuable diagnostic features are already known to exist, such as the variations in the cytological details of infected tissues, the details of which are now being worked out by Miss Isme A. Hoggan in this laboratory.

TOBACCO MOSAIC (*Tobacco virus 1.*) Pl. I, A.

TYPE. Allard, U. S. D. A. Bul. 20, 1914.

HOST FAMILY. Solanaceae.

DIFFERENTIAL HOSTS.

On tobacco, marked mottling, malformation and stunting.

On *N. glutinosa*, stem and leaf necrosis and stunting, no mottling.

On tomato, mottling and stunting, no stem necrosis.

On pokeweed, no symptoms.

RESISTANCE TO AGING *in vitro*. Several years.

THERMAL DEATH-POINT. 90°C. 10 minutes.

RESISTANCE TO CHEMICALS. High (60% alcohol or 1 to 200 HNO_3[1] does not kill in one day).

CUCUMBER MOSAIC (*Cucumber virus 1.*) Pl. III, A and Pl. VII, B.

TYPE. Doolittle, U. S. D. A. Bul. 879, 1920.

HOST FAMILIES. Cucurbitaceae, Solanaceae, and others.

DIFFERENTIAL HOSTS.

On cucumber, chlorosis, mottling, stunting, malformation, necrosis.

On *N. glutinosa*, mottling, malformation, stunting.

On pokeweed, mottling, stunting.

On tobacco, chlorosis, generally no malformation.

RESISTANCE TO AGING *in vitro*. 3 days or less.

THERMAL DEATH-POINT. 60-70°C. 10 minutes.

RESISTANCE TO CHEMICALS. Low, 50% alcohol or 1 to 200 HNO_3, kills in one hour).

SPECKLED TOBACCO MOSAIC (*Tobacco virus 2.*)

TYPE. Johnson, Phytopath. 16: 141, 1926.

HOST FAMILY. Solanaceae.

DIFFERENTIAL HOSTS.

On tobacco, mottling or speckling.

On petunia, mottling, stunting, malformation and necrosis.

On henbane, chlorosis, stunting and malformation.

No symptoms on *N. glutinosa*, pepper or pokeweed.

RESISTANCE TO AGING *in vitro*. 3 or more months.

THERMAL DEATH-POINT. 90°C. 10 minutes.

[1]One part nitric acid C. P. to two hundred parts water.

RESISTANCE TO CHEMICALS. Medium (withstands 50% alcohol 1 day, but is killed by 1 to 200 HNO_3 in 1 hour).

MILD TOBACCO MOSAIC (*Tobacco virus 3.*)

TYPE. Johnson, Phytopath: 16: 141, 1926.

HOST FAMILY. Solanaceae.

DIFFERENTIAL HOSTS.

On *Physalis pubescens.* Marked stunting and chlorosis.

On *N. glutinosa,* mild chlorosis and stunting.

On *N. rustica,* mild mottling and malformation.

No symptoms on *N. glauca* or pokeweed.

RESISTANCE TO AGING *in vitro.* About 6 days.

THERMAL DEATH-POINT. 60°C. 10 minutes.

RESISTANCE TO CHEMICALS. Low (50% alcohol or 1 to 200 HNO_3 kills in one hour).

SPOT-NECROSIS OF TOBACCO (*Tobacco virus 4.*) Pl. I, C.

TYPE. Johnson. Wis. Agr. Exp. Sta. Research Bul. 63, 1925.

HOST FAMILY. Solanaceae.

DIFFERENTIAL HOSTS.

On tobacco, mild mottling and necrotic spots.

On potato, virulent form produces basal leaf necrosis and curling on top leaves.

RESISTANCE TO AGING *in vitro.* About 14 days.

THERMAL DEATH-POINT. 70°C. 10 minutes.

RESISTANCE TO CHEMICALS. Low (50% alcohol or 1 to 200 HNO_3 kills in one hour).

RING-SPOT OF TOBACCO (*Tobacco virus 5.*) Pl. I, B.

TYPE. Johnson, Wis. Agr. Exp. Sta. Research Bul. 63, 1925.

HOST FAMILY. Solanaceae.

DIFFERENTIAL HOSTS.

On tobacco, mottling and ring-like spots.

On henbane, necrotic leaf spots.

RESISTANCE TO AGING *in vitro.* About 14 days.

THERMAL DEATH-POINT. 70°C. 10 minutes.

RESISTANCE TO CHEMICALS, Low (50% alcohol or 1 to 200 HNO_3 kills in one hour).

YELLOW TOBACCO MOSAIC (*Tobacco virus 6.*) Pl. II.

TYPE. This publication.

HOST FAMILY. Solanaceae.

DIFFERENTIAL HOSTS.

On tobacco, mottling and angular yellow chlorotic areas.

On *N. glauca,* round white chlorotic areas.

On petunia, irregular white or yellow chlorotic areas.

The chlorotic areas apparently develop only under special conditions and are imperfectly understood, therefore negative results are not always reliable.

RESISTANCE TO AGING *in vitro*. Three or more months.
THERMAL DEATH-POINT. 90°C. 10 minutes.
RESISTANCE TO CHEMICALS. High (60% alcohol or 1 to 200 HNO_3 does not kill in one day).

MEDIUM TOBACCO MOSAIC (*Tobacco virus 7.*)
TYPE. This publication.
HOST FAMILY. Solanaceae.
DIFFERENTIAL HOSTS.
On tobacco, medium mottling and stunting.
On *N. glauca*, mild mottling.
On petunia, mild mottling.
Very similar to yellow tobacco mosaic except that yellow areas are not produced.
RESISTANCE TO AGING *in vitro*. Three months or more.
THERMAL DEATH-POINT. 90°C. 10 minutes.
RESISTANCE TO CHEMICALS. High (60% alcohol or 1 to 200 HNO_3 does not kill in one day).

BLEACHING MOSAIC (*Tobacco virus 8.*) Pl. III C and Pl. V. B, E.
TYPE. This publication.
HOST FAMILIES. Solanaceae, Phytolaccaceae.
DIFFERENTIAL HOSTS.
On tobacco, mottling and sometimes chlorosis on young plants.
On *N. glutinosa*, mottling and malformation.
On pokeweed, mottling and occasionally necrosis.
On *Physalis pubescens*, chlorosis, necrosis and mottling.
Very mild, if any symptoms on pepper.
RESISTANCE TO AGING *in vitro*. About three days.
THERMAL DEATH-POINT. 75°C. 10 minutes.
RESISTANCE TO CHEMICALS. Medium. (Withstands 50% alcohol but is destroyed by 1 to 200 HNO_3 in one day).

TOMATO STEM-NECROSIS (*Tobacco virus 9.*) Pl. IV.
TYPE. This publication.
HOST FAMILY. Solanaceae.
DIFFERENTIAL HOSTS.
On tobacco, mild mottling.
On young tomato stems, necrosis.
On *Physalis pubescens*, very mild if any symptoms.
No symptoms on *N. glauca* or pokeweed.
RESISTANCE TO AGING *in vitro*. Three or more months.
THERMAL DEATH-POINT. 90°C. 10 minutes.
RESISTANCE TO CHEMICALS. Medium. (Withstands 50% alcohol, but is destroyed by 1 to 200 HNO_3 in one day).

The Isolation of Plant Viruses

The co-existence of two or more viruses in a single plant is not an unusual occurrence. In the case of the viruses described in this bulletin it is frequently possible to separate such combinations, though it is not possible in several other cases with the methods at present available. It is believed, however, that the possibilities in this direction are especially promising along the line of the application of the lethal or inactivating action of chemicals.

The present methods of isolation are, of course, based on the host range and the properties of the various viruses. In order to test the possibility of the separation of certain viruses, combinations of known viruses were made in test-tubes or on plants and subsequently isolated in "pure culture" by such methods. As an illustration, the case of a combination of the ordinary tobacco mosaic virus and the cucumber mosaic virus may be cited. This separation was accomplished by inoculating the mixed viruses to pokeweed. Since pokeweed is susceptible to cucumber mosaic and not to ordinary tobacco mosaic, the cucumber virus was secured free from the tobacco virus. Another portion of the combination extract was aged for four days and then inoculated to tobacco. Since aging *in vitro* for three days or more destroys the cucumber mosaic virus, the inoculation to tobacco resulted in ordinary tobacco mosaic alone. The latter separation could be accomplished in other ways, as for instance, by the use of heat or chemicals destructive to the cucumber mosaic virus but not to the tobacco mosaic virus.

A combination of three viruses may in certain cases be separated. If, for instance, speckled tobacco mosaic, cucumber mosaic, and mild tobacco mosaic are combined, the isolation may be accomplished by heating a portion of the extract to about 75°C. for ten minutes to destroy the infectivity of the two latter viruses. Another portion of the extract may be aged for three days, destroying the cucumber virus, followed by inoculation to *N. glutinosa* plants, which will become infected with the mild mosaic virus but not the speckled tobacco mosaic virus. Finally, a third untreated portion on inoculation to pokeweed should yield cucumber mosaic alone.

In this manner it seems possible to separate combinations of even four or five viruses, but, of course, with greater difficulty and uncertainty of positive results. However, the separation of more than two viruses is rarely of importance in the present state of our knowledge of virus diseases. In the case of the virus diseases of the potato, the development of some such methods of isolation would be of the greatest value at present, but unfortunately several difficulties would present themselves according to our preliminary attempts in that direction. In the first place, the potato viruses are quite generally limited, so far as we know, to the potato as a host, and the absence of a wide host range naturally reduces the possibilities of isolation. Secondly, the potato mosaics, at least, are relatively short lived in extract outside the host, reducing in considerable measure the ease with which they can be handled and isolated on the basis

of their comparative properties. When the properties of the various potato viruses are known in more detail, however, it is quite likely that certain combinations may be separated as readily as "spot necrosis" can now be separated from rugose mosaic of the potato, since the former and not the latter is transmissible to tobacco according to our experience.

Discussion

The evidence for the existence of several specific viruses affecting tobacco has been practically limited to symptomatology on differential hosts in previous contributions on this subject. In this bulletin it has been shown that certain properties of these viruses offer in most cases a sufficient basis for their separation. In the present unsettled state of the plant virus problem there are those who evidently believe that all mosaic diseases of a certain host or group of hosts are caused by a single virus, or on the other hand, that a single virus is capable of such modification in its hosts and properties as to develop forms of a nature which are described as specific viruses in this paper. Walker (9), in a comparative study of the mosaic diseases of cucumber, tomato and Physalis, concludes "that the properties of the mosaic virus of a given plant may be decidedly changed by transferring it to another host. The properties of the viruses from mosaic plants of a certain species also appear to be the same, no matter what source of infection. This fact indicates that there may be a single causal agent for all the mosaic diseases studied here", (namely, cucumber, tomato, and Physalis mosaic). Our results are entirely contrary to this suggestion. We believe the results obtained by Walker could only be obtained by working with mixed viruses in certain experiments. At the time Walker's experiments were conducted, the difference between cucumber mosaic and tobacco mosaic on solanaceous hosts was not clearly recognized. In our experiments, we have carried cucumber mosaic serially through a dozen different plant species, over a period of two years, but as far as our observation goes, we have not changed its behavior or properties in any respect.

The question of virus specificity in another group is best illustrated by the potato degeneration diseases which have concerned the workers on this subject for many years. While much skepticism still exists among the workers themselves as to the possible synonomy of some of the diseases described, there is no doubt whatever of the occurrence of several different virus diseases of the potato in nature, and according to Schultz and Folsom (8), there are at least seven.

The potato virus diseases have been described entirely on the basis of symptoms, on one or another variety of potatoes. The host range of these potato viruses is apparently restricted to the potato, and it still remains to be determined whether or not the properties of these viruses can be used as a basis of separation. An interesting feature of the present studies is the artificial infection of the potato with all the viruses described in this paper, with the exception of ring-spot. It is not likely, however, that these viruses are responsible for any potato diseases in nature. The fact

that they are not readily if at all transmissible from the potato back to other solanaceous species, however, renders this point difficult to determine.

Fernow (3) has recently separated a group of viruses on the basis of differential hosts, but he apparently did not concern himself with their identity in relation to the known or unknown virus diseases.

The probability of a combination of two or more viruses being mistaken for one specific virus always exists. This possibility has been considered from various angles in the separation of the viruses described in this paper, and we believe this possibility has been largely eliminated. That certain of the viruses are so closely related, however, that separation may not be justified in some cases, is granted. In the cases of known attenuated viruses, we believe, of course, that they should retain a designation indicative of their origin. Medium tobacco mosaic may be regarded as an attenuated form of tobacco mosaic, but in the absence of proof of this fact, we believe it should be placed in a class by itself. Yellow tobacco mosaic resembles medium tobacco mosaic as far as virulence is concerned, but it possesses in addition a yellow character, which may or may not be dependent upon a separate virus. In the case of this disease, the classification is dependent entirely upon the yellow symptoms. The occurrence of decided yellow symptoms is very commonly associated with tobacco mosaic in the field, especially on the sucker growth following harvest of the crop. We are inclined to believe, however, that in this case, the yellow symptoms are usually color modifications, brought on by the existing environmental conditions, and that our yellow mosaic is quite different, and that its expression is determined by a special condition or conditions yet imperfectly understood.

The significance of the present investigations does not lie in the economic importance of the viruses described, since perhaps only the tobacco mosaic and cucumber mosaic may be said to be of much concern in this respect. Such importance as they may have lies rather in the demonstration that a number of different viruses exists in nature, and that they can be classified on the basis of the symptoms produced on various hosts and on the basis of their properties. Whether or not we wish to regard some of these viruses as closely related, and to be actually strains or forms of other viruses, is not particularly pertinent in the present stage of our knowledge of the subject. It is evident that workers with plant viruses who are concerned with studies on the nature and properties of viruses should be certain of the strain with which they are working, and that those who are reporting a virus on a new or old host should describe the virus sufficiently to indicate whether or not the causal agency has previously been reported.

The suggested classification of the group of viruses which may affect tobacco and related hosts is of course only a fraction of the entire problem. A great deal of investigation into the host range and properties of the virus diseases of other plant families is necessary before a satisfactory system of classification can be established.

In the meantime it may be of help to speak of a virus disease as due to a specific virus, and to designate this virus by the name of the host

on which it was first described together with an arbitrary number. Thus Physalis mosaic may be written Physalis mosaic (*Cucumber virus 1*), Physalis mosaic (*Tobacco virus 3*), or in other ways depending upon which, if any, of the previously described viruses is determined to be the one concerned. Such a system should eventually aid greatly in reducing the confusion in plant virus literature.

Summary

The existence of eleven different viruses on tobacco and related plants is shown on the basis of their behavior to various factors applicable as tests.

The most useful of these tests are the symptomatology on differential hosts, the longevity *in vitro*, the thermal death-points, and the lethal action of chemicals.

It is suggested that these factors form a basis for the description of a virus, and that some form of classification and nomenclature be established for plant viruses.

On the basis of the behavior of plant viruses to various conditions, it is possible in many instances to separate two or more viruses, where they co-exist in a single plant.

While it is possible to attenuate and to increase the virulence of some plant viruses, the experimental evidence indicates that they are relatively stable and specific entities.

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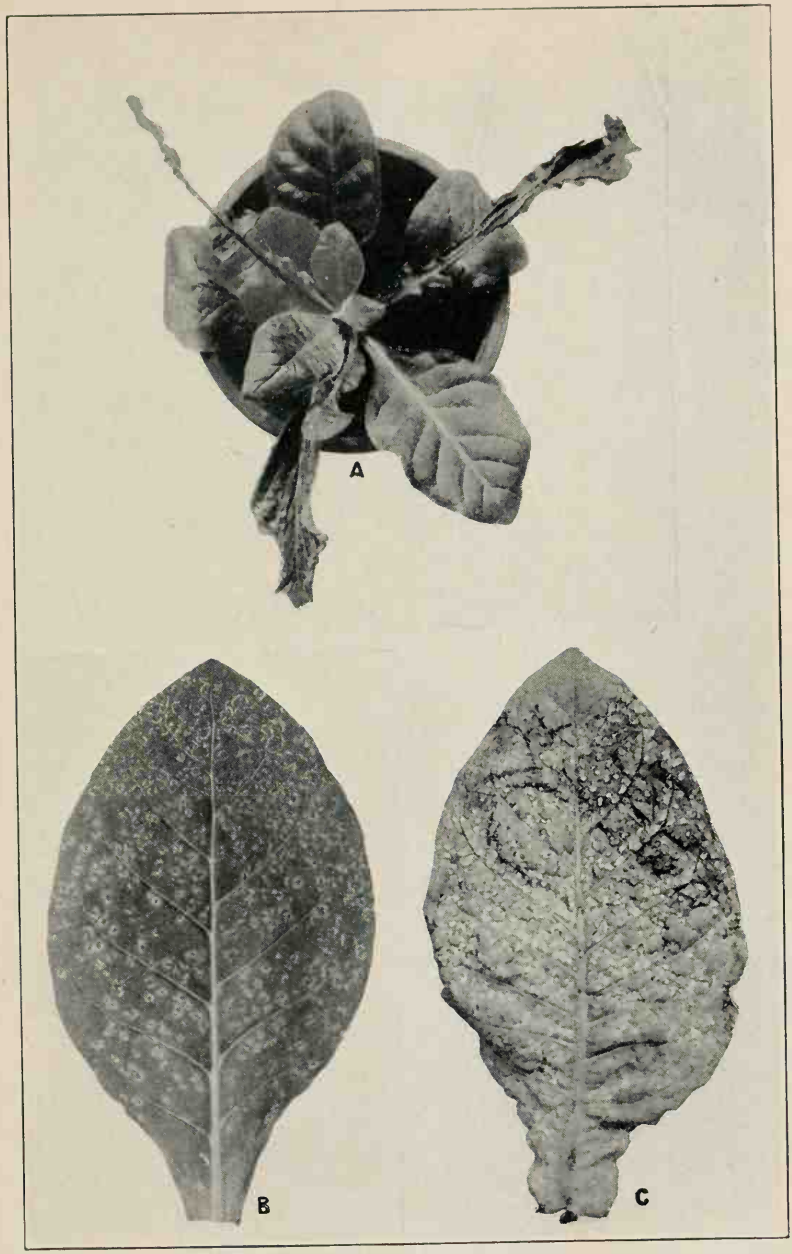


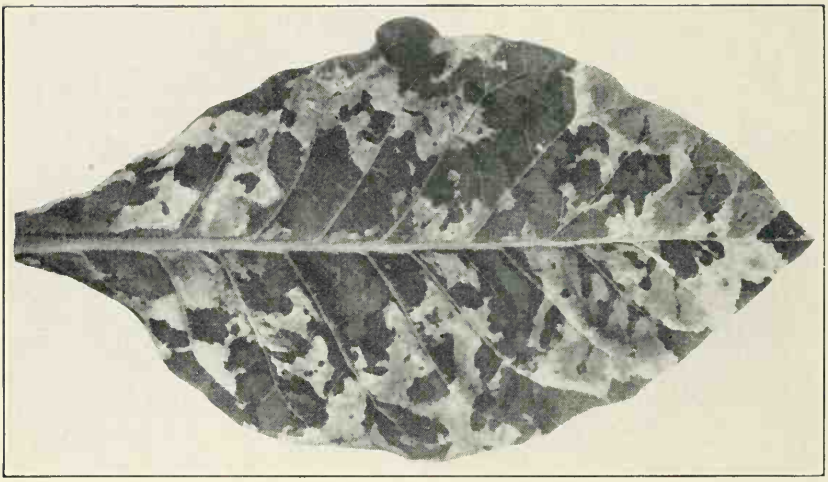
PLATE I.

Variability in Symptoms of Virus Diseases

A. The symptoms of any virus disease may vary remarkably as illustrated by the tendency of this tobacco plant to recover from the malformed condition of the leaves. Symptoms are, therefore, often of little diagnostic value in indicating the specific virus concerned.

B. In the case of certain viruses on certain hosts, the symptoms are however, quite specific as illustrated by the "ring-spot" disease on tobacco.

C. "Spot necrosis" symptoms are often quite specific, but the virus concerned may vary in virulence so that this introduces an additional complication.



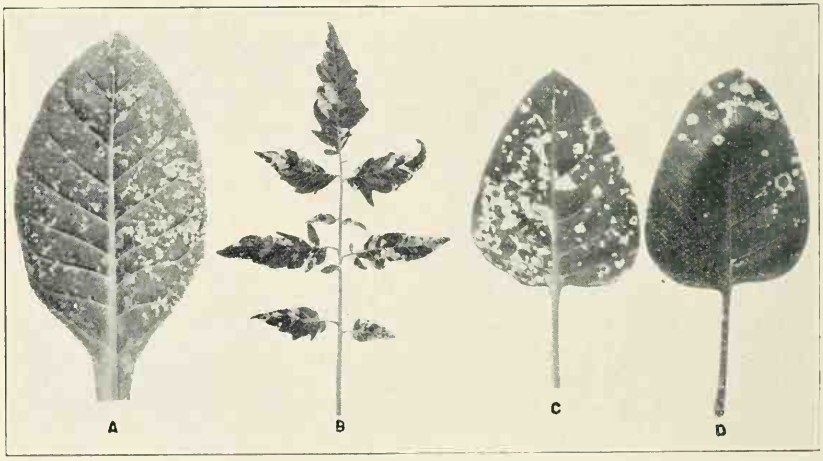


PLATE II.

Yellow Tobacco Mosaic

Upper—Yellow tobacco mosaic is characterized by the development of irregular yellow areas in the leaf in addition to a mottling characteristic of medium tobacco mosaic.

Lower—The yellow character of yellow tobacco mosaic may develop on different hosts as shown on tobacco (A), tomato (B), and *Nicotiana glauca* (C, D). These symptoms were, however, often masked in our trials.

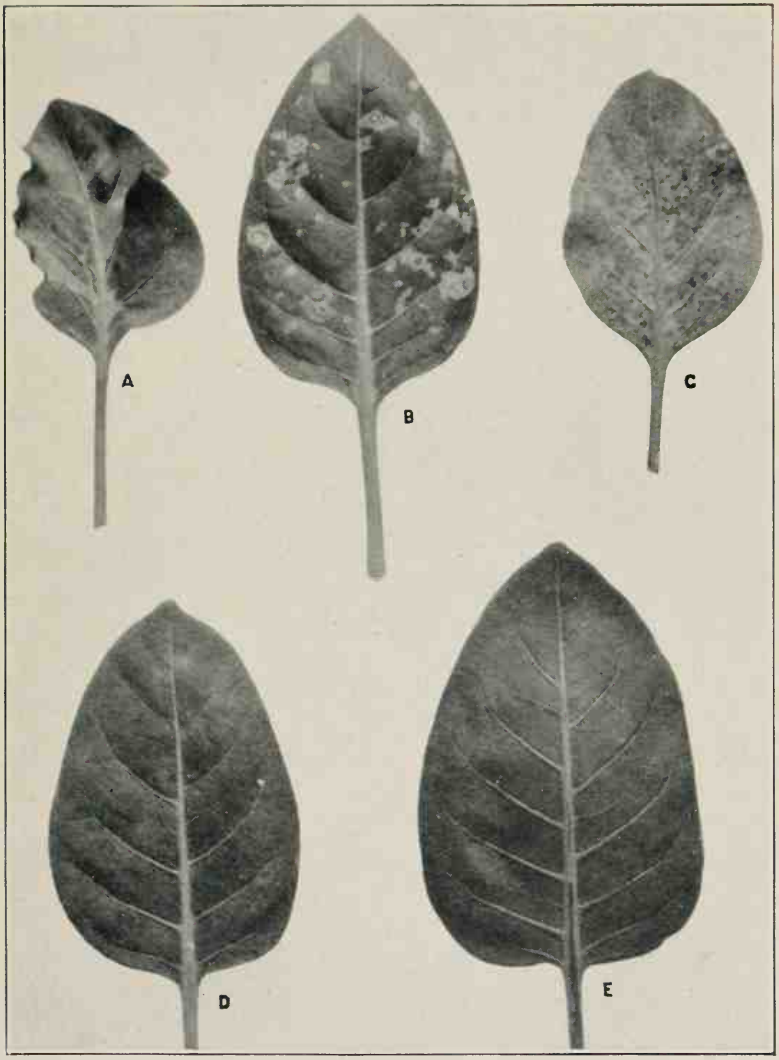


PLATE III.

Specific Viruses on N. Glauca

Nicotiana glauca usually gives a low percentage of infection, but when symptoms are obtained they are fairly distinctive. (A.) Cucumber mosaic. (B.) Yellow tobacco mosaic. (C.) Bleaching mosaic, (D.) Tobacco mosaic. (E.) Control.

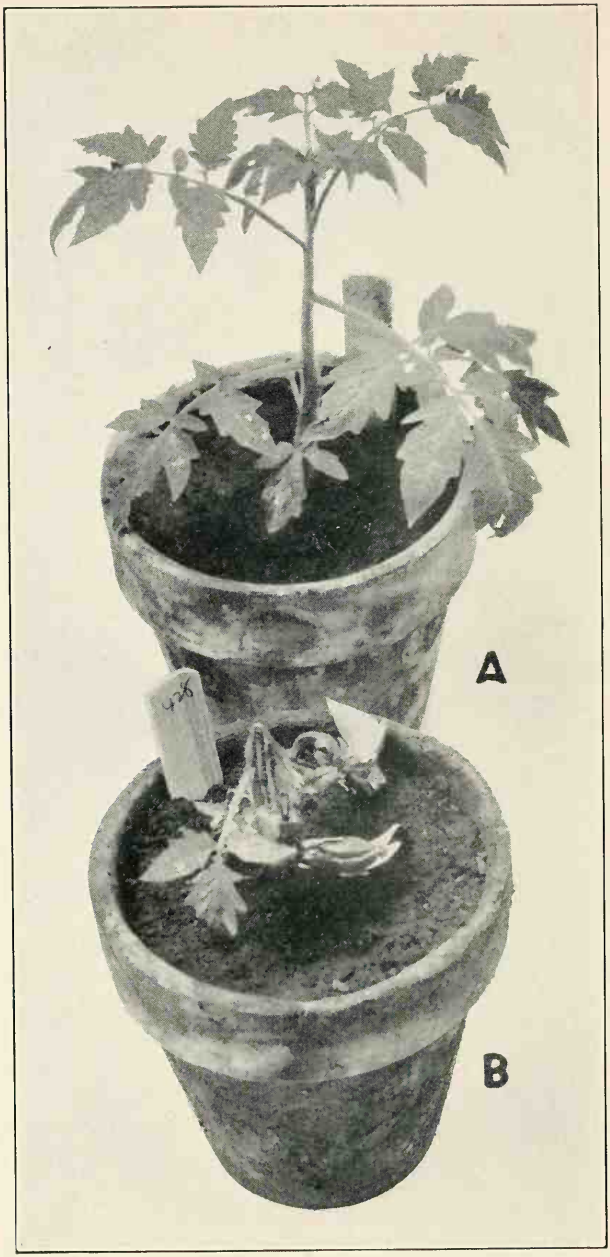


PLATE IV.

Tomato Stem Necrosis

When applied to the stems of young tomato plants the virus causing this disease characteristically causes necrosis and final collapse of the plant. (A) Control plant. (B) Inoculated with the virus of tomato stem necrosis.

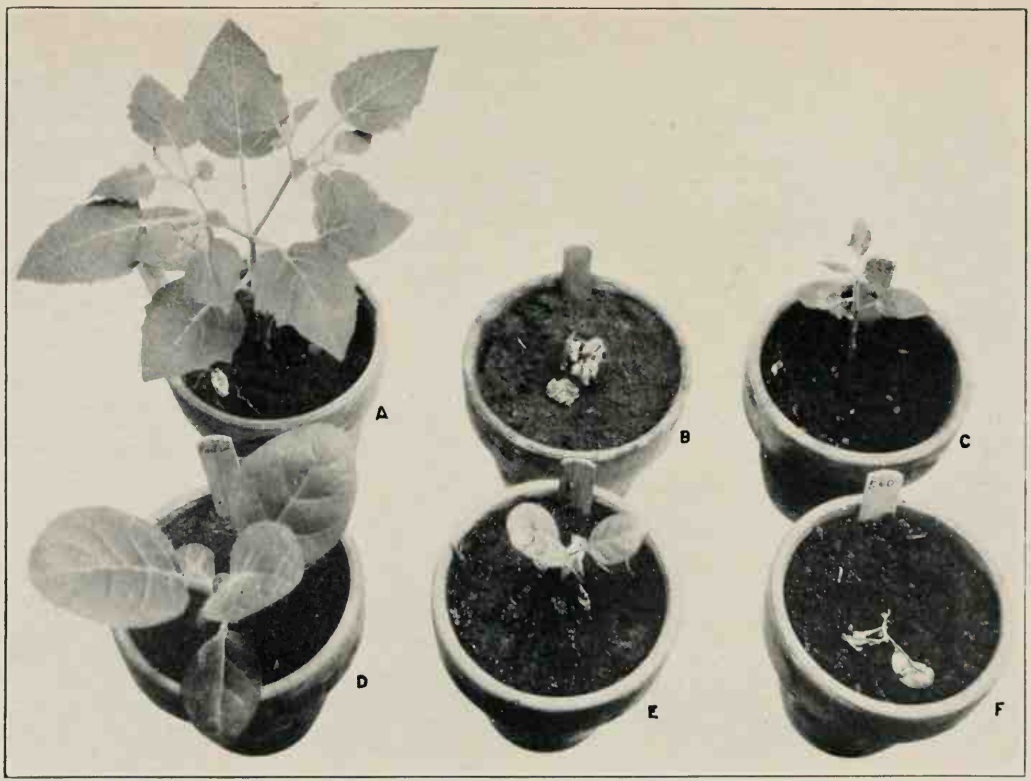


PLATE V.

A Comparative Inoculation With Two Viruses

The symptoms exhibited as a result of inoculating young plants are usually much more striking, and more useful for comparative studies than those exhibited by older plants. (A) *Physalis pubescens* control. (B) Physalis inoculated with the bleaching mosaic virus. (C) Physalis inoculated with the ordinary tobacco mosaic virus. (D) *Nicotiana rustica* control. (E) *N. rustica* inoculated with the bleaching mosaic virus. (F) *N. rustica* inoculated with the tobacco mosaic virus.

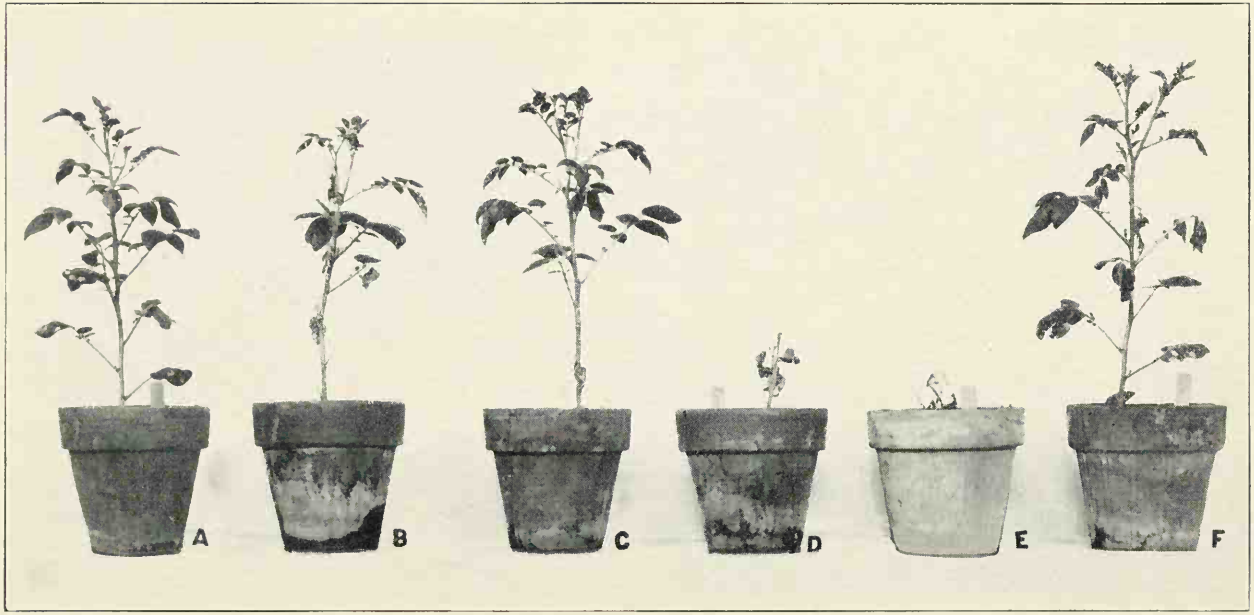


PLATE VI.

Tobacco Viruses on Potato

All the viruses deseribed in this paper give some symptoms when inoculated to potatoes. Bliss Triumph variety used in these inoculations. (A) Control plant. (B) Yellow tobaeco mosaic. (C) Medium tobacco mosaic. (D) Ordinary tobaeco mosaic (E) Bleaching mosaic. (F) Cucumber mosaic. The neerotic symptoms only are illustrated here. Mottling and in the case of cucumber mosaic, striking chlorosis are also characteristic.

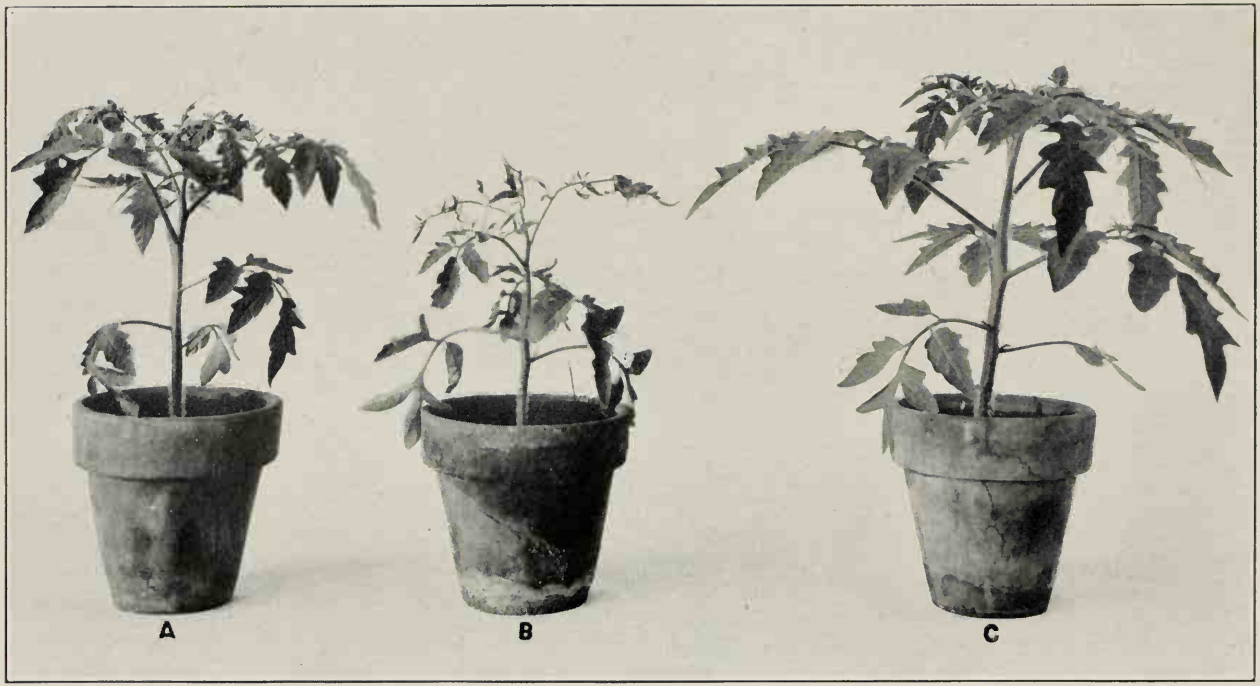


PLATE VII.

Cucumber Mosaic on Tomato

Cucumber mosaic sometimes exhibits very striking malformation symptoms on the tomato which are sometimes referred to as "fern leaf." (A) Inoculated with ordinary tobacco mosaic. (B) Inoculated with cucumber mosaic. (C) Control plant.

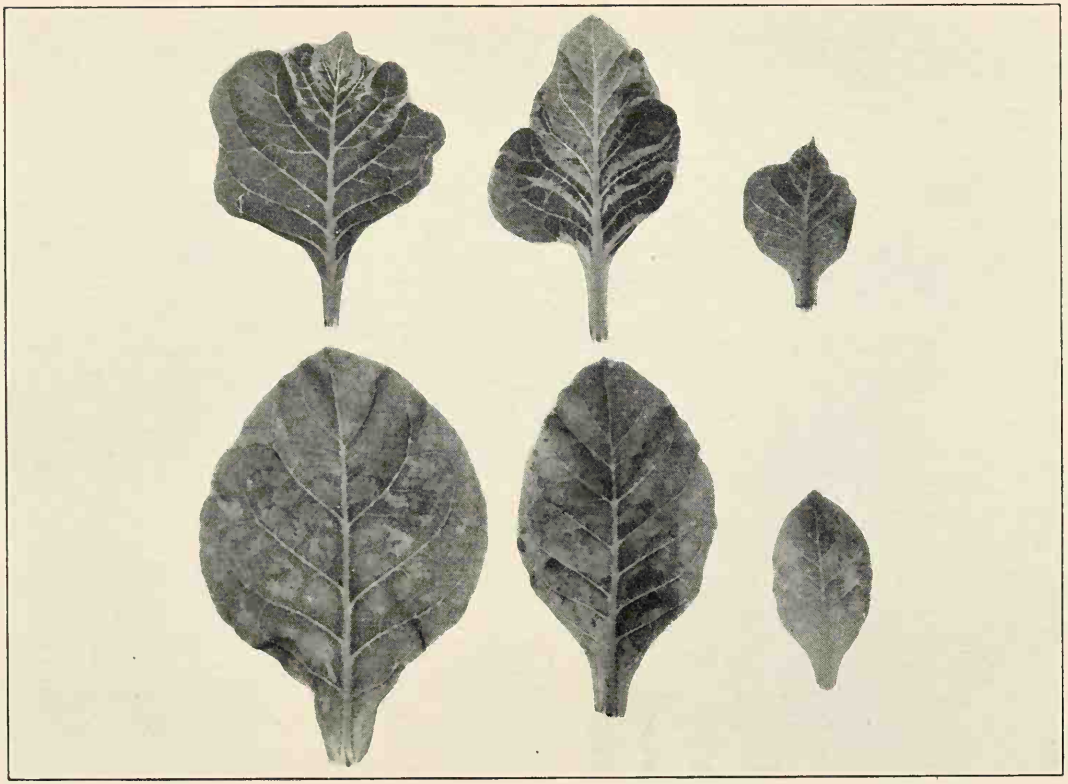


PLATE VIII.

Attenuated Tobacco Mosaic

At least some of the plant viruses can be attenuated by heat. In the upper row are three leaves from a plant with ordinary tobacco mosaic. In the lower row are three leaves from a plant inoculated at the same time with the virus of tobacco mosaic which had been previously developed at a temperature of 35° C. for ten days.

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Spraying Versus Dusting

To Control the Potato Leafhopper in Commercial Potato Fields of Wisconsin

John E. Dudley, Jr., and C. L. Fluke, Jr.
(Co-authorship)

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CONTENTS

Spraying Versus Dusting

To Control the Potato Leafhopper in Commercial Potato Fields of Wisconsin

JOHN E. DUDLEY, JR.,* AND C. L. FLUKE, JR.
(Co-authorship)[1]

THREE YEARS of experiments in Wisconsin on a small scale, from 1919 to 1921, showed that the potato leafhopper[2] can be controlled by liquid Bordeaux mixture (3, 4, and 5).[3] When the agitation for dusting began, commercial experiments were planned to determine the relative effectiveness of liquid Bordeaux mixture and copper-lime dust upon the leafhopper and hopperburn; upon the resulting yield and grade of potatoes; and to compare the cost of each operation when the work was actually done under farming conditions. These experiments extended over a period of four years, from 1922 to 1925.

It has already been shown by Chambers (2) in Wisconsin, Stewart and Parrott (7) in New York, and others, that either spraying or dusting will increase the yield of potatoes considerably above that of untreated acreages.

Most of the results indicate that spraying will increase yields to a greater extent than dusting (1, 6, 7). However, very little attention has been given heretofore to the ease and speed with which dusting can be done as compared with spraying.

Boyd (1) studied the efficiency of copper dusts and sprays in the control of diseases and certain insects, principally fleabeetles.

Folsom and Bonde (6) were experimenting largely with diseases, and they state that the leafhopper was not a factor.

Stewart and Parrott (7) in an experiment with diseases and insects in general, used a hand duster to apply the dusts and an orchard type power sprayer to apply the liquid Bordeaux mixture. They state in effect that the dust did not give satisfactory control in their experiment, while the spray showed high efficiency. They observe, however, that dust may be advisable under certain conditions, where water is difficult to obtain and in small fields where it is necessary to use hand machines.

Chambers (2) secured higher yields with copper-lime dust than with liquid Bordeaux mixture while carrying on demonstration experiments in potato fields in northern Wisconsin.

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[1]We wish to acknowledge the courtesy of J. G. Milward of the Department of Horticulture, University of Wisconsin, who arranged for the co-operative experiment at Spooner, Wisconsin.

Also to express our appreciation to E. M. Searls and T. E. Bronson of the Government force who gave much of their time to assisting with the experiments.

We wish to give special credit to C. J. Schrock, manager of William Culp Farm upon whose land the experiments at Waupaca were carried on for four years. Both Mr. Culp and Mr. Schrock showed unusual appreciation for research work and offered the utmost co-operation in carrying on experiments and taking yields.

[2]***Empoasca fabae*** Harris, order Hemiptera, family Cicadellidae.

[3]Reference is made by number (italic) to "Literature cited," p. 16.

It is not intended in this bulletin to review all the literature on the subject as that has been excellently handled by Folsom and Bonde (6).

A study of resulting yields, grades, and costs as here presented shows little or no difference between dusting and spraying.

Distribution and Importance of the Potato Leafhopper

The potato leafhopper has been known as a pest of potatoes for thirty or more years, with serious outbreaks occurring periodically. It is generally distributed in practically every state in the Union and in parts of Canada and Mexico. Its greatest damage to potatoes occurs in the North Central states, and regularly in Wisconsin.

FIG. 1—FIELD OF EARLY OHIO POTATOES PRACTICALLY DEAD FROM HOPPERBURN

Picture taken on July 17. Leafhoppers were extremely numerous. The field has not been sprayed with Bordeaux.

The economic loss to the potato grower caused by the attacks of the potato leafhopper and the accompanying hopperburn is probably greater than that caused by any other potato insect (Fig. 1). As the insect reproduces very rapidly under favorable weather conditions, it is essential that the grower be equipped and ready to cope with an outbreak before it threatens his crop.

The Experiments

Locations—The experiments were carried on at Spooner and Waupaca, Wisconsin in 1922. During the next three years, they were conducted only at Waupaca. Spooner is the location of a sub-experiment station in Washburn County in the northwestern part of the state. This county has a sandy

soil; a moderate rainfall, averaging 3.34 inches per month from April to September; and moderate temperatures, the normal being 64.3°F. from June to September—the growing period. Waupaca is in Waupaca County, in the east central part of the state, and also has a sandy soil, but the normal rainfall and temperature are slightly higher than at Spooner, the precipitation averaging 3.68 inches per monh from April to September and the temperature 66.2°F. from June to September.

FIG. 2—SPRAYING UNDER HIGH PRESSURE PRODUCES A VERY FINE MIST
With three nozzles per row, it is possible to apply 100 gallons of Bordeaux mixture per acre.

Equipment—A triple pump traction sprayer was used at both places for applying the liquid spray. The boom, especially made for these experiments, covered four rows with three nozzles per row, one above the foliage pointing downward and two nozzles below, one on each side, directed upward. The discs of the nozzles were replaced once a year with new ones having very small apertures in order to secure a fine mist (Fig. 2) and keep the pressure close to 200 pounds.

To apply the dust, an engine-driven, four-row duster was used at Spooner and a four-row traction duster at Waupaca. Each was equipped with two nozzles per row (Fig. 3).

Varieties—In all, five varieties of potatoes were sprayed and dusted: Triumph, Green Mountain, and Early Ohio one year; King three years; and Rural New Yorker four years.

Formulas—1. Home made Bordeaux mixture—4 pounds copper sulfate, 5 pounds hydrated lime, 50 gallons of water.

FIG. 3—TRACTION DUSTER USED IN COMMERCIAL EXPERIMENTS
With two nozzles per row properly set, the entire foliage can be covered with dust.

2. Copper-lime dust—containing either 20 per cent or 25 per cent monohydrated copper sulfate according to whether or not an arsenical was included for the Colorado potato beetle.

This copper-lime dust is not powdered Bordeaux but a mixture of monohydrated powdered copper sulfate, hydrated lime and filler. In the presence of moisture, this combination turns to Bordeaux mixture.

Other insects—As these experiments were carried on primarily to learn the effect of sprays and dusts upon the potato leafhopper and hopperburn, the Colorado potato beetle was kept under control on all plots both treated and check by the use of calcium arsenate, except at Spooner where lead arsenate was used. On the treated plots, the arsenical was combined with the spray or dust whenever beetles were present. On the check plots, the beetles were controlled by spraying or dusting with the arsenicals alone. As beetles were nearly always present, the arsenicals were always used in the first two and often in the third applications. At no time in all four years were the potato flea beetle or potato aphid factors.

Diseases—In these experiments, little or no attention was given to the effect of different treatments upon potato diseases. Two diseases, however, were present in the fields. Early blight (*Alternaria solani*) occurred to some extent each year, especially on the check plots although at no time was it a serious factor in the experiments. Late blight (*Phytophthora infestans*) also made its appearance, but at no time, in the locality where these experiments were carried on, was it a serious factor in potato production. Mention is made of these two principal foliage diseases because

the relation of yield to spraying and dusting is, of course, the net result of these treatments upon all insects and diseases of potatoes which are in any way alleviated by Bordeaux mixture.

Time of day—The spraying was done at any convenient time during the day when little or no wind was blowing. Dusting was performed early in the morning if possible, while the air was still and the plants moist with dew (Fig. 4).

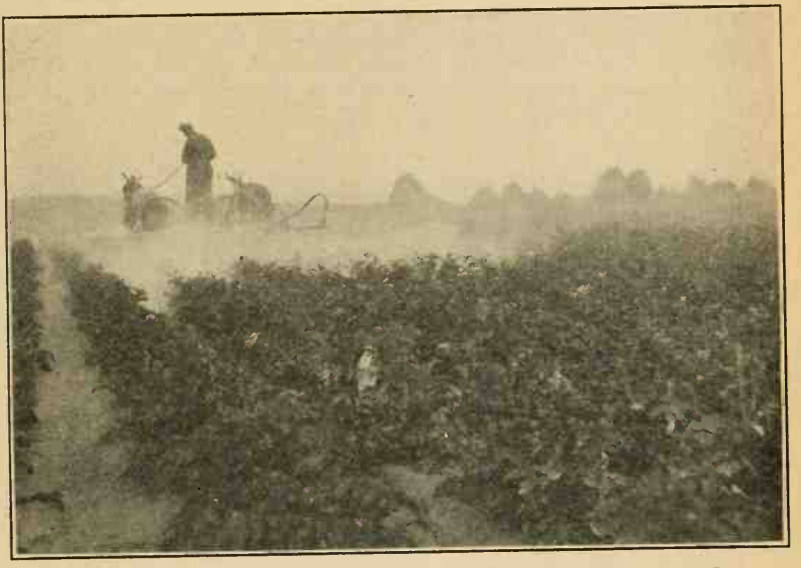

FIG. 4—ABSENCE OF WIND AND PRESENCE OF DEW INCREASE EFFECTIVENESS OF DUSTING

In the absence of wind, the dust cloud hangs low to the ground for many minutes covering most of the vines with fine particles. In the presence of dew, copper-lime dust soon turns to Bordeaux mixture.

Experiments at Spooner

Three varieties were treated at Spooner in 1922: Triumph, Green Mountain, and Rural New Yorker. Sprayed and dusted plots consisted of one-half acre each; the check plots were smaller, averaging about one-fourth acre. Three applications were made, on July 12, 24, and Aug. 7. The amount of liquid Bordeaux mixture (on an acre basis) ran from 80 gallons for the first spray up to 120 gallons for the last.

Applications of copper-lime dust containing 25 per cent monohydrated copper sulfate were altogether too heavy due partly to the use of a power duster and partly to inexperience in the amount of coverage necessary for protection. The amount averaged 40 pounds per acre for the first and 86 to 100 pounds for the last two applications.

The temperature averaged about normal for June and July with abundant precipitation. The infestation of leafhoppers was very light during these two months and hopperburn was scarce. An unusually hot, dry period

occurred the latter part of August, however, continuing into September. Largely on account of this condition, leafhoppers reproduced at a rapid rate with the resulting increase and spread of hopperburn on both treated and untreated plots of the Triumph and Green Mountain varieties. Hopperburn was more general and severe on the checks than on the treated plots.

Very little hopperburn occurred on the Rural New Yorker plots, treated or untreated, although leafhoppers occurred in moderate numbers the latter part of August.

A summary of yields is given in Table I.

Table I.—Potato Yields in Experiments at Spooner, Wisconsin, 1922.

Variety	Treatment	Yield in bu. per acre[1]			
		Total	No. 1	No. 2	Culls
Triumph	Spray—home made Bordeaux	38.4	34.0	No. 2's not separated[2]	4.4
do	Dust—copper-lime dust	43.4	36.7		6.6
do	Check	34.7	30.9		3.8
Green Mt.	Spray—home made Bordeaux	84.85	66.9	15.3	2.7
do	Dust—copper-lime dust	87.5	73.2	12.6	1.8
do	Check	60.4	41.0	16.4	3.0
Rural New Yorker	Spray—home made Bordeaux	111.15	Not graded		
do	Dust—copper-lime dust	121.9	Not graded		
do	Check	89.1	Not graded		

[1]In grading potatoes, the U. S. Standard grade was always employed. This calls for a screen with 1⅞ inch apertures for round varieties and 1¾ inch aperatures for long varieties. All that do not pass through these screens are known as No. 1 potatoes. No. 2 potatoes (of all varieties) are those which do not pass through screens of 1½ inch apertures.

[2]As the Triumphs were wanted for seed stock, they were not machine graded but picked over by hand into two sizes, "Salable" (No. 1's) and "Small" (Culls).

Experiments at Waupaca

A commercial experiment was started at Waupaca in 1922, running four years. The amount of materials applied ran from 65 to 80 gallons of liquid Bordeaux mixture per acre for the first sprays and from 85 to 110 gallons for the last ones. No dusting was done in 1922. It was commenced in 1923 and continued to the conclusion of the experiment. The average rate of application was from 18 to 25 pounds per acre for the first two and from 25 to 32 pounds for the last one or two applications.

Waupaca Results—1922

In 1922 *four* applications of liquid Bordeaux mixture were made, on July 7, 17, 27, and August 5, to one acre each of Green Mountains and Rurals, using the special boom with three nozzles per row. These tests were compared directly with like acres sprayed by the grower *five* times but with only two nozzles per row and both of these pointing downward.

The temperature averaged about normal for June and July with abundant precipitation. A hot, dry spell commenced the latter part of August and continued into September.

On account of the weather conditions the latter part of the summer, hopperburn became general in August on both varieties and severe on the Green Mountains. On August 18 the untreated Green Mountain vines were nearly dead; those sprayed with two nozzles per row, little better; while those sprayed with three nozzles per row were in the best condition of any although showing considrable hopperburn. On the Rurals, hopperburn was much less severe. The Green Mountains were dug on September 14; the Rurals the last of the month.

Table II.—Potato Yields in Experiments at Waupaca, Wisconsin, 1922

Variety	Treatment	Yield in bu. per acre			
		Total	No. 1	No. 2	Culls
Green Mt.	Spray—liquid Bordeaux 3 nozzles per row 4 applications	186.5	144.0	36.0	6.5
do	Spray—liquid Bordeaux 2 nozzles per row 5 applications	136.2	97.5	33.0	5.7
do	Check	83.2	49.5	29.2	4.5
Rural New Yorker	Spray—liquid Bordeaux 3 nozzles per row 4 applications	265.0	Not graded		
do	Spray—liquid Bordeaux 2 nozzles per row 5 applications	250.0	Not graded		
do	Check	180.0	Not graded		

The Green Mountain plot sprayed four times with three nozzles to the row yielded 50.3 bushels per acre more than the one sprayed five times with two nozzles per row.

The corresponding difference in the Rural plot was only 15 bushels per acre in favor of the three nozzles.

Treated plots of both varieties yielded far more potatoes, however, than the check plots.

Waupaca Results—1923

In 1923 it was necessary to make only three applications of spray and dust. These were put on July 13, 25, and August 6 to approximately one-half acre each of Kings and Rurals.

The weather conditions in 1923 were nearly the opposite of those in 1922. The temperature was slightly above normal for June and July with a light rainfall. During August and September it was cool with a rainfall slightly above normal.

The generally cool, wet season was most favorable to the growth of potatoes. At the same time it held back the development of leafhoppers, and consequently hopperburn did not assume serious proportions. An unusually early and severe frost on September 14 killed all the vines before the treated and check plots showed as much difference in the amount of hopperburn as occurred in 1922. The potatoes were dug September 27.

Table III.—Potato Yields in Experiments at Waupaca, Wisconsin, 1923

Variety	Treatment	Yield in bu. per acre			
		Total	No. 1	No. 2	Culls
King	Spray—home made Bordeaux	231.65	209.0	21.25	1.4
do	Dust—copper-lime dust	223.4	198.3	23.5	1.6
do	Check	181.4	157.8	22.6	1.0
Rural New Yorker	Spray—home made Bordeaux	237.4	209.1	24.9	3.4
do	Dust—copper-lime dust	228.6	199.7	26.1	2.8
do	Check	211.1	187.0	22.4	1.7

If the heavy frost had held off another two weeks, it is quite certain that a greater difference in yield between the treated and check plots would have been realized.

Waupaca Results—1924

Kings and Rurals were again used in the experiments of 1924, when four applications of spray and dust were made, on July 6, 17, 30, and August 20, to one-acre plots.

This was an unusually cool, wet year; the monthly mean temperature from June to September averaged 3.8°F. below normal. The rainfall in June and July was slightly below normal but for the next two months together was 7.64 inches above normal.

This year, while too cool and wet to expect any great number of leafhoppers and resulting hopperburn, was also too much below normal for the best production of potatoes. In accordance with what was expected, very little hopperburn appeared even on the checks and the yield of potatoes was below that of 1923. They were dug on October 3.

Table IV.—Potato Yields in Experiments at Waupaca, Wisconsin, 1924

Variety	Treatment	Yield in bu. per acre			
		Total	No. 1	No. 2	Culls
King	Spray—home made Bordeaux	205.85	165.6	35.65	4.6
do	Dust—copper-lime dust	192.2	154.7	33.7	3.8
do	Check	184.3	144.5	37.2	2.6
Rural New Yorker	Spray—home made Bordeaux	183.0	157.2	23.0	2.8
do	Dust—copper-lime dust	210.1	181.7	25.3	3.1
do	Check	188.55	156.4	28.7	3.45

Waupaca Results—1925

In 1925 three varieties of potatoes—Early Ohio, King, and Rural New Yorker—were sprayed and dusted four times; July 7, 16, 25, and August 5.

The summer of 1925 would be called generally a warm, rainy season. The temperature was considerably above normal in June and September and averaged normal during July and August. The rainfall was 2.7 inches above normal in June, 1.2 above in July, and below normal only in August.

Hopperburn was quite prevalent in August and increased until by the middle of September there was a clearly marked difference between the treated plots and the checks. Potatoes were dug on October 2.

Table V.—Potato Yields in Experiments at Waupaca, Wisconsin, 1925

Variety	Treatment	Yield in bu. per acre			
		Total	No. 1	No. 2	Culls
King	Spray—home made Bordeaux	211.1	159.6	51.5	Practically none
do	Dust—copper-lime dust	190.4	143.0	47.4	
do	Check	151.5	92.9	58.6	
Early Ohio	Spray—home made Bordeaux	190.4	141.9	48.5	do
do	Dust—copper-lime dust	162.6	117.7	45.0	do
do	Check	138.5	84.1	54.5	do
Rural New Yorker	Spray—home made Bordeaux	185.7	144.2	41.5	do
do	Dust—copper-lime dust	170.9	128.6	42.3	do
do	Check	124.7	78.8	45.9	

One of the unexpected results of 1925, discovered when potatoes were graded, was the large proportion of No. 2 potatoes to the total yield, of all varieties in treated and check plots alike. There were so few culls that it was not necessary to separate them from the No. 2's. It is evident that the generally warm, moist season resulted in an unusual development of the tubers so that practically all grew to at least No. 2 size.

Table VI.—Percentage of No. 1's to Total Yield of Potatoes in Experiments at Spooner and Waupaca, Wisconsin

Year	Locality	Variety	Percentage of No. 1's to Total Yield			
			Sprayed	Dusted	Check	Difference between check and best treated plot
1922	Spooner	Green Mt.	78.8	83.6	67.8	15.8
1922	Waupaca	Green Mt.	77.2	----------	59.5	17.7
1923	Waupaca	King	90.2	88.7	86.9	3.2
1923	Waupaca	Rural	88.1	87.3	88.5	-0.4
1924	Waupaca	King	80.4	80.5	78.4	2.1
1924	Waupaca	Rural	85.9	86.4	82.9	3.5
1925	Waupaca	King	75.6	75.1	61.3	14.3
1925	Waupaca	Early Ohio	74.6	72.4	60.7	13.9
1925	Waupaca	Rural	77.6	75.2	63.1	14.5
Average percentages			80.9	81.15	74.9	9.4

Bordeaux Mixture Increases Percentage of No. 1 Potatoes

Protection of potato vines from hopperburn, whether by spray or dust, not only consistently increases total yield, but at the same time increases the percentage of No. 1 potatoes, which are the cream of the crop, Table VI.

In these experiments the treated plots yielded a noticeably higher percentage of No. 1 potatoes to their total yield than did the checks to their total yield, with one exception in which the percentage of No. 1's was slightly higher in the check.

The difference in favor of the series of sprayed plots over the checks is 6.0 per cent and the difference in favor of the series of dusted plots over the checks is 6.2 per cent of the total production of No. 1 potatoes.

The average increase in percentage of No. 1's of sprayed and dusted plots taken together, was 6.1 per cent higher than in the checks, which equals 8.4 bushels per acre of No. 1 potatoes.

This increase is for the average year but the increase is much greater, as shown by the table, during severe hopperburn years (1922 and 1925) when fancy No. 1 potatoes usually command a better price.

Table VII.—Total Yields and Percentage of No. 1's in 1922 and 1925 at Waupaca, Wisconsin. (The Two Severe Hopperburn Years.)

1922

Variety	Total yield in bu. per acre				Percentage of No. 1's to total			
	Sprayed 3 nozzles per row	Sprayed 2 nozzles per row	Check	Difference between check and best treated plot	Sprayed 3 nozzles per row	Sprayed 2 nozzles per row	Check	Difference between check and best treated plot
Green Mt.	186.5	136.2	83.2	103.3	77.2	71.5	59.5	17.7
Rural	265.0	250.0	180.0	85.0	Not graded			
Averages	225.75	193.1	131.6	94.15	77.2	71.5	59.5	17.7

1925

Variety	Total yield in bu. per acre				Percentage of No. 1's to total			
	Sprayed	Dusted	Check	Difference between check and best treated plot	Sprayed	Dusted	Check	Difference between check and best treated plot
King	211.1	190.4	151.5	49.6	75.6	75.1	61.3	14.3
Early Ohio	190.4	162.6	138.5	51.9	74.6	72.4	60.7	13.9
Rural	185.7	170.9	124.7	61.0	77.6	75.2	63.1	14.5
Averages	195.7	174.6	138.2	57.5	75.9	74.3	61.7	14.2

Fig. 5 portrays the temperatures and rainfall during two severe and two light hopperburn years.

Thus it is evident that in 1922 and 1925 temperatures were above normal, with scant rainfall—especially during the latter part of the summer. In the other two years, just the opposite occurred.

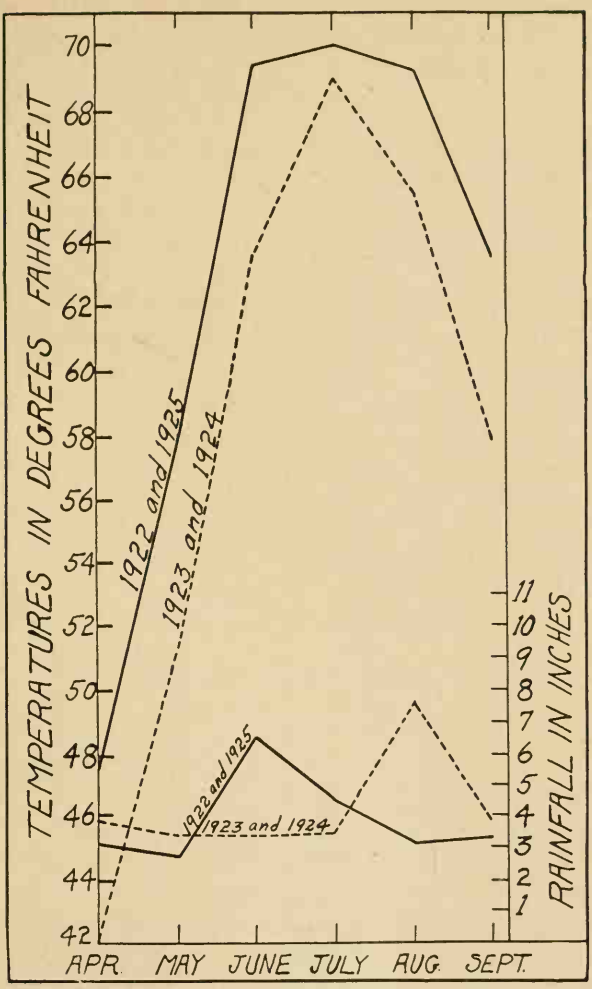

FIG. 5—CHART OF TEMPERATURE AND RAINFALL IN WAUPACA, WISCONSIN.

Comparing the two years of severe hopperburn (1922 and 1925) with the two years of light hopperburn (1923 and 1924), a considerable difference in yield may be noted.

Severe hopperburn years

All treated plots	194.9 bu.
All check plots	135.6 bu.
Difference	59.3 bu. per acre.

Light hopperburn years

All treated plots ..... 214.0 bu.
All check plots..... 191.3 bu.

Difference ..... 22.7 bu. per acre.

A complete summary of the Waupaca experiments, including the four years, is as follows:

All treated plots ..... 203.4 bu.
All check plots..... 160.4 bu.

Difference ..... 43.0 bu. per acre.

Table VIII. summarizes all experiments at both locations.

Table VIII.—Summary of all Spraying and Dusting Experiments at Spooner and Waupaca Wisconsin, 1922-1925

Year	Locality	Variety	Total yield in bu. per acre		
			Sprayed	Dusted	Check
1922	Spooner	Triumph	38.4	43.4	34.7
do	do	Green Mountain	84.85	87.5	60.4
do	do	Rural New Yorker	111.15	121.9	89.1
1923	Waupaca	King	231.65	223.4	181.4
do	do	Rural New Yorker	237.4	228.6	211.1
1924	Waupaca	King	205.85	192.2	184.3
do	do	Rural New Yorker	183.0	210.1	188.55
1925	Waupaca	King	211.1	190.4	151.5
do	do	Early Ohio	190.4	162.6	138.5
do	do	Rural New Yorker	185.7	170.9	124.7
Averages			167.9	163.1	136.4

The experiment at Waupaca in 1922 could not be included in Table VIII for it was concerned with two methods of spraying and not with spraying versus dusting.

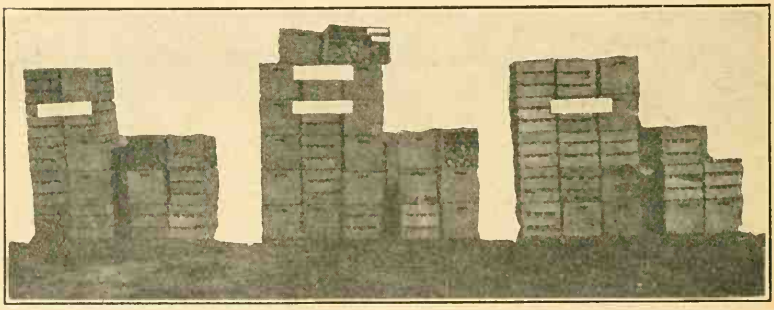

FIG. 6—YIELD OF KINGS FROM SPRAYED, DUSTED, AND CHECK PLOTS
Yields here illustrated from two rows in each plot *Left*, check; *Center*, sprayed; *Right*, dusted. Placards are on stacks of No. 1 potatoes; No. 2's are on the right in each case.

Thus, final results show that spraying is very little if any better than dusting, the difference amounting to 4.8 bushels per acre in favor of the former, which difference, however, is not outside the bounds of experimental error. Both spraying and dusting, however, increased average yields over the checks by 31.5 and 26.7 bushels per acre, respectively (Fig. 6).

A final presentation of all treated plots compared with all check plots, at both locations for the four years on five varieties of potatoes including 14 sprayed plots, 10 dusted plots, and 12 check plots, shows:

All treated plots ..... 171.4 bu.
All check plots ..... 135.6 bu.

Difference ..... 35.8 bu. per acre

In considering these results, it must be kept in mind that the Colorado potato beetle was controlled on all treated and check plots alike.

Relative Costs of Spraying and Dusting

A simplified analysis and statement shows that dusting actually costs very little more than spraying. We have not taken into consideration depreciation, interest, repairs, or other like items because such charges would appear to be quite similar for both machines. The actual labor including team, and cost of materials, for the number of applications given, should form the basis of criteria in the relative cost of spraying versus dusting.

The prices of the sprayers and traction duster were the same.

Tabular Comparison of Spraying and Dusting

Spraying		Dusting	
Unit—one acre Four applications, Amt. per application, per acre 88 gallons. Total—352 gallons		Unit—one acre Four applications, Amt. per application per acre, 24 pounds Total—96 pounds	
Cost		Cost	
Copper sulfate 28.16 lbs. at 9c	$2.54	Copper-lime dust, 20% monohydrated copper sulfate, 25% calcium arsenate 72 lbs. at $11.60 per cwt (3 applications)	$8.35
Hydrated lime 31.68 lbs. at 1c	.32		
Calcium arsenate 13.2 lbs. at 16c (3 applications)	2.12	Copper-lime dust, 25% monohydrated copper sulfate 24 lbs. at $8.80 per cwt (1 application)	2.12
Labor, $1.25 an hour for two men and team, 75 minutes average for all operations for spraying each acre	6.28	Labor, $0.75 an hour for man and team. 28 minutes average for dusting each acre	1.50
Total	$11.26	Total	$11.97

Discussion

Although the experiments recorded in this paper indicate that spraying increases the yield of potatoes to a slightly greater extent than does dusting, particularly in years when the leafhopper and hopperburn are severe, yet the difference shown in average yields is not outside the bounds of

experimental error. It is also apparent that the increase in yield alone is not the only factor which must be taken into consideration when recommendations are prepared for potato growers. A rather surprising reaction was experienced several times. Progressive growers familiar with our detailed experiments and the results thereof became enthusiastic, first, from the decided increase in yields from treatment with Bordeaux mixure and, second, from the greater advantages of dusting over spraying. An analysis of this rather common decision is interesting. Those growers most familiar with our experiments witnessed the whole spraying operation as a rather detailed, complex, and difficult piece of work. On the other hand, the dusting operation could be carried on with only a fraction of the attention to details and immediately appealed to them from the standpoint of the following factors:

Elimination of use of water
Simplicity of preparation
Reduced weight of outfit
Speed of application
Consequent willingness to repeat applications when necessary.

Protection of potato vines from hopperburn whether by spray or dust, not only consistently increased total yield but at the same time increased the percentage of No. 1 potatoes.

Finally it would appear that the net results of the experiments have been, first, to greatly increase the treatment of potatoes with some form of Bordeaux mixture; second, to increase the proportion of dusting to spraying on account of its greater speed and adaptability. Several growers who formerly sprayed but two or three times are now dusting from four to six times, are increasing their yield more than ever before, and appear to be entirely satisfied with this method of hopperburn control.

Table IX.—Mean Monthly Temperatures and Rainfall at Waupaca, Wisconsin, April to September, 1922-1925

Year	Mean temperatures					
	April	May	June	July	August	Sept.
Normal	44.4	56.2	65.7	71.0	67.9	60.4
1922	44.6	63.4	68.2	69.4	70.0	63.0
1923	41.6	54.7	67.4	71.5	64.2	59.0
1924	41.8	48.6	59.7	67.2	66.4	56.5
1925	50.0	52.7	70.0	70.6	68.4	63.6
	Rainfall					
Normal	2.66	4.53	4.43	3.35	3.44	3.83
1922	5.07	3.70	5.86	4.37	3.27	2.63
1923	3.13	1.87	3.06	4.05	4.17	3.68
1924	4.61	5.07	3.84	2.74	11.06	3.85
1925	1.16	1.81	7.13	4.64	2.28	3.99

SUMMARY

Scientific workers are generally agreed that the use of Bordeaux mixture on potato foliage will increase yields.

The potato leafhopper is probably the most injurious insect pest of the potato plant, and it regularly causes serious damage in Wisconsin.

Spraying and dusting experiments were carried on in commercial potato fields of Wisconsin for four years under regular farm conditions.

The object of these investigations was to find out the cost of each treatment and how much each treatment increased the total yield and improved the grade.

Five varieties of potatoes were included in the experiments: Triumph, Green Mountain, Early Ohio, King, and Rural New Yorker.

A definite increase in the percentage of No. 1 potatoes was secured both by spraying and dusting.

The average increase of No. 1 potatoes in the treated plots was 6.1 per cent greater than in the check plots, which equals 8.4 bushels per acre.

With one exception, yields were always increased by spraying and dusting.

The average increases for spraying and dusting combined, varied from 22.7 bushels per acre in the two years of little hopperburn to 59.3 bushels per acre in the two years of heavy hopperburn.

The average yields of *all* sprayed, *all* dusted, and *all* check plots when compared, show the following distinctive results:

All sprayed plots	167.9 bu. per acre
All dusted plots	163.1 bu. per acre
All check plots	136.4 bu. per acre

Computations show the cost of spraying and dusting for four applications per acre to be: Spraying $11.26; dusting, $11.97.

The yields from dusting and spraying were equal within the limits of experimental error.

The cost of dusting was little more than that of spraying.

Dusting appeals more strongly than spraying to many growers on account of its greater speed and adaptability.

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A Fusarium Wilt of Peas in Wisconsin

Maurice B. Linford

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CONTENTS

A Fusarium Wilt of Peas in Wisconsin

MAURICE B. LINFORD

WHERE PEAS ARE GROWN intensively during a period of years, avoidance of disease becomes a problem of major concern. This has proved particularly true in the production of green peas for canning. Since 1910, investigations of the causes of pea failure have been in progress almost continually at Madison, supported by the Wisconsin Agricultural Experiment Station, the United States Department of Agriculture, and by the canners themselves. From these and other investigations it was soon recognized that such failures are generally the result of one or more diseases caused by parasitic fungi and bacteria, and recommendations for their control gave partial relief, but up to the present time it has not been possible to eliminate them entirely.

The diseases that have been found most frequently important are those which attack the underground parts of the plant. In Europe such injuries are generally grouped under the name of "St. John's Disease" (11) which is there attributed to several species of *Fusarium*. In the United States the chief root disease is the rootrot caused by *Aphanomyces euteiches* Drechsler (15), but numerous other parasites are commonly associated with it in causing serious root decay, including: *Fusarium martii* App. & Wr. var. *pisi* F. R. Jones (14); *Mycosphaerella pinodes* (Berk. & Blox.) Stone, and a "micro" form of this same species (25); *Rhizoctonia solani* Kühn; and *Pythium* spp. (16).

During the summer of 1924 in a survey of Wisconsin pea fields (16) for the study of field occurrence and relative importance of these diseases, a Fusarium wilt (24) hitherto unrecognized, was discovered as an additional cause of important pea failures. A study of this disease was begun by the writer during the winter of 1924-25 and has been continued to include field, greenhouse, and laboratory investigations during 1925-26 and 1926-27.

THE DISEASE

Geographical Range

Fusarium wilt has been found generally distributed in the older pea growing districts of southern Wisconsin, but detailed information is still lacking concerning its prevalence in the northern part of the state. Of a total of 59 localities which have been searched, 35 have been found infested. These

The writer gratefully acknowledges the valuable advice and assistance of Fred R. Jones and L. R. Jones throughout this investigation.

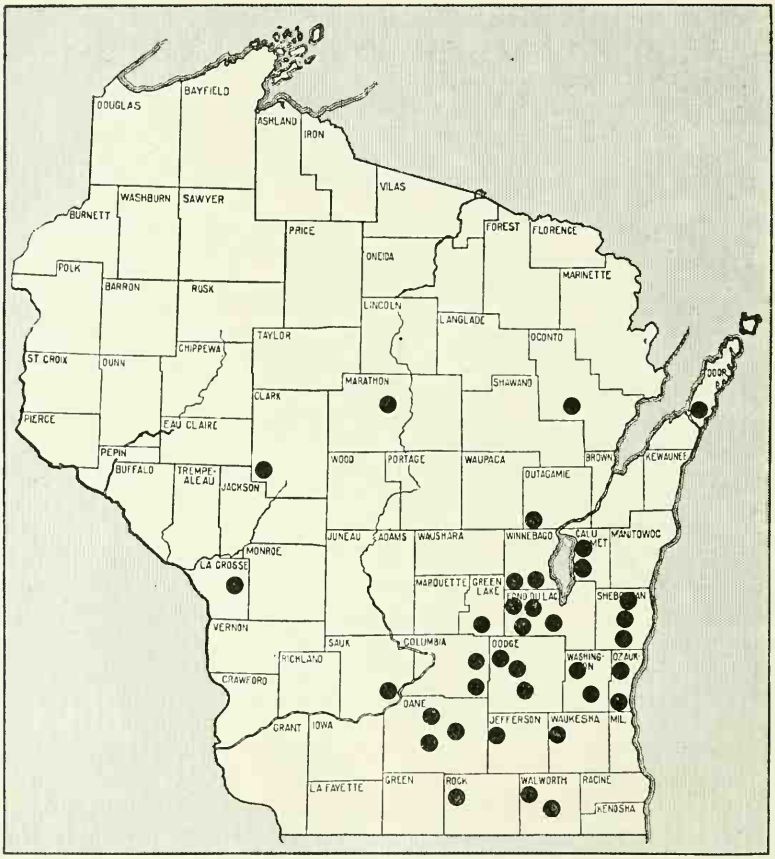


FIG. 1.—GEOGRAPHICAL RANGE OF FUSARIUM WILT OF PEAS IN WISCONSIN AS DETERMINED DURING 1924, 1925, AND 1926.
Black dots represent localities in which wilt has been found.

are distributed, as shown in Figure 1, in 21 counties which represent the most important pea producing areas in Wisconsin.

Outside of this state, wilt has been reported from only two localities. Dr. J. B. Kendrick collected it at Peru, Indiana, in 1926 and sent the writer specimen plants and cultures of the wilt fungus in confirmation. In the same year the writer found it associated with rootrot at McMillan, Michigan. These observations, although narrowly limited, suggest that this Fusarium wilt may be found rather generally distributed at least in the older pea producing regions of the northern United States.

Economic Importance

Fusarium wilt of peas is destructive more frequently in association with several diseases of the rootrot type than as an isolated disease, a fact that

makes difficult a precise statement of its economic importance. In over a dozen of the older cannery districts in southern Wisconsin, however, it caused more important losses during the period of this investigation than all other diseases combined. Here it has probably been, for several years at least, the cause of a considerable part of the injury hitherto attributed to the Aphanomyces rootrot. In Wisconsin as a whole it appears to be less important than that rootrot but more destructive than any of the other pea diseases.

Wilt reduces quantity of yield more than quality of product. Affected plants die quickly and in infested areas the crop may be destroyed completely. During 1925 in a survey of 693 fields with a combined area of 4,564 acres, crop reduction from wilt was estimated at 6.6 per cent. This figure is too high for the state as a whole, but much too low to represent conditions in the most severely infested localities. With the increasing age of intensive pea culture, diseases are gradually rendering unfit for the crop those limited areas best adapted to pea growing. Continued success of the pea industry in regions where it is now established is therefore partially dependent upon the development of adequate means of disease control.

Description of the Disease

Fusarium wilt of peas occurs characteristically in scattered spots of circular outline and variable size which may be distributed in large numbers

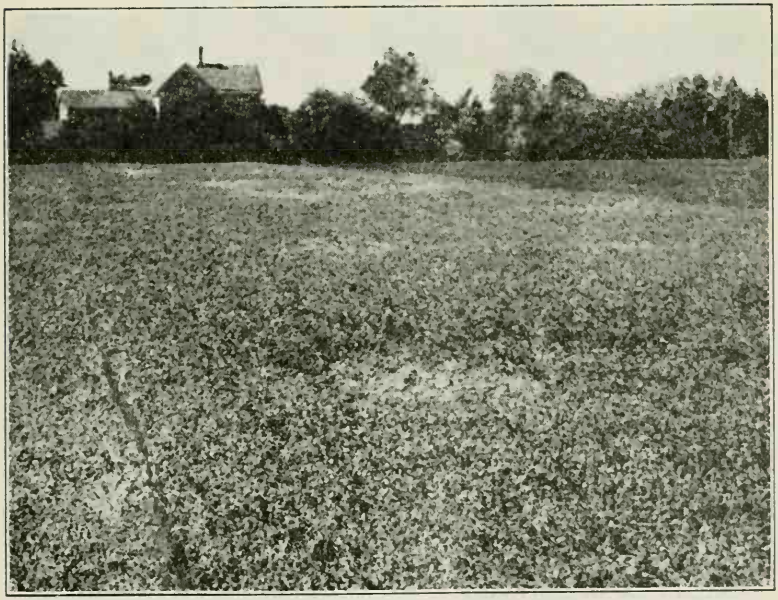


FIG. 2.—FIELD OF WINNER VARIETY PEAS SPOTTED WITH WILT.
The pale areas, distributed through the field, contained plants in various stages of the wilt disease.

through a field (Fig. 2). These spots sometimes consist of only a few plants but they may enlarge both during the season and from year to year until they involve the entire field. They first become discernible through a bluish paleness of the vines which gives way to yellow as the plants die. Plants are affected first at the center of such spots and then progressively outward towards the margins which may continue to advance until harvest or maturity. An apparent radial spread of three feet or more during the season is frequently observed.

Symptoms

The initial and most characteristic symptom of this disease is a recurving of the margins of the younger stipules and leaflets which leads to separation of the tips of the stipules in the terminal bud before their bases begin to diverge, and to rolling of the leaflets (Fig. 3). Simultaneously the upper

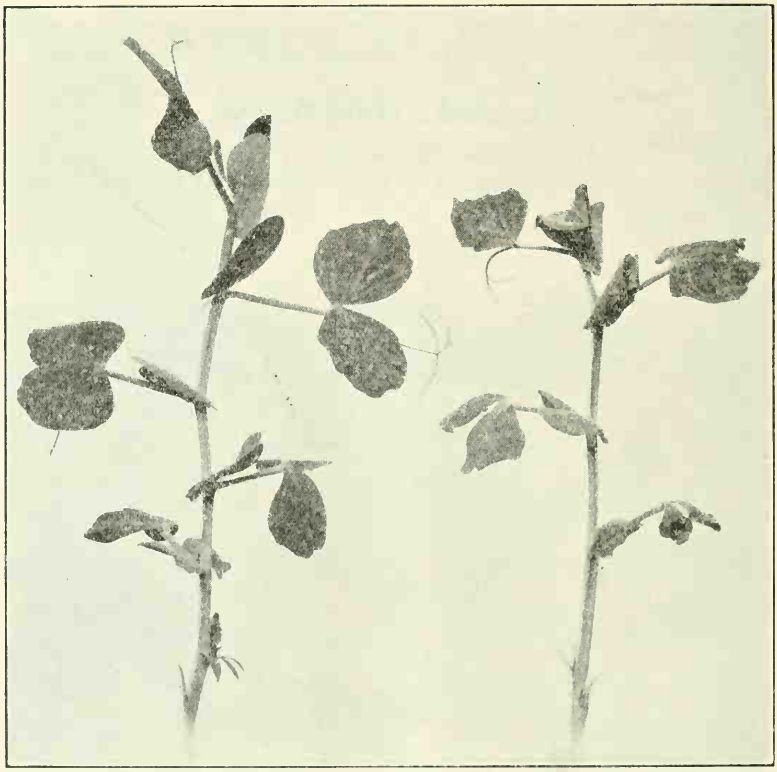


FIG. 3.—EARLY SYMPTOMS OF WILT IN HORSFORD PEA

Affected plant at right shows recurving of margins of stipules and leaflets of the upper leaves, loss of color and withering of lower leaves, retardation of terminal growth, and slightly increased stem diameter at the ground line. Roots of this plant showed no conspicuous external injury. (Slightly reduced.)

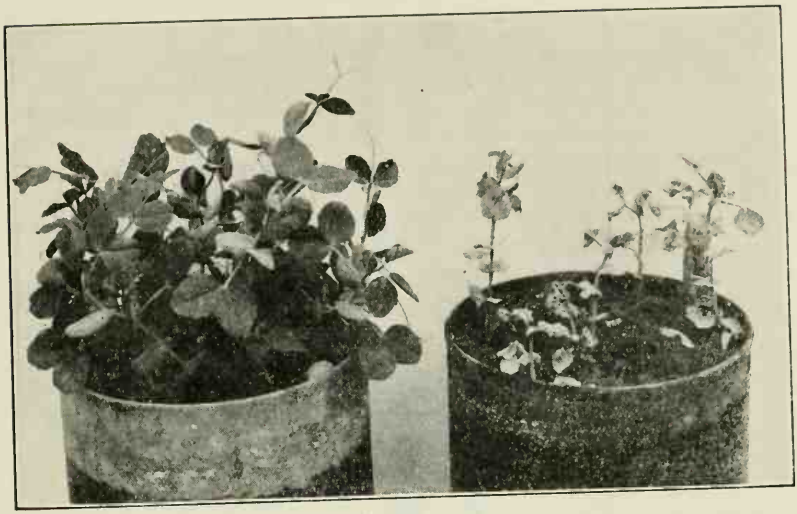


FIG. 4.—LATE STAGES OF WILT IN PERFECTION PEAS.

Healthy plants at left are growing in uninfested soil; diseased plants at right are in naturally infested soil from an old pea field. Grown 36 days in the greenhouse at the variable temperature 16-20 degrees C.

parts of the plant may become pale and develop a bluish sheen, the terminal bud may be checked in its growth, the stem and upper leaves may become more rigid than normal, and the roots more crisp and brittle, while the lower leaves turn pale and commence to wither. Sometimes the entire plant becomes yellowish and the leaves wither progressively upwards to the terminal bud. Characteristically, however, after the collapse of a few basal leaves, the upper part of the plant wilts abruptly and may become dry while still green in color. After such wilting the stem shrivels downward from the tip toward the basal internodes which remain firm and turgid until the end (Fig. 4). The disease may affect one side of a plant earlier than the other, giving rise to a unilateral expression of symptoms which is a useful diagnostic characteristic of this Fusarium wilt as of some related diseases of other plants.

The roots of plants affected with wilt alone characteristically show a few dead rootlets and a limited superficial browning but otherwise are white and clean externally. The dead rootlets which have undergone a dry decay generally point at their base to the most pronounced vascular discoloration to be found in the taproot. Vascular discoloration, varying from an almost imperceptible yellow to a rich orange brown, develops in the upper part of the taproot (Fig. 5) and may extend into several internodes of the stem. General root decay seldom begins until the wilted stem has dried almost to its base. Then, in wet soils, the succulent cortex of the basal internodes may become covered with a white fluffy growth of mycelium.

A plant may wilt within two days after the first symptoms appear, but even under conditions very favorable for the disease, an interval of ten to

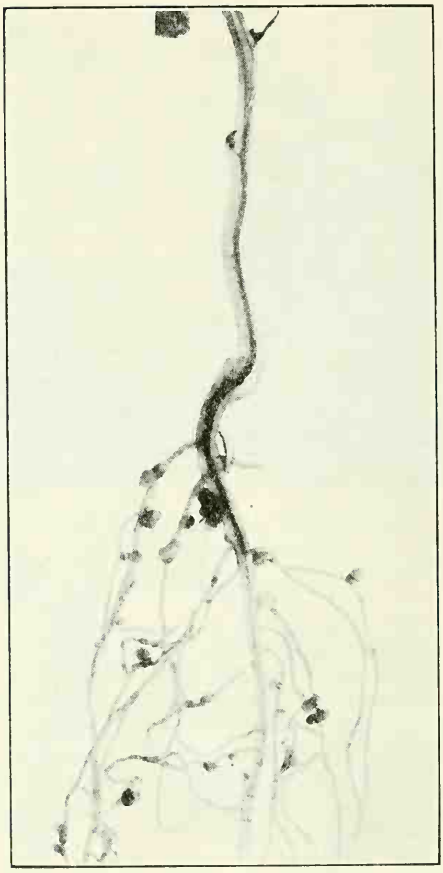


FIG. 5.—VASCULAR DISCOLORATION IN THE BASE OF THE STEM AND UPPER PART OF THE TAPROOT.

This plant, which showed only preliminary wilt symptoms, has been split lengthwise to expose the discolored stele. Note that the root cortex and the nodules even at this stage of vascular discoloration are still white and sound.

twelve days is more frequently observed. During cool weather in the early spring, symptoms develop slowly, but on succeeding bright warm days the plants wilt rapidly. When progress of the disease is slow, plants may die without a definite wilt phase. On such plants, one or more lateral shoots may arise from the basal nodes and grow to a height of several centimeters before each in its turn collapses.

The increased rigidity of the plant in the earliest stages of the disease is associated with a marked change in the water relationships of the plant. The basal internodes, especially near the soil line, become distinctly swollen: one series of measurements showed a 20 per cent greater cross sectional area in the second internode of diseased plants as compared with healthy

plants grown under similar conditions. Such diseased plants are particularly retentive of water and, when removed from the soil, wither much less rapidly than do healthy plants. The swollen basal portion of the stem is especially resistant to drying.

These symptoms collectively are clearly distinct from those of other known diseases of the pea.

Up to the present, the characteristic rolling of leaflets and stipules, and the checking of terminal growth have been seen induced in the greenhouse by only one set of conditions other than wilt infection, and that is calcium deficiency maintained experimentally by Miss Dorothy Day in studies, at the University of Wisconsin, not yet published. In calcium starvation she found rolling of stipules and leaflets and increase in rigidity of leaves and stems indistinguishable from the early symptoms of wilt. The degree of calcium deficiency required to produce this condition is not to be expected in agricultural soils.

Etiology

Preliminary examination of plants affected with this disease revealed the presence of abundant fungous mycelium in the discolored vascular tissues, and numerous isolations have yielded cultures predominantly of one species of Fusarium. The pathogenicity of the fungus has been demonstrated by numerous inoculation and reisolation experiments, several of which are described in following sections of this paper.

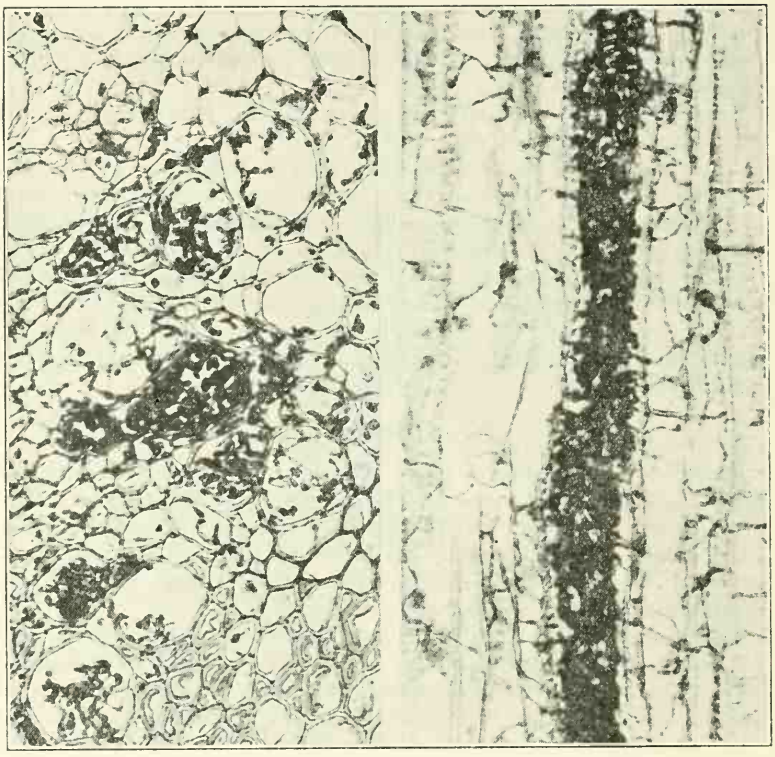
During the summer of 1925 isolations[2] were made from 68 collections of affected plants from many localities. From the cultures thus obtained, 161 were selected for an inoculation experiment in which peas were grown in pots of steamed soil into which the fungus was introduced, in culture on Melilotus stems, at the time of planting. Of the 118 cultures of the pea wilt fungus, 75 produced the disease, while none of the several other species of Fusarium tried proved significantly pathogenic. This and other similar experiments have been accepted as adequate evidence that one species of Fusarium causes pea wilt throughout the known geographical range of this disease.

Pathological Histology[3]

The most conspicuous and apparently most significant development of the pea wilt fungus occurs in the xylem of both roots and stem. The parasite, to attain this position, must pass through cortical and undifferentiated tissue, but, with the exception of the occasional rootlets of entry which are thoroughly invaded, it occurs only sparingly in the cortex, chiefly in the outer

[2]Several methods were found expedient for isolating the fungus from roots and stems, including: surface sterilization in mercuric chloride followed by washing and then cutting into segments with flamed scissors before plating on acidified potato-dextrose agar; cutting the cortex from the stele with a flamed razor blade and plating fragments of the stele; and pulling the xylem core out from the cortex by grasping the tap-root in one hand and the stem base in the other, and plating fragments of the core thus exposed.

[3]These studies were made with stained paraffin sections and with free-hand sections either fresh or stained with cotton-blue in lacto-phenol as outlined by Klebahn (21).



a b

FIG. 6.—MYCELIUM IN XYLEM OF HORSFORD PEA PLANT DYING FROM WILT, SECOND INTERNODE.

a. Transverse section, showing mycelium crowding some vessels and occurring more sparingly in others and in the xylem parenchyma. x 340.

b. Longitudinal section, showing a vessel crowded with mycelium, and hyphae passing through pits in the walls of this vessel and into the surrounding elements. x 375.

layers. No conspicuous cortical lesions such as characterize the *Fusarium martii pisi* footrot are formed by this vascular parasite.

Within the xylem the fungus distributes itself chiefly in the long, continuous tracheae, but it also invades freely the surrounding xylem parenchyma. Thin walls are penetrated directly; thickened walls chiefly if not solely through pits (Fig. 6b). Thorough invasion of the xylem is generally accompanied by a limited invasion of other stelar tissues. The fungus frequently gains access to the cambium where it causes complete disorganization (Fig. 7). From there it may invade the phloem and pericycle, but the endodermis constitutes an effective barrier which prevents the fungus from growing out through the cortex from an invaded stele until very late stages of the disease.

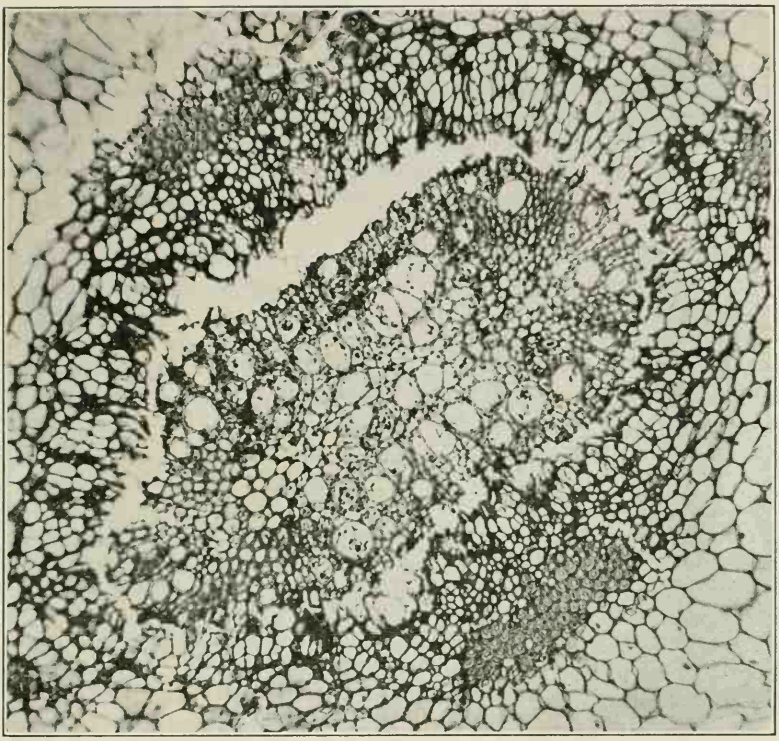


FIG 7—BREAKDOWN OF CAMBIUM REGION IN A YOUNG HORSFORD PEA PLANT DYING FROM WILT.

In this section of the first internode, the xylem is thoroughly invaded and the phloem sparingly as indicated by localized dark areas. x 130.

A few days after the first symptoms appear, the wilt fungus may be found readily in the xylem of the taproot near the base of affected lateral rootlets, and somewhat later throughout the entire upper portion of the root system and in the basal internodes of the stem. In late stages of the disease the fungus usually extends through the lower half of the stem, frequently through five or six internodes, but it has not been found in any instance within two internodes of the lowest blossom or pod, and its entry into even the lowest leaves is rare.

When plants die slowly without a distinct wilt phase, the fungus may be found only sparingly within the xylem. In the usual course of the disease, however, pea wilt is characterized by the accumulation of mycelium in the vessels in greater amounts than has been described in related diseases of other plants. Vessels sometimes become crowded with closely packed masses of mycelium which might, apparently, partially obstruct the passage of water, but this condition does not appear to have any bearing upon the production of the characteristic preliminary symptoms of the disease.

Other Diseases of the Pea Caused by Species of Fusarium

There are numerous reports in literature of the occurrence of species of Fusarium on the pea, but a parasitic relationship has been demonstrated by adequate experimental evidence in only a few instances, and in each well attested case the disease produced is primarily a cortical rot with very limited vascular invasion. The reports of Fusarium species on peas have been summarized so recently by Jones (14) that a complete review will not be necessary here.

In European literature, Fusarium on peas is mentioned chiefly in connection with the "St. John's Disease," a foot disease described by van Hall (11) in Holland in 1903, and attributed by him to *Fusarium vasinfectum* Atk. var. *pisi* (variety not described). Guéguen (10) and Capus (3) in France later found what they considered to be this same fungus associated with the disease but concluded that its entry into the pea roots was secondary to other injury. Schikorra (31) isolated a different fungus which he mistook for van Hall's organism, and concluded on the basis of inadequate inoculation experiments that it was the cause of the disease in Germany. This fungus was later placed by Appel and Wollenweber (1) in the new species *Fusarium falcatum*. Wollenweber (38, 39) while in the United States, described *F. redolens* Wr. as the cause of a wilt and foot disease of peas without presenting any of his experimental evidence. Since then he has mentioned this fungus (41, 42) as probably the chief cause of the St. John's disease in Europe, but has concluded that at least two other species, *F. martii* App. and Wr., and *F. falcatum* App. and Wr. may also cause the disease.

Turreson (37) has given a more detailed statement concerning a foot disease of peas in Sweden caused by *F. viticola* Thüm., characterized by a reddish-brown decay of the base of the stem beginning at a point near the seed.

In the United States, the only species that has formerly been shown to be an important parasite of the pea is *F. martii* App. and W. var. *pisi* F. R. Jones (14). This fungus causes a cortical decay of the stem base and roots and may, at high temperatures, invade the vascular system of the stem for a short distance. Jones made numerous isolations from the vascular systems of plants showing root decay and thereby secured a number of species, but of the following fungi from this and other sources none proved significantly parasitic: *Fusarium oxysporum* Schlecht., *F. solani* Mart., *F. sclerotiodes* Sherb., *F. vasinfectum* Atk., and *F. redolens* Wr.

More recently Togashi (36) has reported wilt of peas in Japan caused by three undetermined species of Fusarium. This also, from his descriptions, is a footrot rather than a typical vascular disease.

All of these reports are concerned with diseases which differ in symptoms, etiology, and pathological histology from the disease under consideration in this paper.

THE PATHOGEN

Taxonomy

Four pathogenic cultures of the pea wilt fungus were submitted to Dr. H. W. Wollenweber of the Biologische Reichsanstalt für Land- und Forstwirtschaft at Berlin-Dahlem, Germany, for determination. He kindly replied that they might be placed as *Fusarium orthoceras* App. and Wr. but observed that they showed some divergence in pigmentation from the type of the species and indeed were not constant in this character among themselves. One of them, showing no bright colors when grown on steamed rice, agreed closely with *F. conglutinans* Wr., the cabbage yellows parasite.

Fusarium orthoceras (38, 40) is a species of the section Elegans that has been isolated by different workers from potato, sweet potato, and other plants including the pea (23) and has been regarded as a cosmopolitan saprophyte. It forms no sporodochia or pionnotes, and the microconidia are always more numerous than the straight, triseptate macroconidia. Chlamydospores are usually abundant.

For comparison with the fungus from pea wilt, the writer obtained subcultures of authentic *F. orthoceras* from four different sources, cultures which had come originally from Europe and Honduras by way of the Fusarium Conference (43) of 1924. The eight cultures thus obtained, when grown on standard substrata (43), were in excellent agreement among themselves, showing only minor distinctions in cultural characters, but all of them were totally different in appearance from any of the numerous cultures of the pea wilt fungus which have been studied in this investigation. With a single exception they were sporulating freely and all of them were producing sufficient triseptate conidia of typical form for purposes of identification.

The pea wilt fungus, on the contrary, sporulates very sparingly. Microconidia may sometimes be found in fair abundance, but even in fresh isolations and on diverse substrata held at different temperatures, the writer has been unable to find sufficient macroconidia of uniform type in "normal condition" (40) to permit satisfactory morphological comparison with *F. orthoceras* or other species.

In the opinion of the writer the pea wilt fungus is distinct from the fungi that have been placed in the species, *F. orthoceras* App. and Wr. In the absence of an adequate morphological basis, however, it can perhaps be best considered a variety of that species because of its predominantly nonseptate conidia, the abundance of thick walled chlamydospores, and the presence of pigmentation in culture on rice. Therefore it is described tentatively as a new variety, with the description based upon growth in culture on standard substrata.

Fusarium orthoceras App. and Wr. var. **pisi** (n. var.).

Differs from *F. orthoceras* in paucity of microconidia and almost complete absence of macroconidia, also in absence of vinaceous colors on potato-dextrose agar, in dominance of other than vinaceous colors on rice, and in absence of salmon colored spore masses on various media. Macroconidia rarely present, irregular in size and form; microconidia continuous or rarely

septate, irregularly ellipsoidal to elongate, somewhat curved, borne on the mycelium either above or within the substratum, usually in very small numbers and sometimes wholly absent; chlamydospores relatively abundant, typical of the species, simple or compound, thick-walled, tuberculate; mycelium well developed, variable in diameter and septation, at first white in mass, becoming tawny to variously tinted; pigmentation of substratum highly variable from strain to strain, but including the following colors (30) in well developed cultures of the strongly pigmented strains grown three weeks on boiled rice: (above, dry growth) hydrangea pink, orange-vinaceous, coral pink, ocher red, dark vinaceous, victoria lake; (below, moist growth) pale pinkish cinnamon, buff pink, glaucous, malachite green, clay color, and Isabella color. Some strains of the fungus remain colorless or develop only the least intense of the colors named above. The cause of a vascular disease of *Pisum sativum* L.

On plain potato agar the cultures remain dirty white or develop small amounts of blue-green pigment. This color is distributed characteristically in tube cultures as a narrow lip where the lower edge of the slant surface touches the glass; in petri dish cultures it occurs in irregular areas in the older part of the colony, most clearly visible from below. The agar remains essentially unchanged in color.

On potato-dextrose agar the characteristic blue-green lip is accompanied by irregular pink areas in the mycelium and by pigmentation of the substratum, ranging from light golden brown to very dark purplish brown. Sometimes pigmentation of the mycelium and substratum are completely lacking.

On oat agar, blue-green is less frequent than on potato. The mycelium is white, pink, or tawny; the substratum strongly colored, brownish, rich vinaceous, or olivaceous.

On *Melilotus alba* stems the mycelium is tawny or dirty white.

On potato plugs the mycelium remains tawny white or becomes spotted blue-green.

Relation of Temperature to Growth in Pure Culture

For comparison with other wilt-producing species, *Fusarium orthoceras* var. *pisi* has been grown on petri dish plates of potato-dextrose (2 per cent)

Table I.—Results of Characteristic Growth-temperature Experiment in Which Fusarium orthoceras var. pisi Was Grown Seven Days on Potato-dextrose (2 per cent) Agar at the Temperatures Specified

Temperature (°C.)	6.5–8	7–10	13–13.5	14.5–16	17–18	19.5–20.5	24.–25	27.5–28	29.5–30.5	31.5–32	34.5–35
Average radial growth of three colonies in seven days (mm.)	0.6	2.4	9.	11.5	15.5	22	[a]	32	29	21.5	1.3

[a]The incubator at this temperature was fumigated with carbon-bisulphide during the experiment, resulting in sharp checking of growth. In another experiment, however, growth at this temperature was intermediate between that at 20° and 27°C.

agar over a range of controlled temperatures, using technique comparable to that generally employed. At each temperature, determinations have been made in triplicate, the three petri dishes being inclosed in a moist chamber to prevent drying of the substratum. The results of two such experiments indicated no important differences from the results obtained by several other investigators (34, 33, 4) working with related species (Table 1). Almost no growth was detected at the lowest temperature tested 6.5—8°, and very little at the highest, 34.5—35° C. The most rapid linear growth under the conditions of these experiments was at 27-30° C.

Pathogenicity

In addition to causing the wilt disease in some varieties of *Pisum sativum*, *Fusarium orthoceras pisi* causes a similar disease in *Vicia gigantea* Hook., a native perennial vetch from California and Nevada. Under some conditions, Sutton's New Giant Broadbean (*Vicia faba*) is also invaded. Plants that have been grown in infested soil at 20-24° C. without developing vascular disease include: cabbage, two strains susceptible to yellows; flax, two strains susceptible to flax wilt; cowpea, New Era, Progressive White, and Early Black varieties; soybean, Hollybrook and Wisconsin Black varieties; bean, Refugee Wax, Bountiful, and California Pea varieties; white lupine; blue lupine; *Cicer* sp.; *Phaseolus aconitifolius; P. angularus; Pueraria thunbergiana; Lathyrus odoratus; L. latifolius; L.* sp.; *Vicia villosa; V. sativa,* five varieties; and *V. pannonica.*

No adequate comparison of the pathogenicity of different isolations of the fungus has yet been attempted, but there is indication of wide variation. In the extensive inoculation experiment described above, only 75 of the 118 cultures of the wilt fungus produced the disease. No search has been made for physiologic specialization.

Dissemination

There are several obvious means whereby the wilt fungus may be disseminated, and field observations have provided numerous examples. Agencies such as the farm operations of plowing and cultivating, the flow of surface water during rain, and possibly heavy wind, which carry soil or crop refuse from an infested to a clean area, will distribute the fungus. Also conidia which are produced sparingly on the tuft of mycelium at the base of plants killed by wilt may be carried in the wind. In commercial pea culture, however, vines from infested fields are of major importance in the dispersal of the parasite. When vines are placed in a silo or silage stack, the curing process presumably kills the fungus, but uncured waste from the outside of silage stacks and from around viner sheds may harbor it. Such waste is frequently spread as a manure. In some localities vines are fed green to stock; sometimes they are cured and used as hay. Such practices appear to account for wilt in the first crop of peas on fields where diseased vines have been fed and fields manured with infested waste.

The characteristic field occurrence of pea wilt in scattered circular patches or sometimes as scattered individual affected plants led early to the view that

the fungus might be carried sparingly on pea seed. Some other vascular diseases (7, 8) caused by species of Fusarum, have been shown to be seed-borne. In the case of peas, however, the fungus has in no case been found within two internodes of the lowest pod, and numerous seeds removed aseptically from the pods of plants wilting when nearly mature and planted on agar have failed to yield cultures of the wilt fungus. It was suspected that the fungus might be among the organisms involved in the molding of pea seed harvested in wet weather from infested fields, and an attempt was made to isolate it directly and indirectly by various means from such molded seed, but without success. These attempts have not been sufficiently extensive to provide convincing evidence that the fungus is never so carried; they only indicate that it is not carried abundantly. To account for the known spread of the parasite it would be expected on seed only sparingly. Seed harvested from infested fields should be looked upon with suspicion.

INFLUENCE OF SOIL TEMPERATURE AND MOISTURE UPON THE DISEASE

In studies of cabbage yellows (33), tomato wilt (4, 6), flax wilt (19) and related vascular diseases caused by species of Fusarium, one of the most significant factors found to limit the development and severity of disease is soil temperature. A corresponding study of the Fusarium wilt of peas was therefore highly desirable.

Greenhouse Experiments

Methods. The following experimental study of the influence of soil temperature upon pea wilt was conducted in the Wisconsin soil temperature tanks following essentially the technique outlined by Jones, Johnson, and Dickson (20). Peas were grown from seed in cylindrical metal containers suspended in tanks of water, each tank maintained by electrical control at the desired temperature. In all experiments the soil temperature was adjusted at the time of planting, so that its influence was effective during both germination and later development of the plant. Air temperature was independent of soil temperature and fluctuated chiefly between 16 and 20 degrees C. Pure cultural inoculation was resorted to in all experiments because of the almost universal occurrence of other fungi parasitic upon the pea in soils naturally infested with the wilt fungus. The soil used was a black silt loam with a water holding-capacity of about 50 per cent of its dry weight. It was autoclaved five to eight hours at 10-15 pounds pressure several days in advance of inoculating and planting. In planting, the containers were filled according to weight, and water was added by weight to maintain the desired soil moisture content. Upon emergence, the seedlings were thinned to ten vigorous and well spaced individuals. A layer of mineral wool or of white sand was spread over the soil for insulation.

Experiment I.—At each of seven soil temperatures (8, 13, 17, 21, 25, 29, and 33 degrees C.) one can was planted with Horsford peas without inoculation and four were inoculated at time of planting, each with a different culture of the wilt fungus from a different locality. These cultures, on

Table II.—Summary of Results of Soil Temperature Experiment I. Influence of Soil Temperature Upon the Production of Wilt in Horsford's Market Garden Peas by Four Different Cultures of the Wilt Fungus

Soil temperature	Plants affected in 36 days				
	Culture number of inoculum				Average
	7	21	251	255	
°C.	%	%	%	%	%
8	0	0	0	0	0
13	50	40	10	10	27.5
17	60	40	40	70	52.5
21	80	70	60	70	70.0
25	80	60	40	40	55.0
29	10	50	50	10	30.0
Average	56	52	40	40	

Melilotus stems, were distributed through the two inches of soil immediately below the seed. The water content of the soil was held in all cans alike at 75 per cent of holding-capacity.

Wilt symptoms were noted first at 21 and 25 degrees C., 23 days after planting. At all times a larger percentage of plants was affected at 21 degrees than at other temperatures, with 25 and 17 degrees C. ranking second and third respectively. At the conclusion of the experiment after 36 days (Table II), no wilt symptoms had appeared at 8 degrees, but at all other temperatures disease had developed in the cans inoculted with all four cultures of the fungus. Differences in wilt production by these cultures were slight. Except at 33 degrees where both controls and inoculated plants died soon after emergence, the controls remained thrifty during the period of the experiment.

Experiment II.—In this experiment the effects of soil moisture as well as temperature were studied. At each of five temperatures (Table III) five cans of Horsford peas were planted, three inoculated with infested soil (culture no. 255 from experiment I above) and two left uninoculated. At

Table III.—Summary of Results of Soil Temperature Experiment II. The Influence of Soil Temperature and of Soil Moisture Upon the Development of Wilt in Horsford Peas

Soil temperature	Plants affected in 31 days				Plants dead in 31 days			
	Soil moisture holding-capacity			Average	Soil moisture holding-capacity			Average
	40%	65%	90%		40%	65%	90%	
°C.	%	%	%	%	%	%	%	%
13	0	10	20	10	0	0	0	0
17	50	80	90	73	10	0	10	7
21	90	90	100	93	40	80	70	63
25	90	100	100	97	20	70	60	50
29	40	20	60	40	16	10	0	7
Average	54	60	74		17	32	28	

each temperature the soil in the three inoculated cans was adjusted to 90 per cent, 65 per cent, and 40 per cent of its water holding-capacity, and in the two controls to 90 per cent and 40 per cent. During the experiment, water was added daily until the daily requirement exceeded 15 grams per can, and then twice daily. In spite of this at the conclusion of the experiment, the uneven distribution of water in the soil made it apparent that these figures were approximate only: soil was wet, medium-wet, or relatively dry. These soil moistures all permitted approximately normal growth of the plants; but the plants came up earlier, produced longer internodes and larger leaflets, and had a brighter green color in the wet and medium-wet soil than in the dry. Control plants remained free from wilt throughout the experiment.

The influence of temperature upon the disease was much as in the first experiment. Disregarding the effects of soil moisture (see averages of the three soil moisture contents, Table III) there were more plants affected after 31 days at 25 and 21 degrees C. than at higher or lower temperatures. This is still more evident in the average counts of numbers dead in 31 days. The only difference from Experiment I is that here 25 degrees appears slightly more favorable than 21 degrees for the early appearance of symptoms but, in conformity with that experiment, 21 degrees appears most favorable for early death of the plants.

Considering now the influence of soil moisture, it is apparent, first, from Table III, that the cardinal soil temperatures for the disease remain essenti-

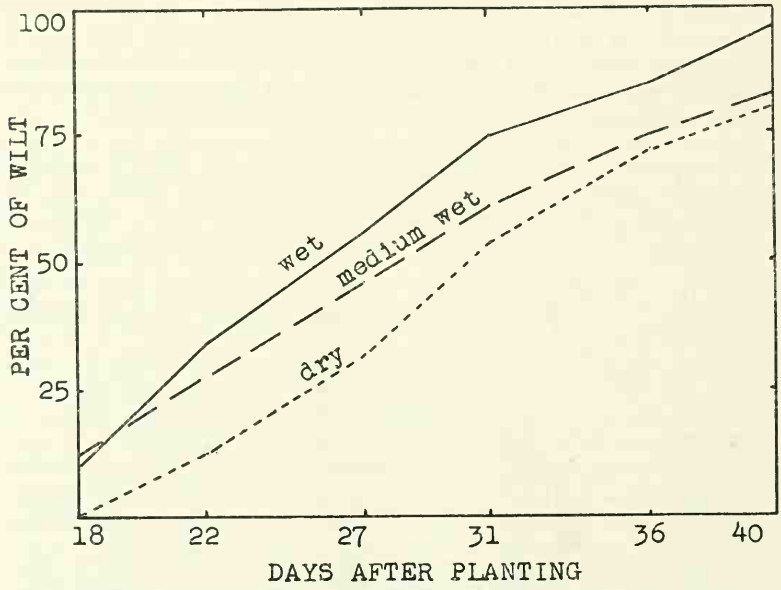


FIG. 8—RELATION OF SOIL MOISTURE CONTENT TO RATE OF DEVELOPMENT OF FUSARIUM WILT OF PEAS.

"Wet" soil contained approximately 90 per cent of its holding-capacity; "medium wet," 65 per cent; and "dry," 40 per cent. Soil temperature experiment II.

ally the same in dry as in wet soil. There are differences, however, in the rate at which the disease develops. As shown in the table, more plants were affected in 31 days in the wet soil than in the medium-wet or dry. Figure 8 shows this to have been apparent also at other times, and much more conspicuous early in the experiment than late. Table III shows, however, that more plants were dead after 31 days in medium wet than in either wet or dry soil. This means that the course of the disease after inception is much more rapid in the medium-wet than in the wet soil. Records of the disease in individual plants showed that in medium-wet and dry soil the average interval between the appearance of first symptoms and the death of the plant was six days, as compared with nine days in wet soil. This experiment thus shows: (a) that soil moisture content does not modify markedly the influence of soil temperature upon the development of pea wilt; (b) that the influence of soil moisture content is relatively slight in comparison with the influence of soil temperature; (c) that wet soil favors the earliest appearance of symptoms; (d) that medium-wet soil favors the earliest killing of plants; and (e) that dry soil, while delaying the appearance of initial symptoms, leads to rapid dying of plants after they show symptoms of the disease. Clayton (5) found that soil dry enough to retard the growth of tomato plants delays the appearance of tomato wilt, but Tisdale (33) found that the driest soil used in his experiments was more favorable for the development of cabbage yellows than either medium or wet soil.

Experiment III.—In this experiment the influence of soil temperature upon the disease was studied in two varieties of peas comparatively: Alaska, an early or short-season variety, and Horsford's Market Garden, a late variety. At each of seven temperatures from 12 to 30 degrees, Horsford peas were planted in two soil-temperature cans of infested soil and one

Table IV.—Summary of Results of Soil Temperature Experiment III. The Influence of Soil Temperature Upon the Development of Wilt in Horsford's Market Garden and in Alaska Variety Peas

Soil temperature	Total plants	Days until first symptom	Days until first death	Plants affected after				Plants dead after			
				20 days	24 days	29 days	36 days	20 days	24 days	29 days	36 days
°C.	No.	No.	No.	%	%	%	%	%	%	%	%
Horsford											
12	20	36	--	0	0	0	10	0	0	0	0
15	"	22	36	0	20	85	100	0	0	0	5
18	"	18	26	40	90	100	100	0	0	25	70
21	"	12	20	85	100	100	100	15	35	70	100
24	"	13	24	70	95	100	100	0	10	25	95
27	"	15	24	20	80	95	95	0	5	25	70
30	"	20	30	5	15	25	40	0	0	0	10
Alaska											
12	10	--	--	0	0	0	0	0	0	0	0
15	"	26	--	0	0	10	80	0	0	0	0
18	"	24	36	0	20	60	90	0	0	0	40
21	"	15	20	60	90	90	90	10	10	60	90
24	"	16	22	20	60	70	80	0	10	40	60
27	"	20	29	20	30	50	60	0	0	10	40
30	"	36	--	0	0	0	10	0	0	0	0

of steamed soil, and Alaska peas in one can of infested soil and one of steamed. Soil from Experiment II, sifted and mixed to insure uniformity, was used as inoculum. Soil moisture was adjusted in all containers alike to 70 per cent of holding-capacity. Detailed records of the progress of the disease from inception to death were kept separately for all individual plants, and similar records were made to trace the development of the healthy control plants.

Table IV, presents the chief numerical results of this experiment, and figures 9 and 10 illustrate the results with the Horsford variety. In both varieties alike the disease developed in fewer days at 21 degrees than at any other soil temperature. Throughout the experiment more plants were affected at this than any other temperature, but after 36 days all the Hors-

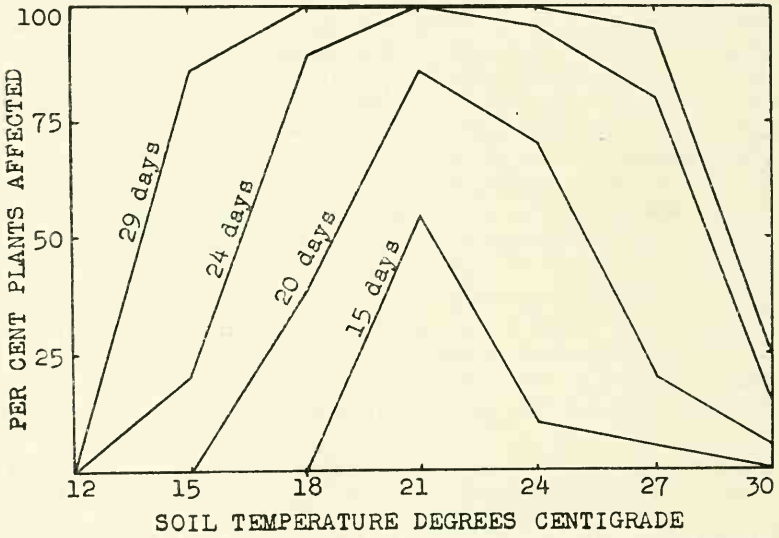


FIG. 9.—RELATION OF SOIL TEMPERATURE TO THE DEVELOPMENT OF PEA WILT.

Per cent of plants affected with wilt at different soil temperatures when grown the indicated number of days in soil inoculated with pure cultures of the pea wilt fungus. Horsford's Market Garden variety; soil temperature experiment III (Table IV).

ford plants were affected at 15, 18, 21, and 24 degrees C. At temperatures above 21 degrees the disease developed more rapidly in both varieties than at temperatures correspondingly below, but it was less regular and ultimately affected fewer plants than in the lower range. As in the first experiment, 24 degrees was distinctly less favorable for the disease than 21 degrees, but more favorable than 18 degrees C.

The disease developed earlier in Horsford than in Alaska peas at the optimal temperature, and likewise developed over a wider range of temperatures. At the close of the experiment, after 36 days, some Horsford plants were dead at all temperatures except 12 degrees, while all Alaska plants were still alive at 12, 15, and 30 degrees C.

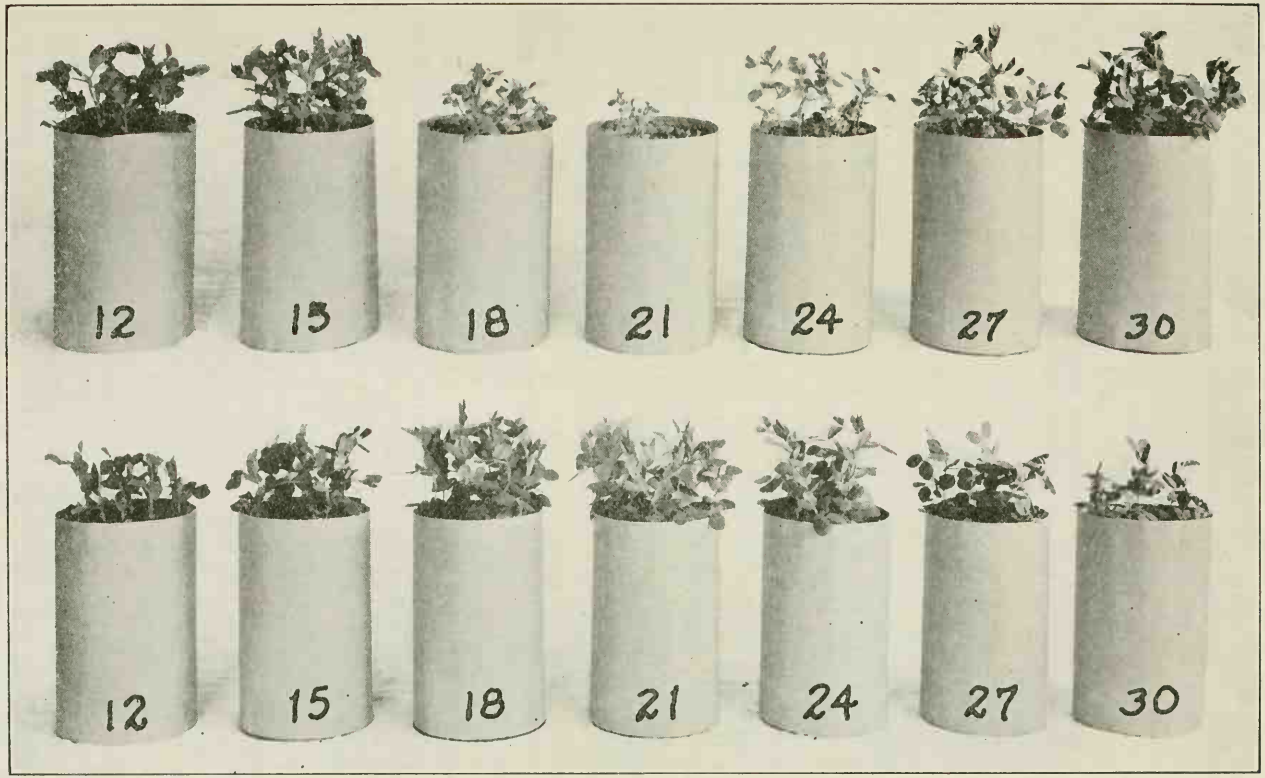


FIG. 10.—RELATION OF SOIL TEMPERATURE TO THE GROWTH OF PEA SEEDLINGS AND THE DEVELOPMENT OF FUSARIUM WILT.

Above, Horsford peas grown in infested soil; below, in steamed soil; soil temperatures as indicated, in degrees centigrade. Soil temperature experiment III, 30 days from planting.

In this as in the former experiments, the most rapid germination and early growth of the control plants occurred at 24 to 27 degrees C. At 30 degrees emergence of the seedlings was only slightly less rapid, but at low temperatures it was much retarded. Judging from the appearance of the plants, both Alaska and Horsford made normal growth from 15 to 24 degrees, and approximately normal even at the extremes of this experiment. Leitch (22) and Jones and Tisdale (13) had shown earlier that continued growth of peas may not be expected at temperatures above 30 degrees, and this was confirmed in Experiment 1 in which peas germinated but soon died at 33 degrees C.

Dry weight determinations made on the control plants in this experiment after 39 days found the greatest weight per plant at 18 degrees in Alaska and at 21 degrees in Horsford. Dry weights of roots alone were greatest at 12 degrees in Alaska and at 18 degrees in Horsford, and were much reduced at the higher temperatures in both varieties. These determinations suggest that the Horsford is adapted to temperatures slightly higher than is Alaska, but this is probably an illusion arising from the fact that the Alaska plants, already in blossom, were much closer to maturity than were the long-season Horsfords which were not yet showing their first blossom buds. Comparison of these results with dry weight determinations made by Richards (29) and Gilchrist (9) on Alaska peas grown shorter times indicates a gradual shift of the greatest dry weight to lower temperatures as the plant approaches maturity. It seems probable from this and other evidence that during a longer time, Horsford peas would develop their greatest dry weight at temperatures below 21 degrees C.

The rate of shoot growth, as measured by the rate at which nodes are expanded from the terminal bud, was not significantly modified by soil temperature where the shoots themselves were exposed to the same air temperature. At the end of ten days, during which all plants came up at even the lowest temperature, there were marked differences in the average numbers of nodes exposed at the different temperatures, but during succeeding intervals until blossoming began in the Alaska and until the close of the experiment in Horsford, the rate of node expansion was equal at all temperatures, and slightly more rapid in Alaska than Horsford. At medium low temperatures, favored by larger and more active root systems, shoots grew coarser and thus, eventually produced a greater dry weight than the more frail shoots produced by the weaker roots in the warm soil.

Since the pea wilt fungus makes its entry into the root system from the soil, and, until the late stages of the disease, develops chiefly in the subterranean portion of the plant, it is probable that the temperature effects upon the host that are most important in the study of pea wilt are the direct effects upon the root system.

Experiment IV.—In a fourth experiment, Horsford peas were grown 50 days at 10, 12, 14, and 16 degrees C. in three lots of soil inoculated each with a separate culture of the wilt fungus, and in one lot of uninoculated steamed soil. One of these strains of the fungus proved less pathogenic than the other two and gave only 70 per cent infection after 50 days at 16 degrees, 60 per cent at 14 degrees, 10 per cent at 12 degrees, and no

infection at 10 degrees C. The other two strains gave 100 per cent infection at both 16 degrees and 14 degrees, 60 per cent and 40 per cent respectively at 12 degrees, and questionable symptoms on a few plants at 10 degrees C. During the 50 days, plants died from the disease at 16 and 14 degrees only; at 12 degrees the course of the disease was extremely slow. Below 16 degrees under the conditions of this experiment, affected plants showed the symptoms of stipule and leaflet roll, yellowing, slow withering, and defoliation, but no true wilting. Plants that showed doubtful symptoms at 10 degrees after 50 days were washed from the soil and their roots subjected to culture studies and microscopic examination. The wilt fungus was observed sparingly in the root cortex but not within the stele. The pathogen was isolated readily from these roots after surface sterilization in mercuric chloride. At 12 degrees the fungus was observed within the stele of the taproot, but not abundantly. The experiment indicates that 12 degrees is an approximate but not absolute minimum soil temperature for the development of pea wilt.

Experiment V.—In a fifth experiment, Horsford peas were grown at 18, 20, 22, and 24 degrees C. in soil inoculated with the same three cultures of the pea wilt fungus as in Experiment IV. In all three alike, the disease developed earlier at 22 degrees than at either higher or lower temperatures.

Discussion of Experimental Results

The relation of soil temperature to the development of pea wilt, determined by these experiments, is strikingly similar to the temperature relations of several closely allied diseases (Table V). In each of these there is a

Table V.—Comparison of the Cardinal Temperatures for Development of Some Vascular Diseases Caused by Species of Fusarium

Host	Pathogen	Cardinal temperatures for disease			Authority
		Minimum	Optimum	Maximum	
		°C.	°C.	°C.	
Tomato	*F. lycopersici*	19	28–29	above 33	(4, 6)
Tobacco	*F. oxysporum* var. *nicotianae*	17–18	28–31	34–35	(12)
Cabbage	*F. conglutinans*	17	26–29	35	(33, 35)
Flax	*F. lini*	14	24–28	34–38	(19)
Pea	*F. orthoceras* var. *pisi*	10–12	21–22	above 30	

critical temperature below which the host develops in infested soil and remains healthy; a distinct intermediate optimal range; and a maximum temperature at which the host escapes the disease. Compare Figure 9 with the corresponding figures of Tisdale (33) and of Jones and Tisdale (19). Pea wilt, however, shows certain individual peculiarities.

The minimum or critical temperature of pea wilt is not sharply drawn. At temperatures progressively below the optimum the symptoms gradually become less complete until at 12 degrees, actual wilting is never observed and the disease assumes more nearly the character of cabbage yellows. Such partial symptoms develop in the Horsford variety in 50 days even at 10 degrees, and an experiment reported below indicates that even

below that the pathogen may gain entrance into the root cortex. A sharply defined critical temperature probably does not exist, but an approximate minimum for the development of disease lies between 10 and 12 degrees in the Horsford pea and probably slightly higher in the Alaska.

No true maximum temperature can be defined, for the disease still develops, though tardily and feebly, at 30 degrees, the highest temperature at which peas will grow. In the Alaska this is very nearly a true maximum.

A clearly defined optimum for the early and severe development of pea wilt lies at 21 to 22 degrees C., but the optimal range for severe development extends about from 18 to 24 degrees C. At 24 degrees the disease develops in fewer days than at 18 degrees, but at the lower temperature the plants become affected while they are in an earlier stage of their development. This is illustrated in Figure 11 in which comparative curves are drawn representing the relation of disease to temperature (a) after 20 days from planting and (b) when the plants at each temperature had developed an average of 5.8 nodes[4] each. This was the stage attained at

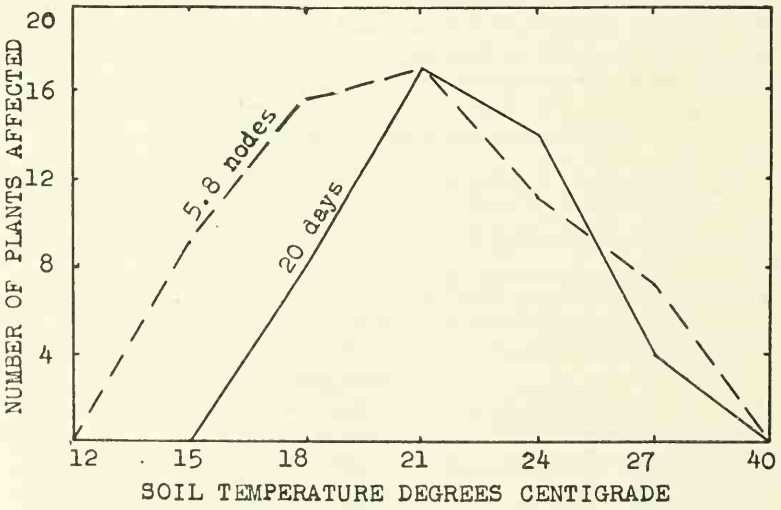


FIG. 11.—INFLUENCE OF SOIL TEMPERATURE UPON THE DEVELOPMENT OF FUSARIUM WILT OF PEAS.

The solid line represents numbers of plants affected 20 days after planting; the broken line, computed numbers of plants affected at the 5.8 node stage. The latter curve is drawn from graphic interpolation of data on rate of node expansion at the different soil temperatures. Horsford's Market Garden variety; soil temperature experiment III.

21 degrees in 20 days. The retarding effect of low soil temperature is thus more marked upon the host than the disease. This interpretation helps to

[4]Peas tend to blossom after developing a rather constant number of nodes, a number which is characteristic of the variety. In Experiment III above, different plants of Alaska set their first blossom at from the tenth to the twelfth node regardless of soil temperature. Thus the number of nodes exposed at any time serves as a fair measure of the stage of development of the pea plant towards maturity.

offset the error introduced in this study by the more rapid germination and early growth of plants at high temperatures.

The cardinal temperatures of pea wilt are distinctly lower than those of the related diseases (Table V): its critical temperature is below that of flax wilt which formerly stood lowest in the series, and its optimum is little above the minimum temperature for tomato wilt. Until this present case, the optimal temperatures for the wilt diseases all were near 28 degrees, and likewise the wilt fungi, when grown on potato-dextrose agar, made their most rapid growth at or near 28 degrees C. Thus it has been concluded, "that the influence of temperature upon the disease development (that of the Fusarium wilt disease in general) is primarily due to its direct effect on the parasite" (20, page 129). The optimum temperature for the pea wilt fungus on this agar, 27 to 30 degrees C., is in close agreement with the related fungi, but is distinctly above the optimal range of temperatures for pea wilt. Thus, while the slow development of the disease below the optimum may probably be explained in terms of the development of the fungus, some other explanation must be found for the retardation of disease development at temperatures most favorable for the fungus. This condition may conveniently be regarded as one of resistance induced in the host by the operation of external factors.

Jones and Tisdale (19) observed that flax wilt develops at lower temperatures than cabbage yellows and tomato wilt and, in correlation, that the healthy flax plant is distinctly a low temperature plant as compared with the tomato. Now the pea is another low temperature plant, as witnessed by the seasonal and geographical distribution of its culture, and pea wilt is favored by still lower temperatures than flax wilt. The healthy pea plant which fails to grow above 30 degrees is injured more quickly by high temperatures than even flax and cabbage which were grown by Jones and Tisdale (19) and by Tisdale (33) respectively at 38 degrees C. This correlation of temperature relations of hosts and diseases suggests the operation of a causal factor, the nature of which is not known.

The temperatures at which the disease declines in severity are those at which germination and early growth are most rapid, leading to early maturity, but also those at which the plant shows a distinct weakening in its later growth. Which of these factors is the more important cannot now be said. The latter fact, correlating with the retardation of the disease in soil too dry for most vigorous growth of the plant as observed in this work with peas and in Clayton's (5) with tomato, appears suggestive. On the other hand, the weakening of pea growth and the decline of the disease at high temperatures may be the result of early maturity of the roots. Without further study, however, it is impossible to say in what way the high soil temperature renders the pea resistant to this disease.

RELATION OF SOIL TEMPERATURE AND MOISTURE TO THE OCCURRENCE OF PEA WILT

The soil temperature relations of tomato wilt, cabbage yellows, and flax wilt as summarized by Jones, Johnson, and Dickson (20), have been found to explain the seasonal and geographical distribution of disease. Cabbage yellows especially is limited in its occurrence to those localities and seasons

in which the temperature is suitably high, and is absent from the cooler cabbage districts.

Pea wilt, however, being favored by lower temperatures than these diseases, would be expected to occur differently. If conditions aside from soil temperature are as favorable in the field as in the greenhouse, it should occur wherever the soil is warm enough for the Aphanomyces rootrot (15), and that disease occurs freely in northern Wisconsin and northern Michigan. A complete survey of northern Wisconsin has not been made, but wilt has been found in the most northerly districts searched. It is most destructive, however, in the older, more southerly pea areas of Wisconsin, a fact which may have resulted from other factors as much as from temperature relations.

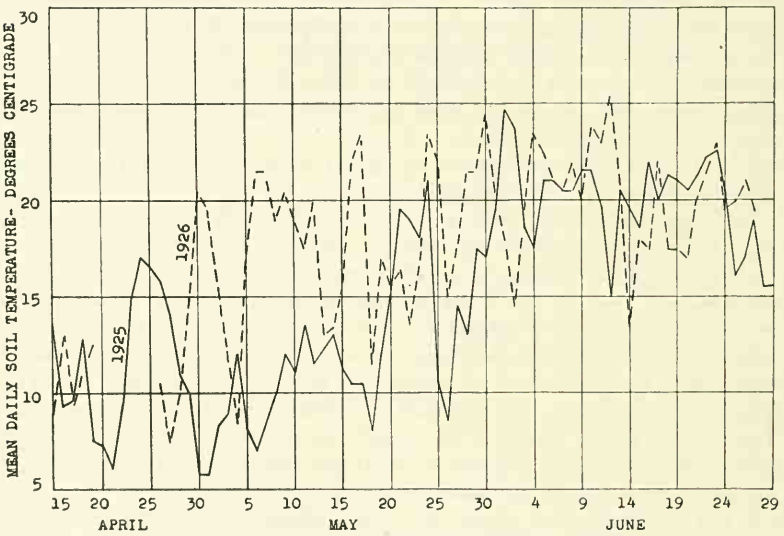


FIG. 12.—MEAN DAILY SOIL TEMPERATURE.

This was taken at a depth of two inches and recorded at Madison, Wisconsin, from April 15 to June 29, 1925 and 1926.

In southern Wisconsin there is no indication of escape from the disease through early planting. The canning crop is planted here chiefly during late April and May. Comparison of the soil temperature records in Figure 12 with similar records presented by other workers for earlier years (28, 33, 15, 16, and 20) reveals the fact that generally the mean daily soil temperature reaches the minimum for the development of pea wilt not later than the middle of May. Harvest rarely begins before the last week of June, and long before this time the soil temperature is practically optimal for the disease. Actually, in both 1925 and 1926 the disease was first observed in this area during the first week of June. Escape from the disease through early planting would seem possible, therefore, only in case peas planted in cool soil were to acquire a degree of resistance which remains effective when the soil later becomes warm enough to favor the disease.

The following experiment was designed to test this possibility.

Four lots of Horsford peas were planted in infested soil at intervals of one week, and were held at the soil temperature of 8 to 10 degrees C. until the first planting was five weeks old. The oldest plants were in the five node stage. Three inoculated and one control can of each age group were then transferred to the soil temperature of 22 degrees, and two inoculated and one control to 18 degrees C. where they were maintained to the end of the experiment.

Table VI.—Comparative Rates of Development of Wilt Symptoms in Plants of Horsford's Market Garden Peas, in Soil Inoculated With Pure Culture of the Wilt Fungus, Grown Two to Five Weeks at a Soil Temperature of 8-10 Degrees and Then Transferred Abruptly to the Temperatures of 18 and 22 Degrees as Indicated; Air Temperature 15-20 Degrees C.

Age in days at time temperature was raised	Soil temperature 18° C.				Soil temperature 22° C.			
	Total plants	Plants affected after			Total plants	Plants affected after		
		9 days	11 days	13 days		9 days	11 days	13 days
	No.	%	%	%	No.	%	%	%
14	20	0	0	0	30	0	13	23
21	20	10	30	50	28	7	43	68
28	20	40	50	80	30	71	90	90
35	20	50	80	100	29	86	97	100

From the sixth day, when first symptoms appeared in the oldest plants, to the end of the experiment, the older plants always showed more advanced stages of the disease than did the younger. The differences were most apparent early in the experiment as shown in Table VI. This experiment does not prove that the oldest plants were the most susceptible (although that possibility merits consideration) for the fungus had already begun to enter the root cortex at the low temperature of 8 to 10 degrees C. The practical importance of the experiment, however, rests on the fact that this low temperature is below that which prevails in the fields in southern Wisconsin after the earliest plantings. Field observations are in direct agreement with this experiment, for the earliest development of the disease in this area has been found in the earliest planted peas.

The single experiment on the influence of soil moisture upon the development of pea wilt suggested a slightly favoring influence of wet soil as compared with dry, but this has not been apparent in the field. Abundant field evidence shows that the wilt fungus develops aggressively in both wet and dry soils within the extremes which favor growth of the pea, but suggests that soils which remain excessively wet for long periods may be less favorable for long persistence of the fungus.

OTHER FACTORS INFLUENCING FIELD OCCURRENCE

Number and Frequency of Previous Crops of Peas

Field observations of limited extent made during the pea disease survey of 1924 (16) indicated that wilt occurs most frequently where peas have been grown repeatedly. In continuing the survey in 1925 an attempt was made to determine this relationship more precisely. Of the fields examined, 599 for which adequate records were obtained were grouped as had been done earlier for rootrot (16) according to soil type, number of crops of peas grown, and the presence and severity of wilt.

Neglecting for the moment the influence of soil type and of intervals between crops of peas, the results of this analysis (Fig. 13) show a close correlation between wilt and the number of crops of peas grown in the field. Only a few fields which were producing their first peas contained the disease, but 57 per cent of all which were growing a fifth crop or more were

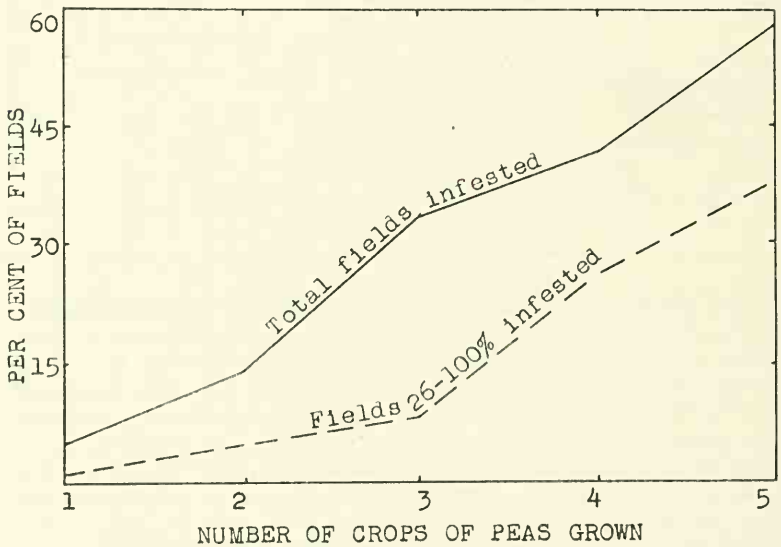


FIG. 13.—INCREASE IN PERCENTAGE OF FIELDS CONTAINING WILT AND FIELDS HEAVILY INFESTED (26-100 PER CENT OF AREA), WITH INCREASE IN NUMBER OF CROPS OF PEAS GROWN.

infested. When the fields which were heavily infested (26 to 100 per cent of their area) are considered by themselves the influence of cropping is more striking. Only one per cent of fields growing their first peas fall in this group as compared with 38 per cent of those growing a fifth crop.

These average figures cannot be taken as absolute for the entire state or as representative of every locality surveyed, for in some regions the disease is rare or absent and in others it is more frequently troublesome than these figures would indicate. In general, however, wilt-free localities contained few fields which were growing more than a second or third crop of peas.

In this analysis no account has been taken of the intervals between previous crops of peas. As in the earlier study of rootrot (16) most of the fields which had grown several crops had not been rotated with any degree of regularity. The majority had grown peas successively or with too short intervals between to be significant in retarding the establishment of the parasite.

Once peas have failed from this disease the fungus appears to persist almost indefinitely, agreeing in this respect with pea rootrot and with many wilt diseases. Several fields have been found thoroughly infested when replanted after intervals of five to 13 years. Whether this is constantly the case is not known, but wherever the fungus persists thus in the soil it seems probable that a long interval between crops of peas in rotation will not eliminate it from an infested field.

Soil Type

When the fields surveyed in 1925 are classified according to soil type, no significant relation is apparent between the wilt disease and any particular soil or group of soils. Wilt, like rootrot (16), was found on almost all soils on which peas were growing, including members from sandy loams to clays of various soil series, calcareous and poor in lime, of both glacial and non-glacial origins. The one soil type on which a considerable number of fields was examined without finding more than a trace of wilt was Colby silt loam. Of 78 fields on this soil, only one contained a single patch of wilt, but since none had grown more than two previous crops, the disease was not to be expected here. Rootrot, however, was devastatingly severe in many of these fields. In general, wilt infestation appeared less frequent on clays and sandy loams than on silt loams (Table VII), which is probably due to the dominance of Carrington and Miami silt loams among pea

Table VII.—Relation of Pea Wilt to Cropping on Light, Medium, and Heavy Soils

	Number of crops of peas grown, including 1925 crop														
	First peas			Second peas			Third peas			Fourth peas			Fifth or more		
	Total fields examined	Infested fields		Total fields examined	Infested fields		Total fields examined	Infested fields		Total fields examined	Infested fields		Total fields examined	Infested fields	
	No.	No.	%	No.	No.	%	No.	No.	%	No.	No.	%	No.	No.	%
Sands and sandy loams	35	4	12	18	3	17	16	5	31	12	0	0	7	3	43
Loams and silt loams	141	5	4	104	11	11	63	24	38	34	19	56	22	14	64
Clay loams and clays	14	1	7	15	3	20	10	3	30	3	1	33	2	1	50
Totals of all soils	234	12	5	159	22	14	116	39	34	53	22	42	37	21	57

soils in the older and most thoroughly infested canning factory districts.

The character of a soil which appeared to influence the disease most significantly is its content of organic matter. Soils naturally rich in organic matter appear to favor more rapid enlargement of infested areas and more uniformly severe injury to plants than poorer soils. The plowing under of large amounts of raw vegetable matter seems also to favor the disease, probably through increase of the fungus in the soil.

RESISTANCE TO FUSARIUM WILT

Wisconsin pea growers have recognized for a long time that certain varieties, notably Yellow (White) Admiral, Green Admiral, and the newer Horal, are less subject to failure from disease than other canners' varieties. In fields where other varieties fail completely these hardy sorts sometimes yield very well; at other times, however, they too may fail. This irregular performance has prevented, up to the present, the widespread planting of these hardy peas on infested fields and has hampered progress in the development of other hardy varieties better adapted to the needs of the canner.

When several pea root diseases were found among the causes of pea failures it was expected that these hardy varieties would prove highly resistant to one or more of them, but, until this Fusarium wilt, this expectation has not been realized. Turresson (37), Jones (14), and Gilchrist (9), found minor differences in varietal susceptibility to footrot diseases, but not sufficient to account for the differences sometimes seen in field trials on naturally infested soil. Important degrees of resistance to the Aphanomyces rootrot were reported by Jones and Drechsler (15) and later by Jones and Linford (16) on the basis of field observations, but these workers recognized that even the most resistant varieties might fail completely under conditions which favor severe development of rootrot. Later, after failing to detect significant resistance to rootrot itself, Jones (17) concluded that the resistance observed under field conditions is probably less resistance to rootrot than to secondary invasion of the vascular system following rootrot, and to Fusarium wilt.

Relative Varietal Susceptibility

The first evidence of resistance to Fusarium wilt of peas came from the observation in 1924 that the Green Admiral remained free from this disease in localities where other varieties were attacked. Again in 1925 this variety and also Pedigreed Extra Early remained unaffected in wilt infested districts, even when directly adjoining other varieties which were dying from this disease. During these two seasons, all the more common canners' varieties were seen to be susceptible to wilt (see list below). Resistance has been found so constantly associated with the related vascular diseases of other plants (2, 26, 18, 27) that with this observational evidence as a basis, greenhouse and field studies were undertaken to determine the extent and importance of resistance to this disease.

In the first experimental demonstration of resistance to pea wilt in January, 1926, the four varieties Alaska, Horsford, Green Admiral, and

Horal, were planted in soil inoculated with pure cultures of the wilt fungus and were grown at a soil temperature favorable for the disease. All the Horsford plants died early from wilt, most of the Alaska plants died but a few remained healthy, while all the Horal and Green Admiral plants were free from wilt symptoms at the end of 46 days.

In a more extensive test, peas of both early and late varieties were grown in a bench of freshly sterilized and inoculated soil. Wilt developed tardily in this planting, but striking differences were observed in varietal susceptibility. The late varieties especially showed clear-cut resistance and susceptibility. Both Green Admiral and Yellow Admiral remained entirely free from any evidence of the disease, while all plants of Horsford and Perfection were affected. Only one of the 23 Horsford plants set seed; and only three of the 71 Perfection even blossomed, and none set seed.

Susceptible early varieties did not develop the disease as early and severely as did the Horsford and Perfection, and, due to their earlier maturity, almost all plants had begun to blossom when symptoms first appeared. In the irregular wilting that followed it was sometimes difficult to distinguish between death from disease and drying from maturity. Varietal differences were therefore less clearly marked than in the late varieties. Eight common stocks of Alaska and two lots of seed obtained from Dr. Wilbur Brotherton Jr., selected from a cross of Rice's 330 on Surprise, proved susceptible, with more than half of the plants dying from the disease. Complete freedom from the disease, indicating high resistance,

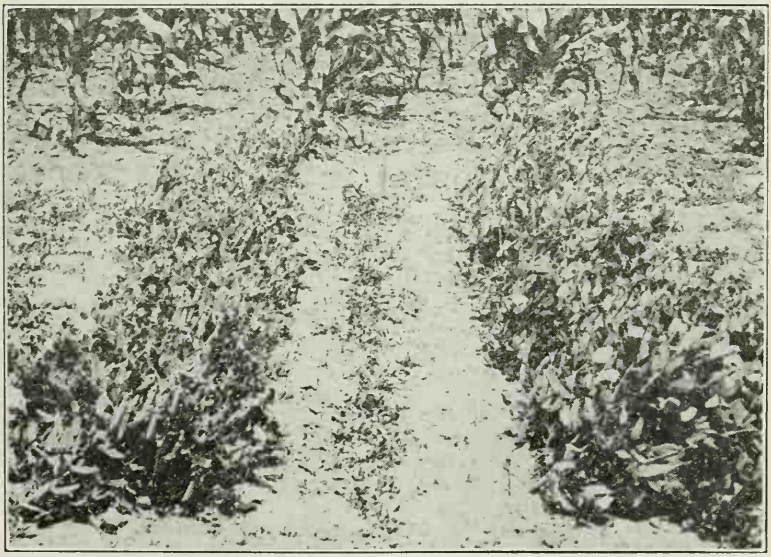


FIG. 14.—WILT RESISTANCE IN PURE CULTURE INOCULATION PLOT, 1926. Horal (left) and Green Admiral (right) show no evidence of injury from wilt, but the susceptible Horsford variety (center) has been killed by the disease.

was found in two early peas, one a strain of Alaska selected for disease resistance by Prof. C. E. Temple of Maryland, and the other a commercial stock of Pedigreed Extra Early.

In the spring of 1926, a resistance test was conducted in a garden plot of well drained Miami silt loam at Madison where peas had not been grown before, again using pure culture inoculation to avoid confusion with rootrot and footrot so commonly met with on naturally infested soil. Eight kilograms of inoculum, consisting of soil inoculated in the greenhouse with a mixture of three different strains of the wilt fungus, were distributed in each 21 foot row before the seed was placed. One row each was planted with the following varieties in the order named: Horal, Horsford, Green Admiral, Pedigreed Extra Early, Brotherton's Selection "A," Alaska, Brotherton's Selection "B," and Rice's 330. Another planting which adjoined this directly and was inoculated in the same manner (described in the following section) included numerous separate plantings of Horsford and Perfection peas.

Wilt developed early and with uniform severity in Horsford (Fig. 14) and Perfection, and more tardily and less regularly in Alaska. By July 6, all Horsford plants were dead and 61 per cent of the Alaskas were affected or dead. Many more Alaska plants died before maturity, but all the other varieties remained uniformly free from the disease to maturity. These varied tests indicate that the more common canners' varieties of peas are either highly susceptible or highly resistant to wilt, with the exception of Alaska which appears to be somewhat intermediate and to contain some highly resistant individuals.

The following lists of susceptible and resistant varieties are based upon: (A), field observations; (B), plot trials on naturally infested soil; (C), pure culture inoculation trials in the open ground; and (D), pure culture inoculation of steamed soil in the greenhouse. The plot trials on naturally infested soil are reported below. Varieties listed as resistant have shown no more than a trace of susceptible individuals.

Susceptible		*Resistant*	
Horsford's Market Garden	A,B,C,D.	Green Admiral	A,B,C,D.
Perfection	A,B,C,D.	Yellow (White) Admiral	B, D.
Advancer	A.	Horal	A,B,C,D.
Gem	A.	Rogers' K.	B.
Rice's 13	A.	Rice's 330	B,C.
Ashford	D	Pedigreed Extra Early	A,B,C,D.
Badger	A, D.	Temple's Alaska	D.
Alaska	A,B,C,D.	Brotherton's Sel. "A"	B.
Winner	A.	Brotherton's Sel. "B"	B.

Resistant Peas Obtained by Selection

Plants which remain free from wilt symptoms on wilt infested soil are found freely in most commercial stocks of Alaska and sparingly in commercial wilt-susceptible late varieties. That such plants might be inherently resistant individuals from which new resistant strains or varieties might

Table VIII.—Results of Test for Wilt Resistance in Progenies of Single Plants Selected for Resistance in 1925; Inoculated at Time of Planting With a Mixture of Three Cultures of the Pea Wilt Fungus; Madison, Wisconsin, 1926

Variety	Total plants	Plants wilted			
		June 22		July 16	
	No.	No.	%	No.	%
Alaska Commercial	157	65	41	--	--
Selections	23	0	0	0	0
Horsford Commercial	183	155	85	183	100
Selections	558	18	3	67	12
Perfection Commercial	161	129	80	159	99
Selections	547	163	30	276	50

be developed was suggested by the findings of numerous other investigators working with related vascular diseases (*2*, *26*, 18, *27*).

To test this possibility, numerous single-plant selections were made from diseased fields of Horsford and Perfection peas at Columbus and Chilton, Wisconsin in 1925. After gathering seed separately from the most promising individuals, a mass selection of each variety was made from the remainder. Survivors were more numerous and more vigorous in Horsford than Perfection.

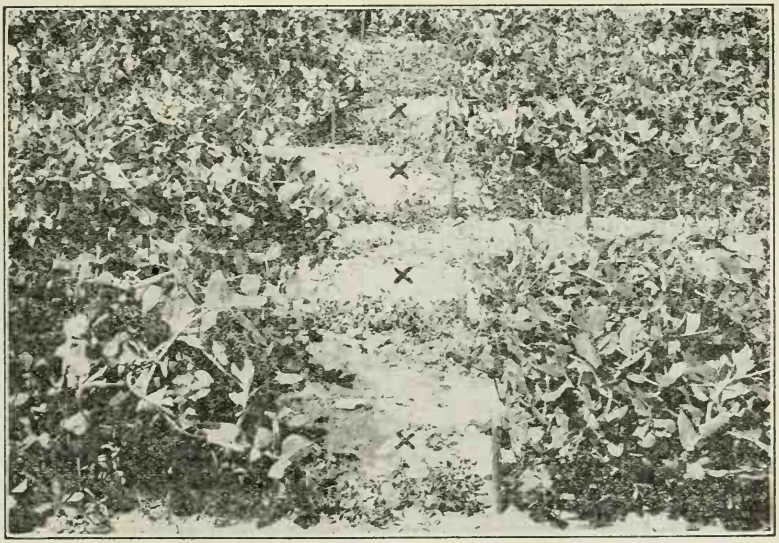


FIG. 15.—WILT RESISTANCE IN THE PROGENIES OF SINGLE PLANTS SELECTED FOR RESISTANCE OUT OF THE SUSCEPTIBLE VARIETY, HORSFORD.

This photograph, which was taken at right angles to the direction of the rows, shows healthy plants in the resistant progenies at left and right, and dead plants of commercial Horsford (indicated by the black crosses) at the center. See Table VIII and IX.

The single-plant selections were planted at Madison, May 12, 1926, in a plot adjoining the last described above and inoculated in the same way. Four single-plant selections of Alaska from the greenhouse were also included in this planting. Ten seeds (rarely fewer) from each selection were allowed one foot of space in the row, and every fifth foot was planted with ten seeds of the commercial variety from which the selection had been made.

Wilt appeared early and uniformly throughout the plot in the commercial variety control plantings. By June 22, many of these plants were dead, and on July 16 very few of them remained alive (Table VIII). Among the selected progenies from Horsford and Perfection, only a few plants were affected as early as the controls. As the season advanced some entire progenies wilted uniformly, and several of them indicated, by the tardiness of their collapse, an intermediate degree of resistance. A few others contained some plants that wilted and others that remained free from the disease. As shown in Table IX, however, all selections from Alaska, 86 per cent of those from Horsford, and 45 per cent of those from Perfection remained uniformly free from wilt (Fig. 15).

Table IX.—Progenies of Single Plants, Selected for Resistance to Wilt in 1925, Grouped According to Their Susceptibility to Wilt as Determined by Trials in Pure Culture Inoculation Plot, Madison, Wisconsin, 1926 (Compare With Table VIII

Parent variety of selection	Single-plant progenies tested	Progenies with—					
		All plants affected		Some plants affected		No plants affected	
	No.	No.	%	No.	%	No.	%
Alaska	4	0	0	0	0	4	100
Horsford	71	7	10	3	4	61	86
Perfection	60	29	48	4	7	27	45

Parallel with this test, thirteen selections from Horsford and seven from Perfection, with appropriate controls, were planted in naturally infested soil which contained a mixed flora of pea parasites. The Aphanomyces rootrot developed tardily and caused little injury. Wilt was therefore the chief disease present but it was less uniformly severe than in the inoculated plot. All plants of commercial Horsford died from wilt. A single selection from Horsford and three from Perfection developed the disease: these same four progenies wilted in the inoculated plot. The remaining progenies developed no wilt, in full agreement with their behavior in the inoculated plot.

The mass selections were tested on naturally infested soil. In the selection from Perfection only two per cent of the plants survived, but in that from Horsford, 85 per cent of the plants remained free from wilt to maturity. It was thus demonstrated that although some of the plants which survive disease on wilt infested soil may do so through the chance escape of infection, many of them are inherently resistant and may serve as a basis for developing new wilt resistant peas.

With the possible exception of one Alaska progeny, all of these selections differed from the parental varieties in some gross morphological characters, and most of them were distinct from all varieties now grown for canning. In general they were off-type plants which, when occurring in canners' peas, are called "rogues." These resistant off-type plants appear to have originated chiefly other than as admixtures from established resistant varieties. In the Alaska pea, resistance is associated with several well marked rogue types, and the greater frequency of resistant individuals here is associated with much more abundant occurrence of off-type plants than in the late varieties. Whether all off-type plants in Alaska are resistant is not known, but in other varieties they are not. In the resistant Horal, susceptible individual plants occur which differ in other respects from the true Horal.

The majority of resistant progenies obtained in the 1925 selections were inferior in type to the resistant varieties at present available, but these results show that by selection resistant stocks may be obtained which may be used either indirectly through hybridization or directly through propagation and possible reselection in the development of new resistant varieties of better quality.

Resistant individuals selected from susceptible varieties are not always of poor type. Field observations in 1926 indicated that the Alcross, an especially uniform strain of Alaska developed by Prof. E. J. Delwiche, contained about 50 per cent of resistant plants of excellent type. Greenhouse and plot tests conducted since that time by Mr. E. J. Renard indicate that these resistant plants breed true for resistance and are indistinguishable in other respects from the susceptible plants within this variety.

Degree of Resistance to Wilt

In the field and greenhouse trials reported above, the wilt resistant varieties showed a degree of resistance which amounted, practically, to immunity. They did not develop wilt symptoms and showed no apparent reduction of vigor. Working with cabbage yellows, Tisdale (33) and Tims (35), found that resistance to that disease breaks down at the temperatures most favorable for the disease. Resistance to pea wilt shows very little break down of this sort, as shown by the following experiments.

Three resistant varieties, Horal, Green Admiral, and Pedigreed Extra Early, were grown in comparison with the three susceptible varieties, Horsford, Perfection, and Alaska, each in three lots of soil inoculated separately with three strains of the pea wilt fungus. All were maintained alike at the soil temperature of 21 degrees C., most favorable for rapid development of disease. The susceptible varieties all failed utterly except a few resistant individual plants in the Alaska variety, but at the close of the experiment when the Extra Early peas were just maturing, none of the resistant varieties had developed wilt, although a few lower leaves had turned yellow and fallen.

In another test, Horal peas were grown 50 days in inoculated soil at 16, 21, and 26 degrees C. without developing the disease. Even at 21 degrees the plants remained as vigorous as the controls grown in steamed soil, but showed some injury in the loss of lower leaves. The fungus was found

sparingly in the cortex of these roots, but not in the stele of the taproot.

Besides such nearly complete resistance there appear to be some intermediate degrees of resistance, as in a few of the single-plant progenies described above and in the Alaska variety. In this early variety the disease requires several days longer to develop and is more sharply inhibited at high and low extremes of soil temperature than in the late variety, Horsford. The Alaska, developing more rapidly, approaches maturity more closely before the disease appears. Such resistance is slight in comparison with the resistance described above, and is not to be confused with the fully resistant plants that occur freely in common stocks of this variety.

Practical Usefulness of Wilt-Resistant Varieties

Since the varieties which are almost immune to wilt are not resistant to the Aphanomyces rootrot it appeared probable that the variable degree of resistance shown in former field trials might have resulted from variation in the relative severities of these two diseases. To test this hypothesis and to determine the extent to which the value of resistant peas may be predicted for a known piece of infested soil, varietal tests were conducted during 1926 in five localities in separate counties of Wisconsin.

On the basis of observations made in 1924 and 1925, fields were selected where rootrot had occurred alone, where rootrot and wilt had occurred together, and where wilt alone had been present. In the selected fields[5] the following varieties[6] were planted in drill width strips, usually in duplicate or triplicate: Alaska, Rice's 330, Perfection, Horal, and Green Admiral.

The trial grounds in two localities were thought to be free from rootrot, but that disease appeared in all five and wilt occurred in all except at Owen. There rootrot alone was devastatingly severe as it had been in 1924 and

Table X.—Summary of Resistance Trials Conducted on Naturally Infested Pea Fields in Different Localities During 1926

Locality	Soil type	Diseases[a] recognized 1924 or 1925	Diseases[a] recognized 1926	Date of planting	Resistance demonstrated
Columbus	Miami silt loam	rootrot[b]	rootrot and wilt	May 7	Marked[c]
Madison	Carrington silt loam	rootrot and wilt	rootrot and wilt	May 11	Marked
Ripon	Carrington silt loam	wilt	rootrot and wilt	May 20	Marked
Owen	Colby silt loam	rootrot	rootrot	May 22	None; all varieties failed
Chilton	Kewaunee clay loam	wilt	rootrot and wilt	May 27	Marked

[a]Other diseases of minor importance were present in some or all of these plots, including Ascochyta blight, Fusarium footrot, bacterial blight, and downy mildew, but none were sufficiently prevalent to have influenced in any important way the outcome of these trials.

[b]The rootrot caused by *Aphanomyces euteiches*, identified microscopically by the presence of characteristic oospores in decayed root cortex.

[c]Varieties susceptible to wilt failed; those resistant to wilt matured a fair crop.

[5]The trial grounds, except at Madison, were provided gratis by the local canning companies.

[6]At Columbus, Yellow Admiral was planted instead of Green Admiral, Rice's 330 was omitted, and Horsford was added.

1925, and the test varieties all failed utterly with no evidence of resistance in any of them. In the other localities, however, there were clearly defined differences between varieties. In each instance, Aphanomyces caused early and extensive decortication of the roots of resistant and susceptible peas, but the plants were little harmed by this. Wilt later destroyed the susceptible varieties, but the wilt-resistant peas remained vigorous to maturity. See Table X.

In each of the trials where rootrot and wilt both occured, it was possible to find plants of susceptible varieties that showed advanced stages of wilt while their roots were still free from rootrot symptoms. It was clear from this that wilt is not to be regarded as secondary to rootrot.

To determine any influence of possible resistance to rootrot upon the outcome of these trials, search was made for varietal differences in earliness and thoroughness of root decay. Early in the season, plants of each

Table XI.—Percentages of Plants With Roots Infected With Aphanomyces Rootrot and of Plants Dead After Specified Intervals, Based on Counts of 100 and 300 Plants Respectively, for Each of Five Varieties of Peas in Resistance Trials at Ripon and Chilton, Wisconsin, 1926

Variety	Varietal type[a]	Chilton		Ripon	
		Plants with rootrot 22 days after planting[b]	Plants dead 42 days after planting[c]	Plants with rootrot 28 days after planting[d]	Plants dead 50 days after planting[e]
		%	%	%	%
Rice's 330	E, R	69	6.6	97	21.3
Alaska	E, S	77	82.0	99	83.3
Horal	L, R	54	2.0	91	11.7
Perfection	L, S	77	98.3	100	99.7
Green Admiral	L, R	73	7.0	98	30.7

[a]E, early; L, late; R, resistant to wilt in pure culture inoculation tests; S, susceptible.
[b]Plants in 5-7 node stage.
[c]Pods set on Rice's 330; Horal and Green Admiral in 11-14 node stage.
[d]Plants in 7-9 node stage.
[e]Rice's 330 four days till fit for canning; Horal and Green Admiral in full blossom.

variety were removed from the soil with care to preserve their roots, and counts were made of the numbers of infected and clean root systems. Table XI shows the results of such counts in two representative trials. Jones (17) had found that Horal sometimes but not always becomes infected with Aphanomyces slightly more tardily than Horsford. In three of the four trials where this count was made the Horal showed this same tendency, but the differences between it and susceptible varieties were slight, and no correspondingly delayed infection could be found in the other resistant peas. This possibly indicates a minor degree of resistance to rootrot in Horal, but too minor to be significant in interpreting the outcome of these trials.

The formation of periderm, described by Jones (17) as a possible factor in resistance to rootrot, was found in resistant varieties but not to any extent in the susceptible in all of the trial grounds except at Owen. There, where rootrot occurred alone, periderm was present in susceptible varieties as well as resistant. Late varieties had developed it somewhat more strongly than the early ones. At Madison, likewise, in plots where rootrot occurred

alone, periderm was developed almost equally in susceptible and resistant varieties, but where wilt and rootrot occurred together it was limited to the resistant. The formation of periderm thus appears to be a response to cortical decay which may be prevented, in varieties susceptible to wilt, by a simultaneous attack of wilt which weakens the plants before the response can begin. A well developed periderm may enable plants to withstand Aphanomyces rootrot, but its formation, under common field conditions, appears to be more an indication of resistance to wilt than to rootrot.

In all of the trials except at Owen the difference between resistant and susceptible varieties was the difference between a fairly satisfactory crop and complete failure. Where the two diseases occurred together, the resistant peas were weakened in proportion to the severity of rootrot and did not yield as well as on clean soil, but plants were killed outright only in wet pockets. The difference between resistance and susceptibility was greater in all cases in late than early varieties. Thinning of the stand from irregular dying of scattered plants was one of the chief weaknesses of resistant varieties in two of the trials. Such dying may have been due in part to the rootrot fungus itself but it appeared to be chiefly attributable to the two footrot fungi, *Fusarium martii* var. *pisi* and *Mycosphaerella pinodes* micro form, which were isolated many times from such plants. Fifteen acres of Horal peas adjoining the trial at Columbus, planted on three successive dates, yielded very satisfactorily in all three plantings even though the roots were extensively decorticated at an early stage.

The outcome of these trials provides a fair basis for judging the practical value and the limits of usefulness of wilt resistance in peas. Resistance to this disease now appears to have been the chief factor in the observed resistance of certain varieties formerly reported, at least in Wisconsin. Wilt has been found with rootrot in the trial grounds in which several of the earlier examples of resistance, supposedly to rootrot, were observed. The behavior of resistant peas in the field, which was formerly baffling in its irregularity, appears to be readily understood on this basis.

If wilt occurred by itself, complete relief could be attained by planting resistant varieties, but this condition is seldom found in old pea fields. Rootrot and footrot usually occur with it and may either destroy a crop independently or thin out the stand. The value of wilt resistance is thus diminished in proportion to the severity of these other diseases, being greatest where they are absent, and negligible where they are severe enough to destroy the crop. Where rootrot occurs destructively, as in the Colby silt loam area of north-central Wisconsin, there appears to be nothing gained by the use of wilt resistant peas. This is true generally of very wet soil types and poorly drained fields in other localities as well. For well drained soils in southern Wisconsin, and elsewhere where wilt is known to be the major factor among pea diseases, they may be recommended. Even here, however, they may fail from rootrot in wet years.

CONTROL OF FUSARIUM WILT OF PEAS

Recommendations for the control of pea wilt follow closely those already given (15, 16) for the control of rootrot. Obviously they must take into

account the control of other diseases which may occur destructively with wilt. The following brief summary seems, therefore, sufficient for present purposes when considered in connection with the previous publications cited.

Recommendations

Rotate crops systematically with as long an interval between peas as practicable, preferably as long as five or six years.

Avoid planting peas where failures from disease have occurred.

Avoid transfer of soil from infested to clean fields.

Avoid planting seed grown on infested fields.

Carefully dispose of pea vines, particularly from infested fields. Vines should be cured in silos or silage stacks; refuse should not be used as manure where peas are even to be grown.

Resistant varieties may be planted where wilt occurs by itself or where other diseases are of minor importance. They are not dependable where rootrot occurs severely with wilt.

In the control of wilt, rotation of crops is advisable in spite of the long persistence of the parasite in infested soil, and for the control of other pea diseases it is an essential precaution. In the case of wilt, even if rotation should not permit more crops to be grown than will continuous cropping before the disease becomes troublesome, it will at least delay the establishment of centers of infestation from which the parasite may be spread to other fields and farms. Cannery districts in which systematic rotation has been followed from the beginning of pea culture are today avoiding much of the trouble from wilt and some other diseases which is being experienced in neighboring districts where rotation has been practiced less diligently.

Losses can be very largely avoided at present by careful selection of fields for pea culture to avoid planting where the crop has once failed. Except in the oldest and most intensive pea growing areas there is still sufficient disease-free land for the production of the pea crop. In such localities the need for suitable wilt-resistant varieties is acute. Elsewhere, if cannery field agents and farmers would keep records of pea failures and, upon the appearance of small amounts of disease, abandon pea culture in infested fields during two or three rotation cycles, important losses could be almost eliminated.

GENERAL DISCUSSION

The recognition of Fusarium wilt marks an important advance towards an understanding of those factors which cause peas to fail when they are planted repeatedly in the same fields. Instead of complicating the problem of pea disease control, the finding of this disease has actually simplified an already complex problem through analysis, and has opened the way to the elimination of considerable loss.

Wilt of peas is probably not a new disease in Wisconsin: its present occurrence suggests that it has been present for years, obscured by its frequent association with the Aphanomyces rootrot. Where the two diseases have occurred together, rootrot has been regarded as the cause of the total injury, and thus has been considered of greater economic importance in

wilt-infested localities than it really is. It is now evident that the severe occurrence of rootrot is restricted to wet soils and wet seasons even more closely than was reported in 1925 (16). At the time of that study the two diseases were still largely confused and the injury from their combined attack was then attributed to rootrot alone. It is now apparent that on medium to light, well drained soils, rootrot may lead to extensive root decortication without causing serious losses if it is not complicated by other diseases.

Special encouragement lies in the significance of wilt-resistant varieties. Their adoption by the canning industry promises to bring wilt fully under control, but since wilt is only one of several factors which jointly cause pea failures, the problem of reducing the damage caused by the other diseases must be attacked with renewed vigor. In extremely wet soils which favor the greatest severity of rootrot, the avoidance of crop failures appears to depend entirely upon withholding pea culture, but elsewhere, where rootrot and related diseases are less destructive, any slight reduction in their severity may be of the utmost importance together with complete control of wilt in permitting the continued production of peas. One of the possible means of accomplishing this which merits close attention is the combining of resistance to these other diseases with resistance to wilt.

While wilt-resistance appears to have been the chief factor in the disease resistance observed earlier in Wisconsin and northern Michigan, there may also be some degree of resistance to rootrot itself. This was not found by Jones (17) in significant degree in the greenhouse, but it has not yet been searched for adequately in the field. The earlier varietal trials (15, 17) in which resistance was sought, were conducted chiefly or entirely where rootrot and wilt were both present, as indicated by the recent isolation of the wilt fungus from plants grown in these trial grounds, and since the presence of wilt would completely obscure any minor degree of resistance to rootrot that might have been present, the failure to detect such resistance in these trials is not conclusive proof of its absence. Minor degrees of resistance to the footrot diseases have been reported (37, 14, 9); they merit further study. Furthermore there is the possibility, not yet tested experimentally, of resistance to secondary invasion following rootrot (17). If such vascular invasion does occur, resistance to it might be expected to correlate with resistance to wilt.

It is probable from earlier investigations that any varietal differences in susceptibility to rootrot or to footrot or to secondary invasion following these will be slight, but any which may be found, even if too slight to be important by itself, may be of considerable value when combined with resistance to wilt. Furthermore, any means other than the developing of resistant peas that may reduce the losses caused by these other diseases will expand markedly the scope of usefulness of wilt-resistant varieties.

In addition to its practical importance, pea wilt is of considerable interest biologically. Comparison with related vascular diseases of other plants caused by allied species of Fusarium reveals several important points of similarity and contrast.

The symptoms of pea wilt are somewhat intermediate between those of cabbage yellows and tomato wilt, with more actual wilting than in the cabbage disease but less than in the tomato. Wilting is not the most characteristic symptom and may, indeed, be entirely absent, as at low soil temperatures where affected plants become yellow and wither slowly, leaf by leaf. At favorable temperatures, the earliest symptoms are directly opposed to wilting and include increased rigidity of the entire plant, rolling of the leaflets and stipules while still turgid, and an increase in diameter of the lower internodes of the stem. These preliminary symptoms are more characteristic of the disease than is wilting alone.

The distribution of the pea wilt fungus through the aerial parts of the plant is relatively limited in comparison with tomato wilt and cabbage yellows. In the pea the upper half of the stem and even the lowest leaves are generally free from mycelium. On the other hand, the fungus appears to accumulate in the xylem vessels in the upper subterranean parts more abundantly than in the other diseases. While this fungus is characteristically an invader of the xylem vessels, as are the allied parasites, it appears less closely limited than some. It damages the cambium rather early and invades the phloem and pericycle at numerous points well in advance of death of the plant.

The earliest symptoms appear at a time when the fungus occurs in the stele in very small amounts, indicating that the pea is highly susceptible to injury. Actual plugging of the vessels with mycelium to such an extent as mechanically to obstruct the passage of water cannot be the cause of any but the final stages of the disease. The most characteristic preliminary symptoms are probably caused by the action on the host cells of injurious products of fungous metabolism.

In its relation to soil temperature, pea wilt is of particular interest because of the detailed study given several related vascular diseases by other investigators who have found remarkable similarity between them. The disease-soil-temperature curve of pea wilt is strikingly similar to those of the allied diseases, but differs notably in that it is transposed to the low temperature side. The most rapid and severe development of this disease occurs at temperatures (21 to 22 degrees C.) several degrees below the optimal range of the other diseases. No detailed study of the influence of temperature upon growth of the parasite on diverse media has been attempted, but on potato-dextrose agar its growth-temperature relation is closely similar to that of the related vascular Fusarium species. In these other diseases, the soil temperature most favorable for rapid development of disease has been almost precisely the same as for most rapid linear growth of the fungus on this substratum. In the pea, however, the optimum for the disease lies distinctly below that for the fungus, and the disease is much reduced in severity at the temperatures most favorable for the fungus on this medium. This case appears particularly favorable for a detailed study of the metabolism and temperature relations of the fungus in relation to composition and physiology of the host.

SUMMARY

A Fusarium wilt of peas which was first observed during the summer of 1924 occurs widely in Wisconsin and at least locally in Michigan and Indiana.

In some localities this is the most destructive disease which affects peas grown for canning. In Wisconsin as a whole it is second in importance only to the Aphanomyces rootrot.

Fusarium wilt of peas is probably not new. It appears to have been present for several years, obscured by its frequent association with other diseases.

This disease typically occurs in scattered patches, approximately circular in outline, but it may infest entire fields uniformly. In infested areas the crop is generally destroyed completely.

Plants affected with wilt show symptoms which are diagnostically specific from other pea diseases in Wisconsin. Some of them are characteristic of vascular diseases of other crops caused by species of Fusarium, while others appear to be associated with no related diseases.

One fungus, described here as *Fusarium orthoceras* App. and Wr. var. *pisi* (n. var.), is associated with this disease throughout its range. This alone, of the fungi tested, causes the disease.

The pathogen invades chiefly the xylem of the roots and the lower half of the stem, but other tissues of the stele may be invaded, and the mycelium may occur sparingly in the root cortex. This is more strictly vascular than other diseases of the pea caused by species of Fusarium.

Early symptoms of wilt appear before the pathogen is present in large quantities within the stele. Later, the mycelium may accumulate abundantly in the xylem vessels. The most significant phases of development of this disease cannot be attributed to obstruction of vessels by mycelium.

Growth of the pea wilt fungus on potato-dextrose agar may occur from 6.5-8 degrees to 34.5-35 degrees C., but it is most rapid at 27 to 30 degrees C. This is in close agreement with the fungi which cause related vascular diseases.

This fungus is pathogenic in some varieties of *Pisum sativum* and in *Vicia gigantea*, and, weakly, in *V. faba*. No hosts have yet been found outside of *Pisum* and *Vicia*.

Dissemination of the pea wilt fungus is accomplished by any agencies which transfer infested soil or refuse. In Wisconsin pea culture one of the most important of these is the improper disposal of pea vines from infested fields.

The influence of soil temperature upon growth of the pea plant and upon development of wilt has been studied in Wisconsin soil temperature tanks.

Peas grow well from 8 to near 30 degrees C. Most rapid germination and early growth occurs from 24 to 27 degrees C., but 18 to 21 degrees C. is near the optimum soil temperature for growth of the healthy pea plant over a long period.

Pea wilt may develop from 10-12 to 30 degrees C., but severe injury occurs chiefly between 15 and 27 degrees C. In less susceptible varieties, with less aggressively pathogenic strains of the parasite, or under conditions

otherwise less favorable for wilt, the total range of favoring temperatures is shortened at both extremes.

At 21 to 22 degrees C. wilt develops in fewer days and affects plants while they are developmentally younger than at any other temperature.

At temperatures slightly above this optimum, wilt develops more rapidly but in fewer plants than at correspondingly sub-optimal temperatures. At the lower temperatures, plants are affected more uniformly and while they are in an earlier stage of their development. Below 16 degrees C., actual wilting is not a characteristic symptom.

The cardinal temperatures for pea wilt are lower than for the related diseases of other plants. The optimum for this disease is below that for rapid growth of the pathogen in pure culture, and very near that for growth of the healthy pea plant.

The retardation of wilt at temperatures which favor fastest growth of the parasite in pure culture probably indicates a condition of temporarily induced resistance in the host.

Soil moisture has less influence than temperature upon pea wilt. Wet soil slightly favors early development of symptoms, but drier soil favors more rapid death of affected plants.

In southern Wisconsin early planting favors the earliest seasonal development of this disease. Pea seedlings grown to the five node stage in soil too cool for the disease acquire no resistance that remains effective when the soil temperature is later raised.

In Wisconsin, low soil temperature may not be expected to permit escape from the disease in late susceptible varieties. Wilt occurs in the most northerly localities yet searched.

Severe losses from wilt occur chiefly where peas have been grown repeatedly. The wilt fungus may persist in the soil almost indefinitely.

Soil type does not restrict importantly the occurrence of wilt, but a high content of organic matter favors most severe development of the disease.

Varietal differences in susceptibility to wilt are clearly defined. The leading varieties of canning peas are subject to complete failure when planted on wilt-infested soil. Several varieties including Green Admiral, Yellow Admiral, Horal, and Rice's 330, are strongly resistant to wilt.

Susceptible varieties usually contain a few plants that are highly resistant to wilt. Selection of such plants has yielded numerous resistant progenies. Such progenies are chiefly not of true varietal type but they include valuable parent stock for hybridization in the production of new resistant peas. Selection by itself promises, in some instances, to yield resistant peas of good quality.

Wilt-resistant peas remain free from wilt symptoms under conditions very favorable for the disease in susceptible varieties. The fungus may invade their rootlets but it does not progress freely into their vascular systems.

Resistance to pea root troubles, formerly observed in practical field trials in Wisconsin, appears to have been chiefly resistance to wilt. Wilt-resistant peas are not significantly resistant to rootrot, and the variations in resistance formerly observed probably resulted from variability in the relative

importance of wilt and rootrot.

Where rootrot and other diseases occur with wilt they reduce the yield of wilt-resistant peas in proportion to their severity. Rootrot is less frequently destructive on well drained, light to medium light soils, than formerly supposed. On such soils, the planting of wilt-resistant peas reduces losses from disease by eliminating wilt.

Further reduction of losses depends upon the control of the rootrot and footrot diseases. Slight degrees of resistance to these diseases may be of much value if combined with resistance to wilt.

The resistant varieties in use by canners at present are less desirable in type and quality than the best susceptible varieties, and therefore can not be recommended for use except under conditions of known wilt infestation.

The possibility of developing new resistant peas has been demonstrated in this work, and it appears to be only a matter of time until, through the perfection of suitable new varieties, the general adoption of wilt-resistant peas for intensive culture will effectively control this disease.

Brief recommendations are formulated for present guidance in the control of wilt.

Comparison of pea wilt with related diseases of other crops reveals interesting and biologically significant points of similarity and contrast, particularly in the production of symptoms, in pathological histology, and in relation to temperature.

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The Classification of Certain Virus Diseases of the Potato

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CONTENTS

The Classification of Certain Virus Diseases of the Potato[1]

THE TRANSMISSION of one or more viruses from apparently healthy potatoes of standard varieties was reported by the writer in 1925 (5). Subsequent investigations in this and other laboratories have confirmed this observation. It naturally became of special interest and importance to investigate this phenomenon further, and particularly to determine the possible relationship of these viruses to other viruses causing disease in potatoes. In this connection it became necessary to make comparative studies with other known potato viruses, and while doing so, an attempt has been made at the same time to contribute some information which may aid in their classification.

The investigations of Schultz and Folsom (10), Quanjer (9), and others have shown that a considerable number of virus diseases affect the potato. The separation of these virus diseases is based largely, if not entirely, upon symptomatology, this being practically the only system available. The numerous disadvantages and difficulties of this system are too well known by pathologists to warrant detailed discussion. The evidence lies in the existing confusion in virus disease literature and in the difficulties encountered by active workers in interpreting the descriptions of others, as well as in the not infrequent difficulty of interpreting their own descriptions. Any clarifying information which may be added to the present descriptions, especially if it is in the form of a measurable characteristic, should, therefore, prove useful in the classification and identification of plant viruses. In a previous investigation (6), this was attempted for certain viruses affecting tobacco in particular, by determination of the properties of the viruses concerned. The present paper deals with a similar attempt with respect to certain of the more confusing virus diseases of the potato.

The Present Status of the Problem

A detailed review of the literature bearing on the description and classification of the potato viruses, to be of most value in the present connection, would need to be presented in an argumentative form. The potato virus problem itself is not, however, actually as complicated as it may appear

[1]In cooperation with the Office of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

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from a brief study of the literature. Some important questions remain to be conclusively established by repeated corroboration in different parts of the world; whereas others may best be solved by bringing material from various sources under one set of conditions. Other difficulties may be remedied by a more general agreement on nomenclature. No doubt, new virus diseases and new problems may arise, but these will be the more readily handled when the confusion in the older problems is reduced.

While potato diseases of the virus type, under the names of "Krauselkrankheit" and "curly-dwarf," have been known for a long time, the literature on this subject has little or no bearing on the development of the present subject of description and classification of the viruses, since these names were probably indiscriminately applied to a single disease or to a group of diseases. It is only within the last fifteen years that serious attention has been given to the potato viruses. During this time the chief interest has centered around recognition of the fact that several different virus diseases exist on the potato, some of which belong to the "mosaic" group, i. e., those which are more or less similar to the well known tobacco mosaic; whereas others exhibit symptoms of quite a different nature, being more comparable to the "yellows" group. Nearly twenty apparently different viruses of the potato are now said to exist, although, fortunately, probably only three or four are economically important.

Schultz and Folsom (10) in this country, and Quanjer (9) and his associates in Holland, were the first to separate and describe a number of virus diseases of the potato. Their unrelated work was carried on almost simultaneously and consequently an agreement in nomenclature was not to be expected. The nomenclature of each group of workers is usually followed on the respective continents. While somewhat inconvenient, this synonomy is not important if the names used can, in each case, be definitely applied to a specific disease. Other workers have reported new virus diseases of the potato, or have added new names, some of which are accepted by one school, while others regard them as synonymous with previously described diseases.

Schultz and Folsom (10) either describe or accept eight different potato virus diseases on the basis of their own work, namely: mild mosaic, crinkle mosaic, leaf-rolling mosaic, rugose mosaic, leaf-roll, streak, spindle tuber, and unmottled curly-dwarf. In addition, they accept aucuba mosaic, yellow top, and giant hill as belonging to the virus group.

Quanjer (9) has described or accepted the following types: common mosaic, interveinal mosaic, aucuba mosaic, crinkle, marginal leaf-roll, leaf-roll, stipple-streak, and leaf-drop streak.

The description of symptoms by these authors is not and probably cannot be made sufficiently adequate for satisfactory comparison to be made as to the identity of the causal agency concerned, for reasons already referred to. The principal confusion exists, however, as regards the mosaic group. It is generally believed that Schultz and Folsom's rugose mosaic is the same as the crinkle of Murphy and Quanjer. Quanjer's common mosaic may conceivably be either the mild or crinkle mosaic of Schultz and Folsom.

The latter's leaf-rolling mosaic may or may not be related to Quanjer's interveinal mosaic or marginal leaf-roll.

As soon as adequate descriptions and comparisons of the various viruses described by the different workers can be made, it should not prove difficult to reach an agreement on nomenclature, at least for the viruses themselves, possibly using some such system as suggested by the writer for the viruses affecting tobacco (6).

Viruses Studied in Experiments

Our first interest in potato viruses in connection with the present investigations developed as a consequence of a study of the influence of environmental conditions on the expression of mosaic symptoms in various plants (4). In these experiments, the type of mosaic occurring very commonly in Wisconsin on the Bliss Triumph variety was largely used. In publishing these results, no mention was made of the specific mosaic concerned. Following continued work on the Wisconsin Triumph mosaic, both with relation to tuber indexing and further environmental work by Tompkins (12), it was decided to refer to this type of mosaic as "rugose" mosaic. This designation unfortunately came to be used by us generally in conversation and in publications from the laboratory. Following the present investigation, in which we have made special efforts to conform in our usage to the nomenclature of Schultz and Folsom, we have come to the conclusion that the common mosaic occurring on Bliss Triumphs in Wisconsin should be called "crinkle mosaic," a term actually introduced in literature by Schultz and Folsom (11) following our first studies on this disease. According to our present conclusions then, in all preceding publications from this laboratory referring to "rugose" mosaic (3, 7, 12), this disease should be designated as "crinkle" mosaic, identical with the "crinkle" mosaic of Schultz and Folsom, but in no way identical with the "crinkle" of Murphy and McKay (8) and Quanjer (9), resembling, however, more closely their simple or common mosaic.

"Crinkle mosaic" is a very serious disease of Triumphs in Wisconsin, commercial stocks often being infected to the extent of 25-50 per cent. Field inspection, roguing, seed certification and indexing are, however, holding the disease in check in a considerable measure. The symptoms are, of course, frequently more or less masked in the field, and crinkle mosaic may often be confused with mild mosaic for this reason. As far as can be judged from very extensive index records in the greenhouse and from observations in the field, no other virus disease commonly occurs on Triumphs in Wisconsin. Leaf-roll, spindle tuber, leaf-rolling and mild mosaic may occur in rare instances or in small percentages of commercial stocks. Crinkle mosaic may also be the more common disease on the Early Ohio variety as grown in the State, but limited studies indicate that mild mosaic is most likely to be found on Green Mountain, Burbank, and Irish Cobbler varieties. The Rural appears to be comparatively free from marked symptoms of virus diseases. This is in accord with the apparent relatively high resistance of the variety to disease.

The main interest has centered around the virus which we have previously described as obtainable from healthy potatoes (5), namely "spot necrosis,"

which is believed to be a virulent form of the "mottle" virus securable from all healthy potatoes of standard varieties. Since both the mottle and spot necrosis forms were found to be transmissible to various solanaceous plant species, previous claims that various virus diseases of the potato were transmissible to other plant species (9) were, therefore, open to question. Following repeated attempts at correlating this problem with that of Schultz and Folsom, we have now been led to conclude that our spot necrosis virus is identical with their rugose mosaic virus. A large and important problem is involved in this connection which will be discussed in some detail in a later chapter. It may help to clarify the subject, however, if the reader will bear this possible relationship in mind throughout the discussion. If we accept rugose mosaic and spot necrosis as synonymous, the relationship of the "mottle" virus to the former naturally assumes a particularly interesting position as far as the control of the rugose mosaic disease of potato is concerned.

Judging from several years of indexing records at Wisconsin with large numbers of potatoes, it appears that no typical symptoms of true rugose mosaic have developed, and we are not acquainted with the disease as such in the field. The virus with which we have worked was, therefore, either secured from apparently healthy potatoes after passage through tobacco, or was secured from Drs. Schultz and Folsom on Green Mountain tubers.

Certain other viruses, especially those of ordinary tobacco mosaic and of potato "streak," have been used in comparative inoculations. The "streak" virus has behaved very erratically in our trials, with the result that its properties could not be definitely determined. The rugose mosaic virus has yielded typical streak symptoms and streak has yielded rugose mosaic symptoms, although more often no infection was secured. It is not unlikely that the "streak" virus, if it exists at all as a separate entity, may be a form of the rugose mosaic virus. Such a possibility has already been suggested by Murphy and McKay (8) and Atanasoff (1).

The viruses with which we have worked have, therefore, been mainly those of crinkle mosaic, mild mosaic, leaf-rolling mosaic, and rugose mosaic. These have been secured in part originally from Wisconsin potatoes, but also in part from material kindly supplied to us at different times for comparative purposes under the above names by Dr. E. S. Schultz and Dr. Donald Folsom. (See Figure 1.)

Experimental Methods

The plan of the investigation of the potato viruses was based largely upon experience gained in an earlier study on the classification of various viruses affecting tobacco (6). The carrying out of the proposed investigation with the potato viruses was found to be much more difficult in many respects than that with the tobacco viruses. Artificial transmission with virus extract is naturally necessary in property studies. Failures to secure infection, or the comparatively low percentages of infection secured by artificial inoculation with certain of the potato viruses, accounts for most of the difficulties preventing rapid progress of the work. The comparative sensitivity of the potato viruses themselves to unfavorable condi-

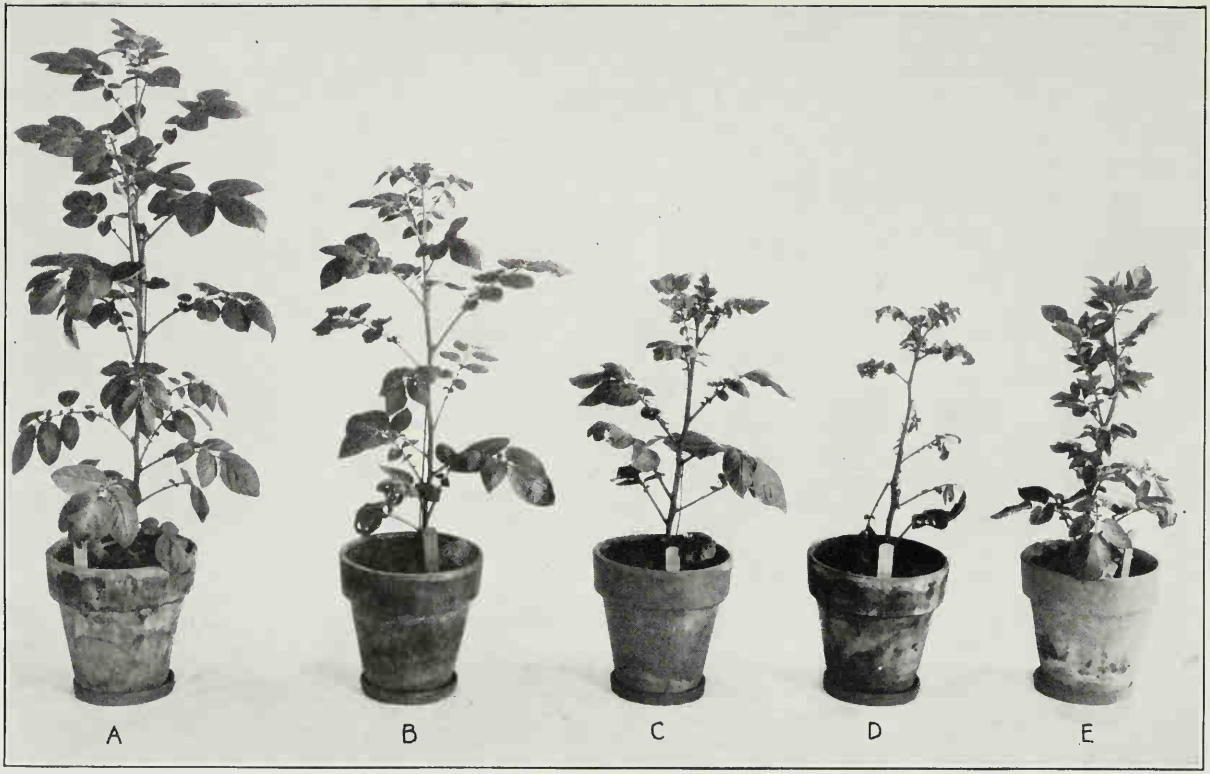

Figure 1

A comparison of the influence of the viruses studied on the general growth and behavior of the Triumph potato plant under greenhouse conditions. Uninoculated control (A), mild mosaic (B), crinkle mosaic (C), rugose mosaic (D), leaf-rolling mosaic (E).

Figure 2

Comparative inoculations with different viruses on Triumph potatoes under greenhouse conditions, illustrating the type of plants used and symptoms secured. Uninoculated controls (A), inoculated with rugose mosaic virus (B), inoculated with leaf-rolling mosaic virus (C).

tions, when in extract, added to the necessity of rapid and careful methods of technique. The requirements in the way of satisfactory plants for inoculation and of desirable environment are in many respects exacting. While these difficulties may in some cases serve as diagnostic features in themselves, they make necessary the accumulation of a considerable mass of data as a basis for reliable conclusions, since negative results cannot be regarded as convincing without very adequate controls.

There are many advantages in the comparison of plant viruses under greenhouse conditions, where a reasonable constancy of environment may be secured, even though such studies do not apply directly to the recognition of these viruses under field conditions. The conclusive determination of a virus may eventually be best accomplished by bringing the virus from the field into the greenhouse for detailed study of its behavior and properties. (See Figure 2.)

The present investigations were, therefore, conducted for the most part during the months from November to May inclusive, and under greenhouse conditions. Everything considered, the months of February, March, and April are most favorable for the work. Two greenhouses were used, one running at a temperature favorable for the normal development of the potato and for the expression of mosaic symptoms (65°-75° F.), the other at a higher temperature (85°-90° F.), favorable for the development of symptoms of mosaic on other solanaceous plants and apparently for the incubation period of the viruses on potatoes. Ordinarily, the potatoes, grown in six-inch pots, were inoculated in the cold house, where they were left for 12-24 hours. They were then transferred to the hot house for ten days, with the purpose of shortening the incubation period, and finally returned to the cold house, where the development and expression of symptoms were observed and recorded at intervals up to four or five weeks. The results are, therefore, all based on current symptoms, following from two to five weeks after inoculation. Tubers were in some cases preserved and grown from inoculated plants, but the added information secured did not seem to justify the additional work involved. It is recognized, however, that secondary symptoms in some cases have diagnostic value, though the interval between inoculation and the development of secondary symptoms is too long to be useful in cases where this method can be avoided.

Inoculum was usually secured from diseased plants by grinding infected leaves in a mortar and straining the liquid through cheesecloth to free it from the pulp. When large amounts of inoculum were needed, the infected plants were passed through a meat grinder before extraction of the virus. In all cases, inoculations were made as soon as possible after extraction, since some of the viruses lose their virulence rapidly outside the host.

The inoculations were all made by dipping a small piece of cheesecloth into the inoculum and rubbing the virus into the leaf until the same was mutilated in several places. Five plants, and sometimes ten, were used for each trial, as a rule approximately one cubic centimeter of inoculum being applied to each plant.

Unless otherwise mentioned, the experiments were conducted with the Bliss Triumph variety of potato. This variety is, on the whole, judging

from our experience, the most susceptible to virus diseases of any of the common American varieties of potato (1).

Diagnostic Features Studied

Symptom expression will, of course, always remain an important diagnostic feature with virus diseases, as it is with other diseases. Symptoms in themselves, however, cannot be regarded as a reliable index of the virus concerned in most cases where diagnosis is required on account of the similarity and overlapping of symptoms produced by different viruses, their extensive modification by environmental conditions and by other circumstances not fully understood, together with the possibility of the existence of viruses in combination. Neither is it likely that any other single diagnostic feature will meet such a requirement. If several unrelated diagnostic features are taken into consideration, however, a process of elimination, or a "key," may be secured which will reduce the possibility of error to a minimum. While other diagnostic features than symptom expression suitable for classification purposes are limited, there are some which will be found very useful in application. (Table I.)

The most reliable diagnostic features of the "property" type are, no doubt, the thermal death-point, the longevity *in vitro*, the effect of dilution, and the influence of certain chemicals. These, together with relative suceptibility, form the basis of the present paper. Several other characteristics more or less useful in classification have, however, been noted in a preliminary way. These are mainly: method of transmission required; length of incubation period; comparative readiness of transmission by a single method, i. e., grafting, insects, mutilation; host range in different species or varieties; relation of source of inoculum to infection; influence of environmental conditions on symptom expression; variation in cytological and histological details; and filterability. These possible diagnostic features have not been sufficiently investigated for all viruses to warrant their detailed discussion in the present paper.

Experimental Results

While recognizing the desirability of presenting experimental data in detail, especially where controversial matters may be involved, it seems justifiable to omit many details in the present instance since the data exist as a large number of separate trials not readily summarized for presenta-

TABLE I.—A COMPARISON OF THE MORE COMMON PROPERTIES OF THE VIRUSES STUDIED AS DETERMINED ON THE POTATO

Virus of	Longevity *in vitro*	Thermal death-point	Tolerance to dilution
Crinkle mosaic	24–48 hours	43–45° C.	1–10
Rugose mosaic	24–48 hours	60–65° C.	1–100
Leaf-rolling mosaic	24–48 hours	70–75° C.	1–200
Mild mosaic	2–4 hours	40–45° C.	1–100

tion. Furthermore, we are more concerned at this time with a proposed method of study than we are with the accuracy of the present determinations. Improved and new technique and greater accuracy of determinations will, no doubt, come in future investigations if the scheme for classification and identification meets with general approval.

Transmissibility by Leaf Mutilation

The experiments conducted have shown that the leaf-rolling mosaic virus is the most readily transmitted to Bliss Triumph potatoes of all the viruses with which we have worked, provided that the plants are exposed to a high greenhouse temperature for approximately ten days after inoculation and subsequently placed at a lower temperature.

The rugose mosaic (spot-necrosis) virus is also readily, but not as reliably, transmitted from potatoes to the Bliss Triumph variety. When transmitted from tobacco to tobacco, however, this virus rarely yields less than 100 per cent infection, although, from tobacco to potato, infection is often not secured (Table II). The "mottle" virus, which can be secured from apparently healthy as well as diseased potatoes, is readily transmitted from potato to tobacco and from tobacco to tobacco, but rarely if ever yields any symptoms on transfer to potato.

Crinkle mosaic can be transferred to Bliss Triumph potatoes by the leaf mutilation method with fair certainty of securing 50 to 100 per cent infection, although sometimes failing to yield infection at all, for unknown reasons.

While 100 per cent infection has been secured on two occasions with the mild mosaic virus, this virus is most difficult to work with because of the low percentages of infection usually secured by the leaf mutilation method, according to our experience.

Incubation Period

The potato may yield the first signs of disease eight to ten days after inoculation with the rugose mosaic virus if incubated at a warm temperature. Usually, however, twelve to fifteen days are required to bring out the first symptoms of disease. The mild mosaic virus appears to require the longest incubation period, twenty to twenty-five days often elapsing before even faint symptoms are evident. The crinkle mosaic virus and the leaf-rolling mosaic virus usually require fifteen to twenty days for the development of symptoms under the conditions employed. On occasional plants, a marked delay of symptom development occurs within a single series of inoculations.

Repeated comparisons have shown that the incubation period can be shortened and a higher percentage of infection secured by an eight to twelve day exposure of the potato plants to a high temperature (85°-90° F.) after inoculation. This temperature is, of course, unfavorable for the normal development of the potato plant, and also for the expression of symptoms, with the exception of those of rugose mosaic. After removal to the cold house (65°-75° F.) the potatoes usually recover rapidly from the high temperature effects, and the disease symptoms develop characteristically.

Aging In Vitro

The determination of this property is fundamental for subsequent experiments on virus properties, since it is important to work with the virus in extract in as virulent a condition as possible. The results show that the potato mosaic viruses as a group are very sensitive to aging *in vitro*, and they probably begin to be inactivated quite soon after extraction. Consequently, it is advisable to perform the treatment to be given to the extract and to inoculate as rapidly as possible. Usually not more than one hour is required between extraction and reinoculation of the viruses in most of the experiments involving trials of thermal death-point, dilution, etc., although two hours or more may be required for trials with filterability.

The extracts were in all cases made from comparatively young potato foliage showing good and typical symptoms. These extracts were merely strained through cheesecloth, placed in stoppered test tubes, and stored at room temperatures for the desired period of aging. It is probably not possible to determine closely the longevity of the viruses *in vitro*, as some variations due to storage conditions may be expected. The data indicate, however, that in the case of the crinkle mosaic virus a large part of the virus is inactivated at the end of six hours, and that it is all inactivated between 24 and 48 hours. In the case of the rugose mosaic virus (extracted from potato foliage) and the leaf-rolling mosaic virus, inactivation is apparently not as rapid, but again little or no infection may be expected from the virus after aging from 24-48 hours. The mild mosaic virus appears to be the most sensitive. Aging for even two hours *in vitro* appears to cause inactivation, although in one case a low percentage of infection was secured after aging for four hours. The mottle form of the virus from apparently

TABLE II.—ILLUSTRATING THE INFLUENCE OF THE SOURCE OF INOCULUM AND THE HOST INOCULATED ON RESULTS SECURED WITH AGING TESTS ON THE RUGOSE MOSAIC (SPOT NECROSIS) VIRUS[1]

Aging of virus	Inoculum			
	From potato		From tobacco	
	To potato	To tobacco	To tobacco	To potato
None	25/8	15/10 (5)	15/15 (0)	15/12
1 day	10/0	10/2 (6)	10/4 (1)	10/0
2 days	25/1	15/0 (2)	15/2 (3)	10/0
4 days	15/0	15/0 (0)	10/5 (0)	10/0
6 days	15/0	10/0 (4)	10/4 (1)	10/0

[1]Upper figure is number of plants inoculated; lower figure number of plants infected; figure in parenthesis represents number showing "mottle" form only.

healthy potatoes (transferred to tobacco) will resist inactivation for ten to twelve days. The rugose mosaic (spot necrosis) virus from tobacco (transferred to tobacco) will also resist aging several days, consequently differing from its behavior when taken from potato (Table II.).

Thermal Death-Point

The thermal death-points were determined by immersing about 5 cc. of the extracted virus in thin-walled test tubes in a well-agitated, constant temperature bath. The time of actual exposure in the bath was ten minutes, after which time the tubes were cooled rapidly in running water. Unheated controls of the same "age" were always used. In most cases, determinations were made only at 5° C. intervals.

The crinkle mosaic virus is inactivated at a temperature of approximately 43° C., although a temperature of even 40° C. is apparently injurious. This conclusion is based on the inoculation of over three hundred plants with virus which had been heated at various temperatures ranging between 40° and 80° C.

The thermal death-point of the leaf-rolling mosaic virus from potato was found to lie between 70° and 75° C., whereas that of the mild mosaic virus lay between 40° and 45° C., this virus being probably more sensitive to heat, however, than that of crinkle mosaic.

The thermal death-point of the rugose mosaic virus, as secured from potato and transferred to potato, lay between 60° and 65° C. The mottle form of virus present in apparently healthy potatoes when taken from tobacco is not inactivated, however, until a temperature of approximately 70° C. is reached; although the spot necrosis form (rugose mosaic) from tobacco may be inactivated at a lower temperature. Here again the source of the inoculum influenced the results obtained.

Tolerance to Dilution

The potato viruses are for the most part relatively intolerant to dilution. A dilution greater than one part of virus extract to ten parts of water usually results in rapid diminution of the percentage of infection secured. The crinkle mosaic and mild mosaic viruses are apparently the most readily inactivated by dilution, withstanding with difficulty dilutions between 1 to 10 and 1 to 100. The leaf-rolling mosaic virus may still be quite infectious at a dilution of 1 to 200. The rugose mosaic virus from potato to potato apparently will not readily stand a dilution much greater than 1 to 100, although infection may occasionally occur up to a dilution of 1 to 1000. The mottle form of the virus from apparently healthy potatoes, taken from tobacco and transferred to tobacco, however, will stand a dilution of at least 1 to 10,000. The rugose mosaic and mottle viruses taken from tobacco will stand similar dilutions more readily than when the inoculum is taken from the potato as host (Table III.).

Influence of Chemicals

The influence of chemicals on the potato viruses has received comparatively little attention thus far. The subject merits more special consideration than we have been able to devote to it. In a general way, we can

TABLE III.—ILLUSTRATING THE INFLUENCE OF THE SOURCE OF INOCULUM AND THE HOST INOCULATED ON RESULTS SECURED IN DILUTION TESTS WITH THE RUGOSE MOSAIC (SPOT NECROSIS) VIRUS[1]

Dilution	Inoculum			
	From potato		From tobacco	
	To potato	To tobacco	To tobacco	To potato
None	20/8	20/12 (8)	20/20	10/0
1–10	10/5	20/14 (6)	20/20	10/0
1–100	15/5	20/8 (11)	20/18	10/0
1–1000	20/1	20/4 (12)	20/11 (5)	10/0
1–10,000	10/0	10/0 (6)	20/4 (7)	10/0

[1]Upper figure is number of plants inoculated; lower figure number of plants infected; figure in parenthesis represents number showing "mottle" form only.

only say that the viruses are comparatively sensitive to such chemical agents as alcohol, nitric acid, and formaldehyde. Twenty-five per cent. alcohol and 1 to 500 HNO_3 (one part nitric acid c. p. to 500 parts of inoculum) inactivate the viruses of crinkle mosaic and rugose mosaic in one hour. On tobacco, the rugose mosaic virus is destroyed by 1 to 200 formaldehyde (1 part 40% formaldehyde to 200 parts inoculum) in one hour, but the "mottle" form remains active. Formaldehyde 1 to 100, however, destroys the mottle form.

Varietal Susceptibility

A marked difference in susceptibility exists among the common American potato varieties to the crinkle mosaic virus. The Bliss Triumph variety is the most susceptible, followed by the Green Mountain, Early Ohio, and Burbank varieties, which show an intermediate degree of resistance. We have not been able to secure definite symptoms on King, Irish Cobbler, or Rural New Yorker varieties in simultaneous inoculations, under the conditions of the experiments.

The rugose mosaic virus shows a similar behavior. In this instance, however, both Bliss Triumph and Green Mountain varieties must be placed in the susceptible class, Burbank, King, Early Ohio, and Irish Cobbler in

the intermediate class, and Rural New Yorker only in the resistant class, although infection may be occasionally secured also on this variety.

Apparently all the varieties named are about equally susceptible to the leaf-rolling mosaic virus, although the experiments have not been carried sufficiently far to determine the finer distinctions in this respect. The results are, however, sufficiently significant to suggest the possibility of using certain differential host varieties for the separation of this virus from such a virus as crinkle mosaic.

The results obtained with mild mosaic are not convincing on account of the small percentage of infection secured, and the difficulty of definitely judging symptoms of the disease on varieties which are not under frequent observation. We are inclined to believe, however, from the studies and observations to date that this virus may affect all of the varieties mentioned, although the symptom expression may in some cases be extremely mild and indefinite. Judging from field observation, it would appear that Green Mountain, Early Ohio, and Burbank are the most susceptible varieties to this disease. The frequency of occurrence of a specific virus under field conditions may evidently be due, however, to other circumstances than host susceptibility.

In this connection it is interesting to note the behavior of the common tobacco mosaic virus on the different potato varieties. This virus, when inoculated to potatoes, produces a more or less marked leaf and stem necrosis as a characteristic symptom, with some mottling and chlorosis. The Rural New Yorker variety is quite as susceptible to this virus as is the Green Mountain. Triumph, Burbank, and Early Ohio varieties are also relatively susceptible, and King and Irish Cobbler most resistant. These varietal studies taken together indicate that resistance or immunity to one virus is not necessarily associated with resistance to other viruses, although some correlation in this respect may be expected to exist (Table IV.).

Symptom Expression

A description of the symptoms produced by the different potato mosaic viruses is in some respects the most complicated and difficult phase of the entire problem. We have previously shown experimentally the striking effect of temperature on certain virus diseases (4) and it is now known that different temperatures affect different virus diseases in a different manner (2). Other environmental conditions, especially those occurring in the field, unquestionably influence the expression of symptoms, and we may expect that such environmental conditions may also have a variable effect depending upon the frequency and relative order in which they occur.

It has, therefore, always been extremely difficult and often impossible to recognize the identity of certain virus diseases by the expression of symptoms as they occur in the field. In our own studies under controlled greenhouse conditions, we have often found such determination equally puzzling. Comparative inoculations with different viruses (as for example with crinkle mosaic and rugose mosaic viruses) on the same variety and under identical conditions frequently have yielded symptoms so similar in

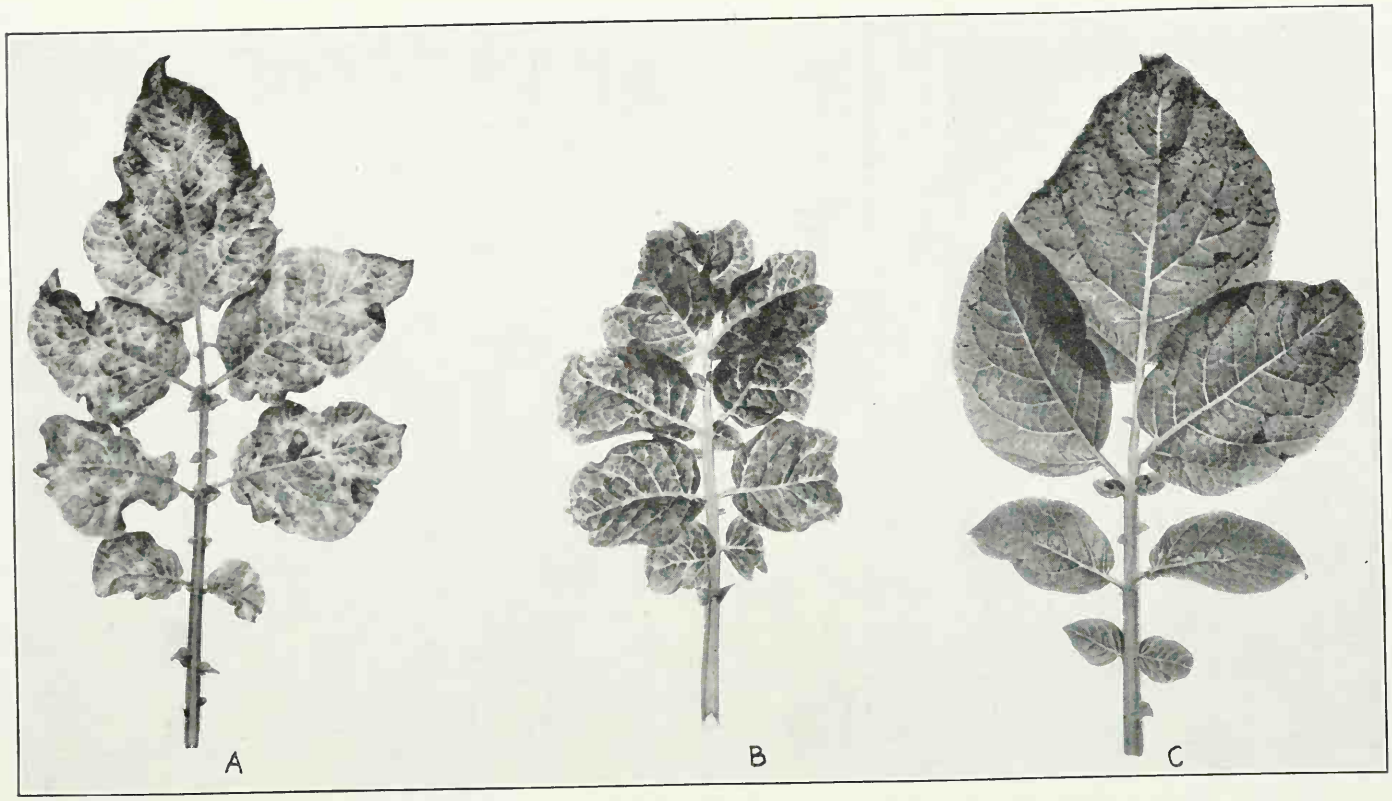

Figure 3
Leaf symptoms on Triumph potato of crinkle mosaic (A), rugose mosaic (B), and "streak" (C).

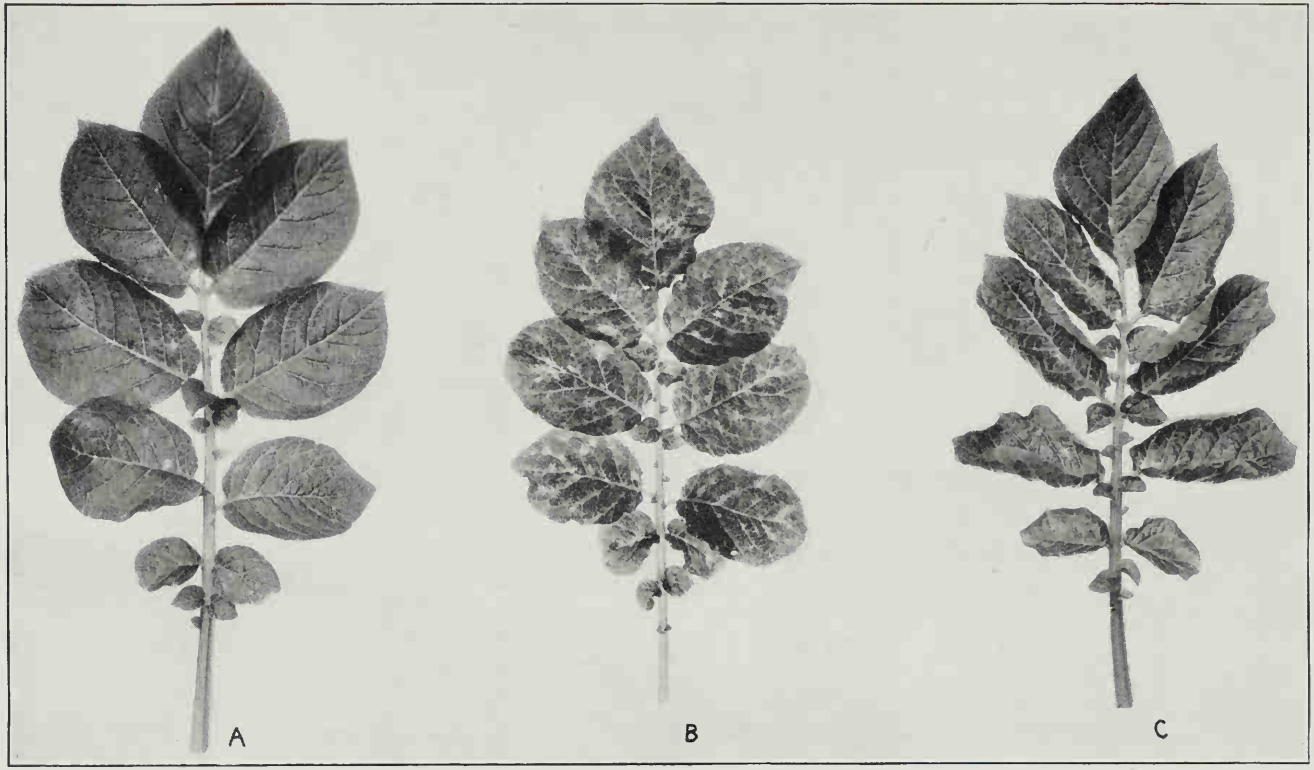

Figure 4

Leaf symptoms on Triumph potato of mild mosaic (B) and leaf-rolling mosaic (C) in comparison with normal foliage (A).

appearance that one disease might readily pass for the other. It is for this reason that any distinctions are of value which may be made on bases other than that of symptomatology.

TABLE IV.—SUMMARIZED RESULTS OF CURRENT INFECTION SECURED WITH VARIOUS VIRUSES INOCULATED TO DIFFERENT VARIETIES OF POTATOES[1]

Potato variety	Virus of				
	Crinkle mosaic	Rugose mosaic	Leaf-rolling mosaic	Mild mosaic	Tobacco mosaic
Triumph	55 / 21	15 / 14	20 / 7	30 / 2	15 / 6
Green Mountain	55 / 6	20 / 19	15 / 4	25 / 1	10 / 8
Burbank	40 / 6	20 / 6	20 / 8	20 / 0	15 / 6
Early Ohio	25 / 4	10 / 3	10 / 5	10 / 4	15 / 4
King	25 / 0	15 / 7	5 / 5	15 / 0	10 / 1
Irish Cobbler	25 / 0	15 / 6	10 / 2	15 / 1	15 / 1
Rural New Yorker	25 / 0	10 / 1	15 / 6	15 / 1	20 / 10

[1]Upper figure is number of plants inoculated; lower figure number of plants infected.

It has furthermore been shown that potatoes normally carry a virus without any symptoms being evident, though we are not certain whether this virus may not manifest itself under certain special environmental conditions. This occurrence of a specific virus in all apparently healthy as well as in diseased potatoes actually means that all of the common potato viruses with which we are working exist only in combination. Consequently, if we still regard the "mottle" virus as distinct from the potato rugose form (or spot necrosis), we are led to the assumption that the rugose mosaic virus can not as yet be separated from the mottle form, although the mottle form can be, and usually is, separated from the rugose mosaic form as it exists in apparently healthy potatoes.

It is admitted, however, that a discussion of potato virus diseases would be quite impossible without reference to the symptoms produced. On the other hand, it is not believed that minor and overlapping details with respect to symptomatology add to the description, and they may result in unnecessary confusion. The following brief descriptions, therefore, apply to the principal current symptoms as observed particularly on the Bliss Triumph variety, under greenhouse conditions favorable to the development of the potato plant:

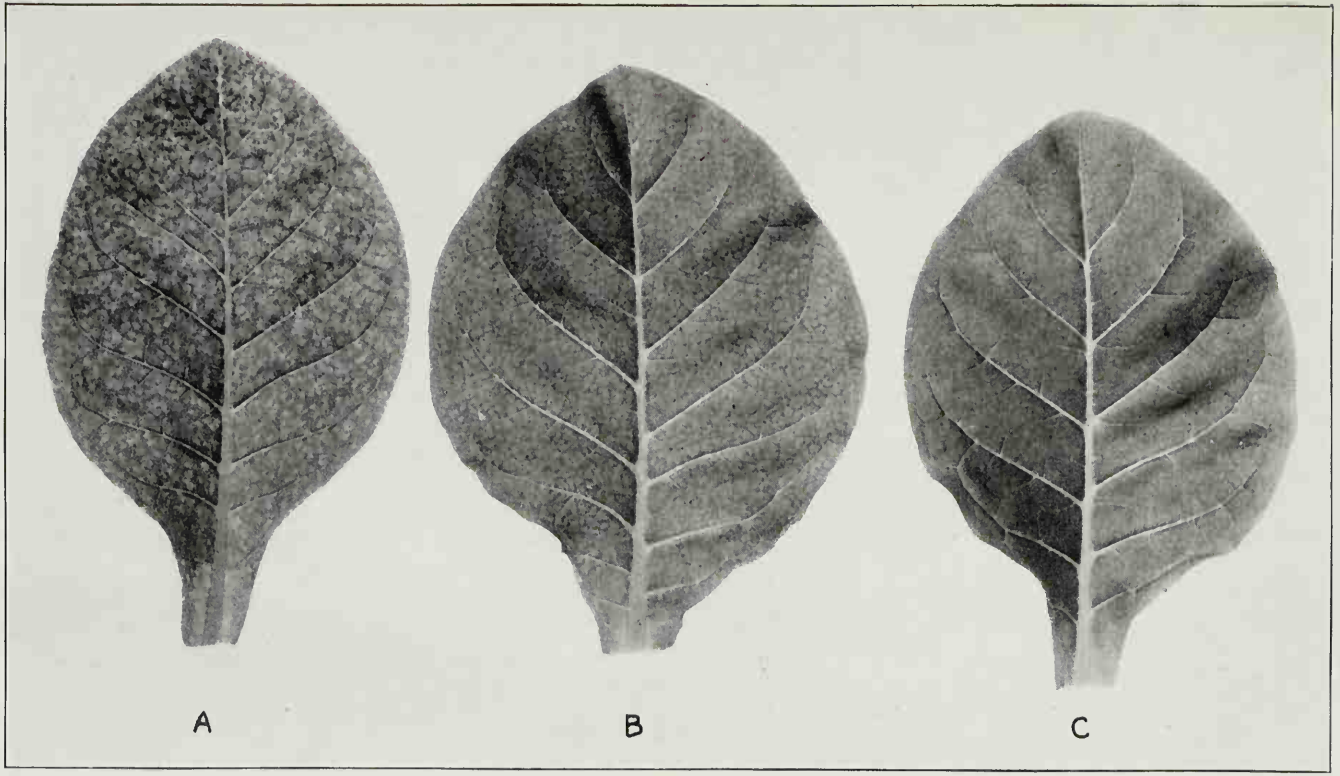

Figure 5

The "mottle" form of virus secured from apparently healthy potatoes. Sometimes this may be perpetuated in tobacco in different degrees of virulence which appear to be fairly stable. A marked form of mottle approaching "spot necrosis" (A), an intermediate form (B) and a mild form (C).

Crinkle mosaic. Distinct mottling of foliage followed by wrinkling[3] or ruffling and dwarfing of leaflets and generally stunting of growth. Leaves may sometimes curl or roll to some extent. No necrosis. Symptoms masked by high temperatures (See Figure 3-A).

Rugose mosaic. Symptoms commonly of two types, i. e., with and without necrosis. The characteristic and distinctive symptom is leaf and stem necrosis, often resulting in death of the entire plant. Frequently the progress of necrosis ceases before death and a dwarfed, mottled and wrinkled foliage continues to develop. In the absence of stem necrosis, mottling and wrinkling similar to crinkle mosaic, but often associated with distinct downward curling of leaflets and leaves; sometimes an upward rolling or rugosity of the leaflets. Streaking on veins, petioles, and stems without serious necrosis frequent. Brittleness of leaves and petioles, followed by leaf-drop characteristic. Occasionally first symptoms may suddenly appear as a yellowing or chlorosis of the foliage, with one-sided leaf or stem necrosis, or streaking. The yield of tubers is very greatly reduced if not entirely prevented. Symptoms not masked by high temperatures (See Figure 3-B).

Leaf-rolling mosaic. The characteristic symptom is a distinct rolling of the young upper leaves of the plant, the disease being different in this respect from the leaf-roll disease, where the rolling more commonly occurs on the lower and older leaves. It is, of course, very different from leaf-roll in other characteristics as well. Mild and diffuse mottling usually associated with the rolling of the leaf. Leaves dwarfed in size and plant as a whole somewhat stunted. No necrosis observed. Symptoms may be partly obscured at high temperatures (See Figure 4-C).

Mild mosaic. Mild mottling, frequently diffuse, and at other times as distinct spots. Mild wrinkling or ruffling of the leaf may or may not be present. The plant is only slightly, if at all, dwarfed. Symptoms masked by high temperatures. This disease may easily be confused with partially masked crinkle mosaic (See Figure 4-B).

The Identity of the Rugose Mosaic Virus and the Spot Necrosis Virus

In the previous discussion we have treated the rugose mosaic virus of Schultz and Folsom as identical with spot necrosis of tobacco and potato. This conclusion as to their identity has been reached only very gradually, and it is difficult to present convincing evidence in this direction. In our earlier paper from this laboratory it was shown that the "mottle" virus could be secured from all healthy (or diseased) potatoes tested (5). The spot necrosis form of virus was occasionally secured directly from healthy (or diseased) potatoes, but more often was secured by repeated transfer of "mottle" through tobacco. It was suspected early in the work that the virulence of the viruses concerned was variable and that mottle and spot necrosis might be one and the same virus in different degrees of virulence. Many difficulties lie in the way of conclusive proof of this contention.

[3]Following in general Schultz and Folsom's definition of "unit symptoms."

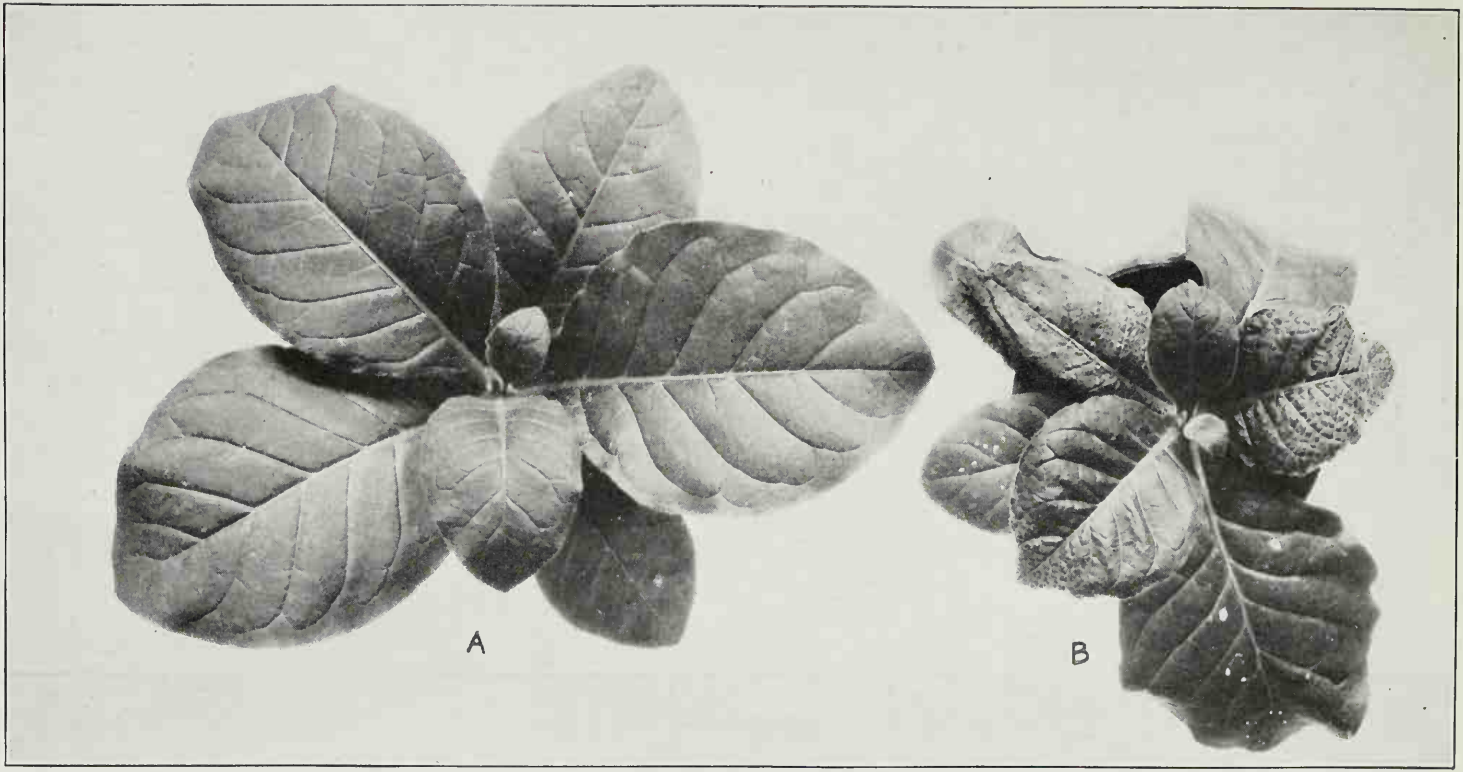

Figure 6
Typical "spot-necrosis" or potato rugose mosaic (B) on Havana tobacco. Uninoculated control (A).

The mottle form from healthy potatoes cannot ordinarily be changed to spot necrosis at will. As far as is known at present, spot necrosis can never be secured separate from the mottle virus, although the mottle form may presumably exist separately from spot necrosis. There is some reason, therefore, for the assumption that we are dealing with two viruses in this case. Nevertheless, following repeated experiments which demonstrate changes in virulence and attenuation of these and other viruses, it was finally concluded that "mottle" was only a mild form of spot necrosis, and both forms were included in one category (6).

Prior to this work, Quanjer (9) and Schultz and Folsom (10) had claimed that certain of the potato viruses were transferable to tobacco and other solanaceous plants. The investigations with viruses from apparently healthy potatoes naturally led us to question those results. Repeated trials with the transference of viruses from potatoes affected with various diseases to other solanaceous species yielded on the whole results similar to those from apparently healthy potatoes. However, in the fall of 1927 a lot of rugose mosaic Green Mountain potatoes were received from Dr. Folsom which yielded in every case infection on tobacco of the spot-necrosis type. At about the same time we were forced to the conclusion that the common mosaic occurring on Bliss Triumph in Wisconsin was not rugose mosaic but "crinkle mosaic," according to Schultz and Folsom's descriptions and specimens. We were, therefore, able to follow Schultz and Folsom's claim that the rugose mosaic virus could be transmitted to tobacco, and we found that it produced symptoms very similar to, if not identical with, our spot necrosis virus. It was consequently possible that Schultz and Folsom's rugose mosaic virus, Murphy's and Quanjer's crinkle virus, and our spot necrosis virus were identical.

Returning now to the viruses secured from apparently healthy potatoes, it is possible to accept one of two different explanations; namely that the "mottle" virus is a specific virus, different from any previously described virus, or that it is a mild or attenuated form of potato rugose mosaic, existing in all apparently healthy potatoes, but under certain special conditions increasing in virulence and causing the disease known as "rugose mosaic." The virulent form being practically self-exterminating, however, is not commonly perpetuated, and consequently is not generally economically important in the more susceptible varieties. We have at least not seen it occurring naturally on Wisconsin Triumph potatoes, and it is reported as rare from other important potato-growing states as well. If the latter explanation (i. e., origin from apparently healthy potatoes) should be the correct one, it is at least interesting to speculate as to the significance of control measures for a disease of this type.

In our experiments we have started on various occasions with the mottle form only from healthy potatoes, and by repeated transfer through tobacco have secured the spot necrosis (or rugose mosaic) form of virus on tobacco. (Figure 5.) We have also been able to secure various intermediate forms of mottle between the extremely mild form and the spot necrosis form, perpetuating themselves true to type (Figure 6). As previously stated, however, we have on other occasions frequently failed to increase the virulence.

It cannot be positively stated, however, that the spot necrosis form was not accidently transmitted in some unknown manner to the experimental plants in the cases where this sudden change in virulence was noted, although we are personally satisfied that such was not the case. The spot necrosis form sometimes may be secured directly from healthy potatoes, which furthermore remain healthy to all appearances for weeks following the use of portions of them for inoculum (5). Furthermore, the occurrence of spot necrosis (rugose mosaic) on tobacco following inoculation from rugose mosaic (spot necrosis) potatoes is the exception and not the rule. In one series of trials, twenty-six successive sets of inoculation from rugose mosaic potato to tobacco yielded only the "mottle" form and no typical "spot necrosis" symptoms, whereas the same virus from tobacco to tobacco always yielded "spot necrosis." It is uncertain whether such differences are due merely to the source of the inoculum (Tables II., III.) or to differences in the viruses.

Turning now to another type of evidence, we find the rugose mosaic virus to be extremely sensitive to external conditions, and readily inactivated, attenuated or localized in the plant according to the explanation that best fits the results obtained. As previously stated, we have only observed this virus in association with the mottle form. Certain treatments of the extract, such as aging, dilution, heating, chemicals and filtration, or a change in environment of the infected growing plant may readily remove the spot necrosis form of the virus from the "combination" and leave only the "mottle" form. A growing tobacco plant showing a virulent form of spot necrosis (rugose mosaic) on the lower leaves, placed in a different environment (usually a somewhat higher temperature) will outgrow the necrotic symptoms and the new mildly mottled or symptomless leaves will yield only the "mottle" form, whereas the older leaves from the same plant with necrotic symptoms will yield the spot necrosis form.

If the plants infected with spot necrosis are exposed to different temperatures or to certain temperatures for different lengths of time, it is possible to isolate the virus in various degrees of virulence between the two extremes. Several explanations may be offered. We believe we are dealing here with a virus extremely subject to attenuation as compared with tobacco mosaic, which requires quite extreme measures for the development of attenuated forms. In this respect, the spot necrosis virus would resemble certain of the human and animal virus diseases in which mild and virulent forms of the disease are said to occur commonly.

If we accept the explanation that the "mottle" form of virus which is normally present in healthy potatoes is an attenuated form of potato rugose mosaic, the theory previously suggested that the potato protoplasm may be the cause of the disease is no longer tenable. If the "mottle" virus is regarded as distinct from the rugose mosaic virus, or if the rugose mosaic virus is regarded as a more virulent form of "mottle," this theory may still remain as a possibility. It may yet be argued that the spot necrosis virus on tobacco and potato and the rugose mosaic virus on tobacco and potato are not identical, but we have not observed sufficient evidence in our experiments to justify serious consideration of this possibility.

Discussion of Results

It is hoped that the investigations reported in this paper may be of some material aid in eventually clearing up questions of classification and nomenclature of potato virus diseases. We are confident that these studies have at least been of value as far as Wisconsin conditions and the Bliss Triumph variety of potato are concerned. Fortunately, the investigations have tended to decrease rather than to increase the number of names to be applied to specific viruses affecting the potato, and the problem on the whole seems in some respects less complicated now than at the beginning of the studies. From an international viewpoint, much remains to be done in the way of corroboration and agreement on nomenclature, but this should not be difficult as soon as an agreement can be reached as to the specificity and description of the viruses concerned. It is hoped that the property and behavior studies such as described in this paper under controlled environmental conditions which can be approximately reproduced upon definite potato varieties may materially aid such mutual understanding and agreement. There is probably no need, for instance, of regarding rugose mosaic, Murphy and Quanjer's crinkle and spot necrosis as due to different causes, and until some one shows more convincing data than has heretofore been presented, the "streak" disease of potatoes may also well be included in this category. Particularly promising too is the possibility that the causal agency of these diseases may be connected up with the virus commonly associated with apparently healthy potatoes.

Even more important than agreements on description and nomenclature of a specific virus, is the recognition of virus combinations and their separation into the constituent entities, thereby reducing confusion by avoiding the publication of new descriptions and names of uncertain and unreliable significance. An understanding of virus properties may be expected to be of particular help in this respect. By the use of certain treatments of the extracts based on their respective properties, it is certainly feasible to separate certain combined viruses from each other. In other cases, the use of the selective action of some varieties of potatoes or other host species for particular viruses may be used to separate certain combinations of viruses into their component parts.

An outstanding feature of these investigations has been the recognition of the fact that although we have used the symptoms exhibited as a partial criterion in judging the presence or absence of a specific virus disease, the limitations of this method have become increasingly evident with the progress of the investigation. Symptoms on several plants resulting from trial inoculations with any individual case of disease are, of course, more reliable, but it is to be hoped that eventually the knowledge of the properties of a virus may serve as a more satisfactory basis for classification and determination of specific viruses.

Finally, it has been a source of satisfaction to conform our earlier work on the viruses secured from healthy potatoes with the existing work relating to potato rugose mosaic. It remains to be seen whether or not our interpretation in this regard is wholly correct. Investigations along this line should be extended, especially in those regions where it is believed that

rugose mosaic is an economically important disease of potatoes. If our present conception of the problem is correct, the control of this disease by eradication will prove to be peculiarly perplexing.

Summary

1. The investigations reported in this paper were undertaken primarily for the purpose of securing further information on the nature and identity of the virus normally present in apparently healthy potatoes. At the same time, an attempt has been made to develop a method of study which would aid in the description, isolation, and classification of certain other viruses affecting the potato.

2. The studies reported are especially concerned with a determination of the properties of the common potato mosaic viruses. Symptomatology alone has been practically the only basis for the separation of the potato viruses in the past, and this system is confronted with many serious disadvantages.

3. The following virus diseases, according to Schultz and Folsom's nomenclature, were studied in particular: crinkle mosaic, rugose mosaic, leaf-rolling mosaic, and mild mosaic. Particular attention was given also to the study of the "spot necrosis" virus, its relation to "rugose mosaic," and to "mottle" as secured from healthy potatoes.

4. Attention was directed mainly to the following characteristics of the viruses studied: longevity *in vitro;* thermal death-point; tolerance to dilution; varietal susceptibility; and symptom expression.

5. The viruses as a whole were found to be very sensitive to unfavorable conditions as compared to the tobacco mosaic virus. They lose their virulence rapidly *in vitro*, they are destroyed by relatively low temperatures, showing an inactivation range between approximately 40° and 70° C. The tolerance to dilution is also comparatively low, falling off rapidly in most cases beyond dilutions of 1 to 10 or 1 to 100. A marked difference in the susceptibility of different varieties of potatoes exists in some cases, but is less marked with certain other viruses. The expression of symptoms is very variable, in some cases overlapping so seriously as to make determinations on the basis of symptoms impossible even under reasonably constant environmental conditions of the greenhouse.

6. In previous literature from the Wisconsin laboratory referring to "rugose mosaic" of Triumph potatoes, (3, 7, 12) this name should read "crinkle mosaic." The present studies have shown that the common Wisconsin disease of Triumph potatoes agrees in practically all details with "crinkle mosaic" as described by Schultz and Folsom.

7. Evidence is presented which points toward the identity of true "rugose mosaic" with "spot necrosis," and the probable existence of this disease in an attenuated form in practically all apparently healthy potatoes of the standard varieties.

8. It is believed that a knowledge of the properties of these potato viruses will aid not only in their classification, but may also help in the separation of combination virus diseases of the potato into their component parts.

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The Overwintering of the Tobacco Mosaic Virus

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CONTENTS

The Overwintering of the Tobacco Mosaic Virus[1]

MOSAIC ON TOBACCO occurs so commonly and universally that one rarely finds a field entirely free from the disease. Fortunately the percentage of infection is usually low, or the actual damage produced so small that it is not regarded as an important disease by most growers. On the other hand, certain growing districts as a whole, or many individual farms in other districts, frequently suffer serious losses from the disease, and the total annual loss is consequently considerably greater than is generally recognized.

A knowledge of the source or origin of this mosaic disease[2] each season is, of course, of major importance in relation to the development of preventive measures. While it is of considerable importance to discover the origin of the scattered individual infections which may occur in a field, the greatest economic interest centers around an explanation of the epidemics of the disease. Is a part or all of an epidemic due to the overwintering of a minor amount of virus and slight direct infection from this followed by wholesale dissemination by one or more agencies, or is it due largely to direct infection of the plants from virus-carrying materials? Since previous investigations indicate that overwintering and dissemination of the virus may actually or possibly occur in a number of different ways, the answer to this question cannot be a simple one. These conclusions have, however, been based chiefly on somewhat fragmentary experimental data, with little or no extensive field observations. In the present investigation it has been attempted particularly to base our experimental work and conclusions upon conditions as they occur in the field. While the actual cause of the mosaic disease is not known, infection can originate only either directly or indirectly from previously infected plants. The control of the disease is, therefore, largely a matter of preventing infection of the new crop from the overwintered virus. The present investigation deals particularly with the soil as a factor in the overwintering of the tobacco mosaic virus.

Previous Investigations

The history of the suggestions for the control of tobacco mosaic is interesting in that scientific opinions on the subject have varied greatly, while the growers on the other hand have neither attempted

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Office of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

[2] The ordinary tobacco mosaic disease (*Tobacco virus* 1) (8) is referred to throughout this paper.

nor accomplished much in the way of reliable control measures. It is especially interesting in the present connection to note that the early students of the disease, Mayer (9), Iwanowski (6), Beijerinck (2), Raciborski (10), and others were inclined to the belief that the soil was an important source of infection. The subsequent trend of investigation has been away from the conception of the soil as a source of infection. This has been due partly to the discovery of other supposedly important modes of overwintering and dissemination of the virus, such as overwintering in perennial host plants and dissemination by aphids, and partly to the negative results secured by some investigators in soil transmission trials. Allard (1) concluded from his trials that mosaic material in the soil did not infect plants growing therein. His trials indicated that the virus was quickly destroyed in the soil and that infection through the roots was infrequent or dependent upon other factors. According to Allard (1), the occurrence and spread of tobacco mosaic could generally be accounted for by overwintering of the virus in wild perennial hosts and subsequent dissemination by aphids.

Clinton (3) concluded that soil infestation was likely to cause mosaic infection in the seed-bed, and that the seed-bed infection was probably primarily responsible for most of the mosaic developing in the field. Infection from the field soil after transplanting was considered rare, and the amount of mosaic each year was believed to have little or no relation to the amount occurring the preceding year. The belief that the soil had no relation to mosaic of tomato and tobacco (assuming the ordinary tobacco mosaic virus to be concerned in both cases) became so strong that in 1912 Gardner and Kendrick (4), in a paper on the overwintering of tomato mosaic, do not suggest such a possibility.

An important characteristic of tobacco mosaic in relation to overwintering is the extreme resistance of the virus to unfavorable conditions. That the tobacco mosaic virus may live for several years either in plant extract or in dried plant material has been repeatedly shown. This behavior places tobacco mosaic in quite another category in relation to control from certain other virus diseases, such as cucumber mosaic and potato mosaic, the viruses of which diseases are notably short lived outside of the living host. Another important characteristic in this connection is the extreme infectiousness of the tobacco mosaic virus, both with respect to the minute quantity of virus sufficient to cause infection, and the readiness with which infection occurs when the virus is introduced into the plant.

Plan of Experiments

The possible original sources of overwintering tobacco mosaic virus are primarily of two types, namely, (a) living plant tissue, that is, infected perennial host plants, and (b) dead plant material,

that is, material from dead plants infected during preceding seasons. In the present paper the possibility of the common occurrence and epidemics of tobacco mosaic as resulting from the overwintering of the virus on perennial host plants and its subsequent dissemination by aphids will not be considered in detail. While this phase of the subject may warrant more study than has been given to it in the present connection, field observations have not suggested its importance, and the negative experimental evidence secured by Dr. Hoggan in our laboratory (5) relative to aphid transmission of tobacco mosaic has not encouraged this study. The experimental work of the present bulletin is, therefore, concerned largely with the relation to the tobacco crop of infected plant material from dead host plants grown in preceding seasons. The material in question, of course, usually consists of tobacco or tobacco refuse, but may be refuse from other host plants of tobacco mosaic, as, for example, the tomato.

With this in mind, a series of experiments was planned to test the actual survival of the virus in such material after exposure to different environmental conditions, such as may exist on tobacco farms, and it is with the results of these experiments that the present bulletin is concerned. This tobacco plant material, which may be infected with mosaic and which normally is cured or dried out or partially decayed before the following spring, may fall into one of several categories as far as its relation to subsequent infection is concerned. It may first be classified as "curing barn refuse" and "field refuse," both of which may lead to soil contamination or infestation. The curing-barn refuse is primarily the tobacco stalks and leaf refuse following stripping from the stalk. Secondary sources of this material may, of course, be refuse from tobacco warehouses, such as "stems" from leaf stripping and trash from assorting warehouses, or refuse from commercial tobacco, all of which may eventually be returned to tobacco seed-beds or fields either purposely or accidentally. The field refuse includes the tops and suckers broken from the plants prior to harvest and the stalks or stubble and the roots left in the field after harvest, together with the usual secondary sucker growth. This field material either freezes and dries up in the field and is subsequently distributed by wind about the farm or is plowed under and undergoes more or less gradual decay.

In general farm practice the mosaic-infested material which is removed from the field (i. e., cured tobacco and tobacco refuse) may presumably be transferred to the seed-beds in various ways. It is, for instance, not uncommon for growers to sprinkle tobacco seed-beds with decoctions of tobacco refuse for insecticidal purposes. Much infested material may be transferred to the seed-beds in the form of chaff in the seed or tobacco refuse used as fertilizer. Other material may be accidentally transferred to the seed-beds through

the agency of wind, water, farm animals, tools, or other equipment and by man himself. Some infection of field plants may occur in a similar manner. There is, consequently, no lack of possible sources and modes of dissemination to account for scattered infections in the field in such a manner. In addition to such obvious possible sources of mosaic infection, there remains the possibility of infection from the soil as a consequence of overwintering virus, originating from diseased plants previously grown on the land. Since part of either seed-bed or field infection may possibly arise directly from such a source, or infection may develop from both sources simultaneously together with some mechanical transfer from plant to plant, the situation may often be very complicated. In most of the experimental work it has been possible to avoid such complications by using known sources of virus and known healthy seedlings.

The methods of testing various materials and soils for the presence of the mosaic virus for the most part have been very simple. Several of the conclusions are based upon direct inoculation of plants in the greenhouse with extracts of the material or soil in question after this had been exposed to various environmental conditions for different lengths of time. On the other hand, considerable data have been secured from regular field plantings where the previous history of the soil and the condition of the seedlings were definitely known.

Experimental Results

Overwintering of the Virus

The first tests for the overwintering of the virus were conducted for the purpose of determining the behavior of the virus in different parts of the plant. Infected stalks, roots, and leaves were taken at harvest time and placed in the tobacco-curing shed. Similar leaves were dried down rapidly in the laboratory. Portions of these mater-

TABLE I.—THE OVERWINTERING OF TOBACCO MOSAIC IN DIFFERENT PARTS OF THE TOBACCO PLANT. (MATERIALS COLLECTED OCT. 10, 1923.)

Inoculum	Storage	Number of plants infected of ten inoculated		
		December 24, 1923	January 21, 1924	April 5, 1924
Leaves, dried	Laboratory	10	10	10
Leaves, cured	Curing shed	0	0	0
Stalks	Curing shed	7	—	8
Roots	Curing shed	10	—	8

ials were tested at intervals during the year for the presence of mosaic virus by means of extraction with distilled water and inoculation to healthy plants in the greenhouse. The data of the first trial are presented in Table I and illustrate a rather unexpected result, namely, the rapid inactivation of the virus in the cured leaves as compared with the dried leaves and with the other parts of the plant. In subsequent trials (Table II), cured leaves were found to retain their infectiousness in some cases, but on examination, "mosaic leaf-spots" were found on some of the leaves. Since the spots were formed in the field and were consequently dried out before the curing process, it was to be expected that such portions of the leaf would contain the virus. In further trials, the data of which are not presented here, the virus was found to be inactivated in leaves

TABLE II.—THE OVERWINTERING OF TOBACCO MOSAIC IN DIFFERENT PARTS OF THE TOBACCO PLANT, ATTACHED AND UNATTACHED TO OTHER PARTS DURING THE NORMAL CURING PROCESS. (MATERIAL COLLECTED SEPT. 4, 1924.)

Inoculum	Attached to	Number of plants infected of ten inoculated				
		Sept. 4, 1924	Dec. 28, 1924	March 11 1925	June 1, 1925	Aug. 13, 1925
Leaves, cured	Stalk and root	10	1	0	10[1]	10[1]
Stalk	Leaves and root	—	9	9	10	—
Roots	Stalk and leaves	10	—	9	9	—
Leaves, cured	Stalk	10	0	0	0	10[1]
Stalks	Leaves	—	9	9	9	—
Stalks		10	9	8	—	4
Leaves, cured		10	4	0	0	0
Roots		10	7	6	10	9
Leaves, dried		—	—	9	10	10
Fresh virus (control)		10	10	10	10	10
None (control)		0	1	0	0	0

[1] Result doubtful; leaves used showed necrotic spots which had developed in the field. Virus would naturally not be inactivated in such areas.

which cured out with a normal chocolate color, but not in leaves which cured out green. The curing of tobacco is essentially an enzymatic process involving various chemical changes, and it is not surprising that under these conditions all or a part of the virus should be inactivated. We do not consider, however, that this inactivation is sufficiently complete in curing tobacco to destroy a sufficient amount of the virus to eliminate the danger of overwintering in cured leaves or in refuse from such leaves. The almost universal occurrence of some necrosis following mosaic makes complete inactivation in the curing process very improbable. The persistence of the virus in stalks and roots when placed under the conditions of the curing shed is sufficiently evident from Table II.

Infected tobacco refuse of various sorts may frequently become lodged on seed-bed frames, sash, cloth covers, or other material, and may eventually be transported directly to the seed-beds the following spring. In order to secure some experimental evidence as to whether the mosaic virus may overwinter on such materials and in soil, 50 pieces of cloth and 50 two-inch squares of wood, split into

TABLE III.—THE OVERWINTERING OF TOBACCO MOSAIC ON OR IN DIFFERENT MATERIALS INFESTED WITH LIQUID EXTRACT FROM GREEN MOSAIC LEAVES. (MATERIALS INFESTED ON SEPT. 20, 1923).

Infested material used as inoculum	Stored	Number of infected plants of ten inoculated				
		Sept. 20, 1923	Oct. 22, 1923	April 9, 1924	Feb. 10, 1925	Sept. 30, 1925
Seed-bed cloth	Indoors	10	9	8	6	8
	Outdoors	10	10	9	3	2
Seed-bed frame, board	Indoors	10	7	3	1	4
	Outdoors	10	9	10	2	0
Soil, moist	Indoors	10	4	0	0	—
	Outdoors	10	2	0	0	—
Original inoculum	In flask	10	5	8	—	—

10 pieces and tied in bundles, were soaked in mosaic tobacco extract for two minutes and then allowed to drain and dry. At the same time two kilograms of field soil were finely sifted and mixed with 500 cc. of mosaic extract and stored in a moist condition. Another portion of the mosaic extract used for the infestation of these materials was stored as liquid extract. In order to determine whether the conditions of storage influenced the overwintering of the virus, one-half of each of the above lots was stored out-of-doors in a small "weather house," whereas the other lots were stored indoors in a dry heated room. At intervals of one or two months a sample was taken from each lot and tested for its infective power by inoculating a water extract of the sample into five or ten tobacco plants. The results obtained at selected periods only are shown in Table III. As

might be suspected, the virus of tobacco mosaic may not only live over winter on cloth and boards, but may do so for two years, and no doubt for a considerably longer period. On the other hand, it was apparently quite rapidly inactivated in the moist soil. Although not shown in Table III, this particular lot of soil was not infectious after two and one-half months. We were not able to draw any satisfactory conclusions as to the difference in effect of indoor and outdoor exposure on the inactivation of the virus. The data in Table III, however, indicate that the virus was, on the whole, more rapidly inactivated out-of-doors, and if so, this was probably on account of the greater humidity of the air, the presence of moisture even in small amounts apparently favoring inactivation. It was found difficult to obtain uniform extractions from some of the materials, which together with the limited number of plants used in each inoculation series, may account for the variation in results.

A similar experiment was conducted using powdered dry mosaic leaves as the source of inoculum. The cloth and board were dipped into a water suspension of the infectious material in order to obtain more uniform adherence. In the soil, however, the inoculum was added dry, one lot of soil remaining air-dry and the other being moistened. The results (Table IV) are similar to those presented

TABLE IV.—THE OVERWINTERING OF TOBACCO MOSAIC ON OR IN DIFFERENT MATERIALS INFESTED WITH POWDERED DRY MOSAIC LEAVES. (MATERIALS INFESTED ON SEPT. 22, 1923.)

Infested material used as inoculum	Stored	Number of infected plants of ten inoculated				
		Sept. 22, 1923	Oct. 24, 1923	April 18, 1924	Dec. 28, 1924	Sept. 30, 1925
Seed-bed cloth	Indoors	10	6	4	2	6
	Outdoors	10	9	9	5	1
Seed-bed frame, board	Indoors	10	8	3	1	1
	Outdoors	10	10	10	6	6
Soil, dry	Indoors	10	10	10	10	10
	Outdoors	10	10	10	10	—
Soil, moist	Indoors	10	0	0	—	—
	Outdoors	10	0	—	—	—

in Table III. Dry infested soil remained uniformly infectious, while the moist soil lost its infectiousness in one month. The above experiments were exactly duplicated in a series started on October 5, 1923, and the results obtained were again essentially similar.

In order to obtain some further evidence on the relation of the humidity of the air to the inactivation of the virus on cloth and wood, a series of experiments was started on September 4, 1924, in which infested materials were placed in a cool storage cellar with a high relative humidity and compared with similar material placed in

a dry room somewhat above ordinary room temperature. Owing to the accidental loss of some of the material, the data are not complete, though they show a striking rate of inactivation of the virus at a high as compared with a low humidity. More evidence would be needed on this subject, however, before any definite conclusions could be reached, although it seems apparent from what is known relative to moisture and inactivation that a more rapid destruction of the virus will occur under outdoor conditions than in material remaining under continuous air-dry conditions.

Experimental evidence that tobacco seed chaff may carry the virus into the seed-beds at the time of sowing the seed has also been secured. Sprinkling the seed-beds with an extract of dried mosaic leaves has also given heavy infection. These and other methods by which the virus may be transferred directly to the seedlings are, however, too obvious to warrant detailed discussion.

The influence of the soil and its physical condition on the infectiousness of the virus is suggested in Tables III and IV. The mosaic virus was found to die out quite rapidly in moist soil as compared with dry soil. In the first case, where the soil was moistened with the virus but later dried, the virus was also inactivated, but less rapidly than in soil remaining moist. Air-dried soil to which dry powdered mosaic leaves were added showed no inactivating action on the virus. The apparent rapid destruction of the virus in moist soil and its persistence in dry soil invited further studies along this line in particular.

In Table V are shown the results of a preliminary trial in which two different quantities of virus extract were introduced into radically different types of soils and into other materials which were tested

TABLE V.—THE EFFECT OF ARTIFICIALLY INFESTED MOIST SOILS AND MATERIALS ON THE LENGTH OF ACTIVITY OF THE TOBACCO MOSAIC VIRUS. (INFESTED MARCH 3, 1926.)

Inoculum	Number plants infected of five inoculated					
	Series 1 1 part virus extract to 10 parts soil			Series 2 1 part virus extract to 100 parts soil		
	March 3	March 17	March 31	March 3	March 24	April 7
Sandy loam	5	1	0	5	3	1
Carrington silt loam	5	2	0	5	5	2
Red clay	2	4	0	2	4	1
Fine sand	5	0	0	5	0	0
Coarse sand	5	0	0	5	0	0
Kaolin	4	5	0	4	0	1
Talc	5	1	0	5	1	0
Fresh mosaic virus (control)	5	5	5	5	5	5
None (control)	0	0	0	0	0	0

at various times for the virus. The two series are not strictly comparable, since ten times as much soil was used in Series 2 as was used in Series 1, with consequent differences in water, and possibly in biological relations. The experiment shows, however, that a more rapid inactivation of the virus occurs in sand than in clay or silt loam. In kaolin and talc, finely powdered inert materials, the virus was inactivated somewhat more slowly than in sand. In general, it appears that some relation exists between the size of the particles of the medium and the rate of inactivation of the virus. The experiment was consequently repeated (Table VI), using various soil types and including ground quartz of about the fineness of medium sand, but naturally "sterile" and inert in character. The virus was inactivated most rapidly in the ground quartz and in a peat soil, and least rapidly in a clay loam and a silt loam soil, in which it remained active between four and five months.

In the preceding trials the soils were stored in the laboratory. Another trial was now conducted exposing the infested soil to greenhouse conditions favorable to the growth of tobacco plants (Table VII). Artificially infested manure was also included in this experiment. Again the virus was inactivated most rapidly in the quartz (in 20 days or less). Destruction was also rapid in the manure and less rapid in the various soils used.

These experiments seem to demonstrate that the tobacco mosaic virus may remain active much longer in some soils than in others. Furthermore, as shown in Table VI, the virus may be in intimate association with the soil for as long as four months in some types of soil without becoming inactivated even under temperature conditions favorable for plant growth. There is some reason to believe that

TABLE VI.—THE EFFECT OF DIFFERENT ARTIFICIALLY INFESTED MOIST SOILS AND MATERIALS ON THE LENGTH OF ACTIVITY OF THE TOBACCO MOSAIC VIRUS. (INFESTED OCT. 22, 1925. STORED IN LABORATORY.)

Inoculum	Number of plants infected of five inoculated			
	Oct. 22, 1925	Dec. 9, 1925	Feb. 19, 1926	March 9, 1926
Ground quartz	5	0	0	0
Carrington silt loam	5	2	2	0
Clay loam (dark)	5	2	0	0
Clay loam (red)	5	2	4	0
Sandy loam (dark)	5	4	0	0
Silt loam	5	3	0	0
Peat	5	0	0	0
Fine sandy loam	5	2	0	0
Medium sand	5	3	0	0
Fresh virus (control)	—	—	5	5
None (control)	0	0	0	0

the virus can remain active in the soil for considerably longer periods under winter conditions than at warm temperatures. However this may be, it is evident that much of the field refuse does not become intimately associated with the soil during the winter, and some not until late spring or early summer following plowing. In any case the virus, existing as it does in comparatively large portions of the plant material, does not come in close contact with the soil until considerable decomposition of the refuse has occurred. Furthermore, much of the material and the surrounding soil may remain dry for a relatively long period. It is, therefore, quite possible that the virus may survive the winter in certain types of soil and still be active at the beginning of the next tobacco season. While it is yet probably too early to conclude that the rate of inactivation of the virus in the soil is correlated with the physical character of the soil, it is at least logical to conclude that different soils and the conditions to which they are exposed may result in a wide difference in the rate at which the mosaic virus is inactivated.

TABLE VII.—THE EFFECT OF DIFFERENT ARTIFICIALLY INFESTED MOIST SOILS AND MATERIALS ON THE LENGTH OF ACTIVITY OF THE TOBACCO MOSAIC VIRUS. (INFESTED JAN. 22, 1926. STORED IN GREENHOUSE.)

Inoculum	Number of plants infected of five inoculated			
	Jan. 22, 1926	Feb. 10, 1926	March 10, 1926	April 10, 1926
Medium sand	4	3	1	0
Silt loam	5	5	4	0
Peat	4	4	1	0
Ground quartz	5	0	0	—
Barnyard manure	2	0	0	—
Fine sandy loam	5	5	2	0
Fresh virus (control)		4	5	5
None (control)		0	0	0

In a previous experiment we have shown that the virus can remain active for many months in roots in the dry condition (Table II). In a later experiment it was found that the virus could remain active in roots in dry soil for nine months or more, but that it was destroyed more rapidly when the roots were in moist soil, although even in this case the virus was still active after six months. During the spring of 1926, tobacco roots and leaves and soil were collected at random from fields badly affected with mosaic the preceding year and in which the virus was believed to have overwintered. The positive results of inoculation with extracts from these materials are shown in Table VIII. The soil in these cases probably contained small pieces of partially decomposed infected roots, but no definite roots were visible. There can consequently be no doubt about the overwintering of the virus in plant parts and in the soil in these

cases. Similar evidence of the persistence of the virus in roots and soil selected at random in 1927 is shown in Table IX and from previously located roots in Table X. Since it has been shown in previous experiments that the virus lives for a considerable time even

TABLE VIII.—THE OVERWINTERING OF TOBACCO MOSAIC IN TOBACCO SOILS FROM DIFFERENT WISCONSIN LOCALITIES. (MATERIALS SELECTED AT RANDOM IN APRIL 1926 FROM FIELDS SHOWING HEAVY MOSAIC INFECTION THE PRECEDING SUMMER.)

Inoculum	Number of plants infected of ten inoculated		
	Cambridge	Windsor	Madison
Leaves	2	3	3
Roots	8	10	9
Soil	3	3	2
Fresh virus (control)	10		
None (control)	0		

TABLE IX.—THE OVERWINTERING OF TOBACCO MOSAIC IN TOBACCO SOILS FROM DIFFERENT WISCONSIN LOCALITIES. (MATERIALS SELECTED AT RANDOM IN MAY 1927 FROM FIELDS SHOWING HEAVY MOSAIC INFECTION THE PRECEDING SUMMER.)

Inoculum	Number of plants infected of five inoculated		
	Albion	Edgerton	Madison
Roots	4	2	5
Soil	3	2	2
Fresh virus (control)	5		
None (control)	0		

TABLE X.—THE OVERWINTERING OF THE TOBACCO MOSAIC VIRUS IN THE FIELD AT MADISON, WISCONSIN, IN PREVIOUSLY SELECTED ROOTS. (ROOTS COLLECTED ON FEB. 26, 1927.)

Source of roots	Number of plants infected of five inoculated
Mosaic root No. 1, Field 1	4
Mosaic root No. 2, Field 1	5
Mosaic root No. 1, Field 2	4
Mosaic root No .2, Field 2	5
Healthy root No. 1, Field 3	0

after being thoroughly incorporated into the soil, there can be little doubt about the infestation of the soil at the time of transplanting and later. The observational evidence of the preceding year was practically conclusive that the infection in the fields from which these data were secured was due to soil infestation, since other fields planted from the same seed-beds yielded little if any mosaic. In the Cambridge soil (Table VIII) the 1925 infestation was practically 100 per cent and occurred relatively early in the growth of the plants.

About a bushel of soil from each of the three fields shown in Table VIII was brought to the greenhouse, placed in flats together with control soils, and planted with healthy young tobacco plants. In two cases, the soil was stirred in such a way as to injure the roots in some such manner as may occur in field cultivation, since there was good observational evidence that mosaic infection was sometimes favored in this manner. The data given in Table XI, while quite

TABLE XI.—ILLUSTRATING THE INFECTION OF TOBACCO WITH MOSAIC THROUGH THE SOIL IN GREENHOUSE TESTS AS A CONSEQUENCE OF TRANSPLANTING SEEDLINGS INTO OVER-WINTERED, NATURALLY INFESTED SOILS FROM DIFFERENT LOCALITIES AND INTO SOIL ARTIFICIALLY INFESTED WITH ROOTS FROM MOSAIC PLANTS.

Soil	Infestation	Number of plants transplanted April 14, 1926	Number of mosaic plants, May 18, 1926
Cambridge	Natural and overwintered	29	4
Windsor	Natural and overwintered	20	2
Madison	Natural and overwintered	9	1
Cambridge	Natural and overwintered and cultivated	9	5
Madison	Natural and overwintered and cultivated	9	0
Greenhouse compost	Artificial with mosaic roots	20	8
Greenhouse compost and steam sterilized	Artificial with mosaic roots	20	7
Greenhouse compost	None	40	0
Greenhouse compost and steam sterilized	None	40	0
Greenhouse compost	Artificial with mosaic roots, then steam sterilized	20	0

limited, since only 12 out of 76 plants became infected, are sufficient to illustrate the fact that infection of tobacco may occur from virus naturally overwintering in the soil when seedlings are transplanted into the soil. The evidence from this experiment that cultivation favors infection is too limited to be significant. In Table XII it is again shown that tobacco transplanted into infested soil may become heavily infected, 54 out of 130 plants becoming infected in this case.

TABLE XII.—ILLUSTRATING THE INFECTION OF TOBACCO WITH MOSAIC FROM SOIL AS A CONSEQUENCE OF TRANSPLANTING SEEDLINGS INTO NATURALLY INFESTED SOIL IN GREEN HOUSE TESTS.

Soil	Number of plants transplanted	Number of mosaic plants
Uninfested and steam sterilized	70	1
Infested and steam sterilized	130	1
Infested	130	54

TABLE XIII.—SHOWING THE PERCENTAGE OF TOBACCO MOSAIC OCCURRING UP TO THE TIME OF TOPPING IN FIELDS WITH DIFFERENT CROPPING HISTORIES, 1926. ALL PLANTS FROM STEAM-STERILIZED BEDS LOCATED ON NEW LAND.

Field	Number of preceding tobacco crops	Amount of mosaic on preceding crops	Number of plants in count	Percentage of mosaic		
				July 13	July 28	Aug.12
1	3	Heavy	850	2.7	23.8	35.1
2	23	Heavy	1,200	4.5	8.1	13.8
3	0	None	1,500	0.8	1.5	2.5
4	0	None	16.000	0.2	0.2	0.6
5	2	Considerable	188	3.3	16.0	24.0

In Tables XIII, XIV, and XV is shown even more conclusive evidence of the overwintering of the mosaic virus and infection through the medium of the soil under actual field conditions. Table XIII shows the percentage of mosaic occurring in five different fields planted entirely with seedlings from "new" and steam-sterilized soil. The origin of the small amount of infection occurring in fields 3 and 4 on a farm where tobacco had not previously been grown, and two miles from any other tobacco field, cannot be given. It is quite impossible with our present knowledge of the mosaic problem to account in a satisfactory manner for small and scattered infections of this sort. Table XIV gives similar data for these same fields for the following year and, in addition, the results from fields 5 and 6, which were not in tobacco the preceding year. Finally, in Table XV, the percentage of mosaic is given for these same fields in 1928. The correlation between the amount of mosaic present in the preceding and the succeeding crops is admittedly not good in many cases, nor

TABLE XIV.—SHOWING THE PERCENTAGE OF TOBACCO MOSAIC OCCURRING IN FIELDS WITH DIFFERENT CROPPING HISTORIES, 1927.[1] ALL PLANTS FROM COMPARABLE SEED-BEDS.

Field	Number of preceding tobacco crops	Percentage of mosaic on preceding crop	Number of plants in count	Percentage of mosaic		
				July 8	Aug.12	Sept.15
1	4	35.1	1,270	—	15.2	41.6
2	24	13.8	3,570	2.4	16.8	46.1
3	1	2.5	1,500	0.8	2.2	4.3
4	1	0.6	16,000	1.6	5.2	10.7
5	23		3,600	0.5	5.4	19.5
6	0	0.0	6,500	—	—	1.5

[1] Fields 1 to 4 inclusive same as shown in Table XIII.
Field 5 is a part of Field 2. In 1926 it was in rotation plots and in 1927 it again raised tobacco. Field 6 was new tobacco land.

TABLE XV.—SHOWING THE PERCENTAGE OF TOBACCO MOSAIC OCCURRING IN FIELDS WITH DIFFERENT CROPPING HISTORIES, 1928.[1] ALL PLANTS FROM COMPARABLE SEED-BEDS.

Field	Number of preceding tobacco crops	Percentage of mosaic on preceding crop	Number of plants in count	Percentage of mosaic		
				July20	Aug.2	Sept.19
1	5	41.6	615	1.1	44.0	73.5[2]
2	25	46.1	6,020	8.3	32.1	99.0
3	2	4.3	7,800	1.4	9.3	17.1
4	2	10.7	16,000	0.7	4.0	28.6
5	24	19.5	4,800	13.8	57.8	98.8
6	1	1.5	6,630	0.3	1.9	4.6

[1] Same fields as shown in Table XIV.
[2] The last count of this field was on suckers just before harvest, while the last counts of the others were made on stubble suckers.

TABLE XVI.—THE DISTRIBUTION OF PERCENTAGE OF MOSAIC IN SUCCEEDING ROWS ON AN ACRE OF LAND IN 1928 IN RELATION TO THE 1927 MOSAIC DISTRIBUTION COUNTS.

Rows (inclusive)	Percentage of mosaic	
	1927	1928
1- 5	26.0	7.7
6-10	16.2	16.8
11-15	16.8	15.2
16-20	6.4	4.7
21-25	4.0	1.0
26-30	5.1	6.8
31-35	4.1	4.8
36-40	9.5	7.7
41-45	5.2	3.1
46-50	19.4	7.2

TABLE XVII.—A COMPARISON OF THE RATE OF INACTIVATION OF THE TOBACCO MOSAIC VIRUS IN MOIST, COARSE SAND UNDER AEROBIC AND ANAEROBIC CONDITIONS. (EXPERIMENT STARTED APRIL 7, 1926.)

Inoculum	Conditions	Number of plants infected of five inoculated		
		April 7	April 26	April 28
Infested moist sand I	Aerobic	5	0	0
Infested moist sand II	Aerobic	5	0	0
Infested moist sand I	Anaerobic			5
Infested moist sand II	Anaerobic			5
Fresh virus (control)		5	5	5
None (control)		0	0	0

would we expect it to be so on account of the number of complicating factors which determine overwintering and infection. As a matter of fact, our laboratory data would lead us to expect that in many cases overwintering in the soil would not occur and consequently no correlation would exist. We have occasionally noted mosaic infection in a field to be more or less localized without respect to any definite factor. In such cases, localized soil infestation seemed to be the most likely explanation of the occurrence of the disease. During the summer of 1927, an opportunity presented itself for studying such a case of unequal distribution of mosaic on an experimental field one acre in size. The infection in 1927 was heaviest on the two sides of the field and comparatively light in the middle. Detailed notes on the location of the infected plants were therefore made in 1927 and likewise in 1928. An effort has been made to condense these notes in Table XVI, dividing the field into plots of five rows each, and recording the percentage of mosaic just prior to topping. It is quite evident that a considerable correlation existed in this field between the 1927 and 1928 infections, except for the two outside plots. The high count on the two outside plots (Rows 1-5 and 46-50) in 1927 was due in part to one section of each of these plots being transplanted to Burley tobacco which was known to be partly infected in the seed-bed. No defiinite reason, however, is known for the comparatively low percentage of infection in Rows 1-5 in 1928 as compared with the neighboring plots.

Everything considered, however, the field data are convincing that the soil may harbor the virus, and that it is a source of infection which should not be overlooked in considering the epidemiology of the disease. Although we do not wish to minimize the importance of subsequent mechanical dissemination as also accounting for a high percentage of infection, we have tried to use comparable means of controlling such dissemination throughout the experimental trials.

The Influence of Aeration on the Virus

The relatively rapid destruction of the virus in moist soil, and the more rapid rate of destruction in ground quartz, sand, and sandy soil than in heavier soils, is of considerable interest. Several possibilities present themselves in accounting for the inactivation of the virus in soil, including physical or chemical absorption, toxic action, oxidation, and biological activity.

In the present experiments we have been led to examine in particular the relation of aeration to this phenomenon. Some evidence also exists that both physical and biological factors may play a part in the inactivation of the virus. As will be shown, oxygen plays a considerable part, but whether through direct chemical reaction or indirect action on the biological processes of the soil is not yet clear.

When it was discovered that the virus was inactivated more rapidly in ground quartz and coarse sand than in field soils, aeration was naturally suspected of being a controlling factor. An experiment was therefore conducted with infested sand under anaerobic and aerobic conditions. In one series, the oxygen was removed from desiccators in which the infested sand was placed, by means of the pyrogallic acid—potassium hydroxide method. The coarse sand was in this case infested with dry powdered mosaic leaves and then moistened. The results are shown in Table XVII. The rapid rate of destruction of this comparatively resistant virus under aerobic as compared with anaerobic conditions is striking.

TABLE XVIII.—A COMPARISON OF THE RATE OF INACTIVATION OF THE TOBACCO MOSAIC VIRUS IN MOIST, COARSE SAND STORED UNDER AEROBIC AND ANAEROBIC CONDITIONS. (EXPERIMENT STARTED OCT. 6, 1926.)

Inoculum	Storage condition	Number of diseased plants of five inoculated					
		Oct. 6	Oct. 19	Nov. 2	Nov. 9	Nov. 23	Nov. 29
Infested moist sand	Aerobic		5	1	0	0	0
Infested moist sand 1	Anaerobic				4	4	5
Infested moist sand 2	Anaerobic					5	5
Infested moist sand 3	Anaerobic					5	5
Infested moist sand 4	Anaerobic						5
Original dry inoculum (control)	Aerobic	5	5	5			5
None (control)		0	1	0	0	0	0

This experiment was repeated under similar conditions as shown in Table XVIII, with similar results. It was again repeated, using extract from green mosaic plants mixed with fine sand, and inactivation again occurred in about twenty days under aerobic conditions, while there was apparently no inactivation after forty days in the oxygen-reduced atmosphere.

Since the tobacco mosaic virus will live for years in liquid extract of mosaic tobacco plants under conditions of decomposition and fermentation, but undoubtedly under anaerobic conditions, it was thought worth while to determine the effect of air and oxygen on such an extract. In the first experiment, air was merely bubbled through the extract for about one hour daily in one case; every third day

in the second case; and every sixth day in the third case. The control consisted of a non-aerated sample. Five inoculations were made with these differently treated extracts over a period of forty days. The first three tests during the first twenty days indicated an inactivation of the virus in the extract aerated daily, but not in the other extracts. In the last two tests the virus aerated daily seemed to have regained its infective power. During the summer months these extracts were set aside without any treatment, but were tested after six months, when they all showed normal infective power. They were again subjected to the same amount of aeration as previously, and tested ten different times during a period of two months. After forty days the virus aerated daily showed reduced infective power, but the others remained normal. However, in a dilution experiment to test the amount of virus present, the virus aerated daily gave infection of only one plant out of five at a dilution of 1-100, whereas the control, aged for the same time but not aerated, gave infection of five plants out of five inoculated with a dilution up to 1-10,000. The mosaic virus was unquestionably inactivated, though slowly, by aeration in this liquid extract. Since a tobacco mosaic virus extract permits of such high dilutions without reduction of the infective power, it was believed that the slow rate of inactivation may have been due to the escape of part of the extract from exposure to air under the particular experimental conditions used. In a subsequent trial, therefore, glass tubes eighteen inches long and about one-half inch in diameter, filled with small glass beads, were used as containers for the virus. The extract was placed in these tubes, and air was bubbled through one tube from the bottom and oxygen from a tank through a second. A third tube was untreated and served as a control. In the first trial the virus showed striking inactivation after one and two weeks in both treated tubes as compared with the control, but after three weeks the extracts seemed to regain their infectiousness. The re-

TABLE XIX.—THE INFLUENCE OF DAILY EXPOSURE OF THE TOBACCO MOSAIC VIRUS IN LIQUID EXTRACT TO AIR AND OXYGEN ON ITS INFECTIOUSNESS. (TREATMENT STARTED NOV. 4, 1926.)

Inoculum	Number of plants infected of five inoculated			
	Nov. 4	Nov. 11	Nov. 18	Nov. 27
Untreated virus	5	4	3	5
Air-treated virus	—	2	0	4
Oxygen-treated virus	—	1	0	5
None (control)	0	0	0	0

sults secured in this experiment are shown in Table XIX. This experiment was repeated, twelve tests for infectiousness being made over a period of three months or more. Again marked inactivation occurred after about two weeks, but after three or more weeks the virus was practically as infectious in the air and oxygen treated tubes as in the control. When, however, these extracts were tested by the dilution method, it was found that the extracts treated with air and oxygen were not infectious above dilutions of 1-10, whereas the control extract was infectious at dilutions of 1-1000 (Tables XX and XXI). No significant difference has been noted in these trials between the inactivating power of air and of oxygen.

An interesting feature of these experiments has been an attenuation of the virus which appeared in occasional plants inoculated with the air and oxygen treated extracts. We have previously shown that tobacco mosaic virus may be attenuated by growing inoculated

TABLE XX.—THE INFLUENCE OF DAILY EXPOSURE OF THE TOBACCO MOSAIC VIRUS IN LIQUID EXTRACT TO AIR AND OXYGEN ON ITS INFECTIOUSNESS. (DAILY EXPOSURES FROM NOV. 11, 1926, TO FEB. 14, 1927.)

Inoculum	Number of plants infected of five inoculated							
					By dilution April 7			
	Nov.11	Dec.11	Jan.22	Feb.14	None	1 to 10	1 to 100	1 to 1,000
Untreated virus	5	4	5	4	5	5	5	5
Air-treated virus	5	3	5	3	5	4	0	0
Oxygen-treated virus	5	2	5	4	3	5	0	0
None (control)	0	0	0	0	0			

TABLE XXI.—THE INFLUENCE OF DAILY EXPOSURE OF THE TOBACCO MOSAIC VIRUS IN LIQUID EXTRACT TO AIR ON ITS INFECTIOUSNESS. (DAILY EXPOSURES FROM JANUARY 27 TO MARCH 15, 1927.)

Inoculum	Number of plants infected of five inoculated							
				By dilution, April 15				
	Jan. 27	Feb. 4	Mar. 15	None	1 to 10	1 to 100	1 to 1,000	1 to 10,000
Untreated virus	5	5	5	5	5	5	5	5
Air-treated virus	5	3	5	3	3	0	0	0
None (control)	0	0	0	0				

plants at temperatures around 37°C for ten or more days (7). The attenuation secured in these aeration experiments has been of a similar type, and remains stable through several generations of subsequent transfers. Further work needs to be done upon this subject to determine whether or not complete inactivation is a result of gradual attenuation, or whether, as appears to be the case in some instances, the virus is killed suddenly with no intermediate condition between the living and the dead virus. The tobacco mosaic virus has been kept for three and one-half years in tobacco extract in our laboratory without any apparent change in virulence of the type described as attenuation, although we have noted some small change in the type of symptoms produced as compared with freshly extracted virus. We have also secured attenuated forms directly from material overwintering in the soil, showing that this phenomenon also occurs in nature and probably as a result of oxidation.

The inactivating effect of aeration on the virus is no doubt connected in some way with biological activity, since when aseptic conditions are maintained in moist, virus-infested soil, marked inactivation does not occur. This experiment was conducted by adding filter-sterilized virus to heat-sterilized soil. The evidence from two such trials is shown in Tables XXII and XXIII. In Table XXIII it is also shown that when soil is treated with a small excess of the virus extract, i. e., flooded instead of moistened, inactivation is slowed up or prevented. We are inclined to believe that, under field conditions,

TABLE XXII.—THE RATE OF INACTIVATION OF THE TOBACCO MOSAIC VIRUS IN MOIST, DRY, AND STERILIZED SOILS. (SOIL INFESTED WITH VIRUS ON SEPT. 4, 1924.)

Inoculum	Stored	Number of plants infected of ten inoculated			
		Sept. 4, 1924	Dec. 28, 1924	Feb. 10, 1925	Mar. 10, 1925
Mosaic extract in soil	Dry	10	6	4	0
Mosaic extract in soil	Moist	10	2	0	0
Dry powdered mosaic leaves in soil	Dry	10	9	10	10
Dry powdered mosaic leaves in soil	Moist	10	0	0	0
Filter-sterile mosaic extract in sterile soil	Moist	10	9	6	9
Filter-sterile mosaic extract in unsterilized soil	Moist	10	3	0	2

TABLE XXIII.—THE INFLUENCE OF WATER-LOGGED AND ASEPTIC CONDITIONS ON THE RATE OF INACTIVATION OF TOBACCO MOSAIC VIRUS IN SOIL. (EXPERIMENT STARTED NOV. 23, 1927.)

Inoculum	Moisture conditions in soil	Number of plants infected of five inoculated	
		Dec. 29, 1927	Feb. 1, 1928
Mosaic extract in soil	Moist	4	0
Mosaic extract in soil	Moist	2	0
Mosaic extract in soil	Water-logged	5	5
Mosaic extract in soil	Water-logged	5	4
Filter-sterile mosaic extract in sterile soil	Moist	5	5
Filter-sterile mosaic extract in sterile soil	Moist	5	5
Filter-sterile mosaic extract in sterile soil	Water-logged	5	5
Filter-sterile mosaic extract in sterile soil	Water-logged	5	5
Original virus extract		5	5
None (control)		0	0

flooding or waterlogging of the soil may result in the overwintering of more virus in some parts of the field than in others. The action of such flooding on the virus may probably be explained on the basis of interference with aeration.

The evidence presented is believed to show satisfactorily that soil aeration has an influence on the persistence of the mosaic virus in the soil. It is interesting to note that twenty-eight years ago Sturgis (11) wrote as follows: "The disease occurs abundantly in some localities, notably on the close, clayey soils on the east side of the Connecticut River, sparingly in other localities, where the soil is open and porous."

The variations in different soils with respect to aeration and the factors which influence it are fairly well known and in some cases too obvious to be discussed here. Whether or not any close correla-

tion exists under actual field conditions between the aeration of soil and the persistence of the mosaic virus in infested soil remains to be determined.

Discussion of Results

The overwintering of the tobacco mosaic virus under farm conditions in tobacco or tobacco refuse, in either the cured or the dried condition, is sufficiently evident. The part played by such material in epidemics of the disease, however, is quite uncertain. It is naturally dependent upon the amount of this infectious material which is transferred to the seed-bed or to the field. If it is assumed that in most cases only occasional primary infections result from such a source, subsequent dissemination in the case of epidemics must be due to a very active agency. Certain cultural operations, especially topping, of course, are responsible for much of this dissemination, as is shown by the experiments of others as well as our own (Table XXIV), but this does not by any means account for heavy infection occurring before topping in cases where the seedlings were known to be healthy at the time of transplanting. That aphids are responsible for such dissemination appears very doubtful, and Dr. Hoggan has already shown that the aphid commonly suspected (*Myzus persicae*) does not transmit the tobacco mosaic virus (5).

If, on the other hand, tobacco plants may become directly infected from virus carried to or overwintered in the soil, a disseminating

TABLE XXIV.—SHOWING THE INFLUENCE ON DISSEMINATION OF TOPPING MOSAIC AND HEALTHY PLANTS IN A ROW SEPARATELY AS COMPARED WITH TOPPING BOTH AT THE SAME TIME, IN A FIELD WHERE A RELATIVELY HIGH PERCENTAGE OF MOSAIC INFECTION WAS DEVELOPING FROM SOIL INFESTATION.

Season	Mosaic and healthy plants topped	Row	Percentage of mosaic at time of		Percentage of increase between time of topping and suckering
			Topping	Suckering	
1926	Separately	1*	24	36	50
		2	24	46	91
	Together	3	16	76	375
		4	4	40	900
	Separately	1	20	44	110
		2	18	38	111
	Together	3	12	82	583
		4	20	72	260
1927	Separately	1	12	12	0
		2	14	16	14
	Together	3	12	74	516
		4	6	80	1233

*Each row represents a total of fifty plants.

agent of the insect type is not necessary to explain the observed conditions. The investigations presented in this paper, as well as numerous confirmatory field observations in Wisconsin and certain other tobacco districts, seem to prove conclusively that infection frequently occurs from virus overwintering in the soil. Heavy infections occurring in the field may then result from (a) transplanting of plants infected in the seed-bed; (b) infection from the field soil; and (c) spread of the original infection by one or more disseminating agencies, particularly cultural practices. The infection of plants in the seed-bed may result from one or more of several different sources. The soil may be infested as a consequence of the previous growth of mosaic plants on the soil, or refuse from curing barns may have been used as fertilizer or accidentally carried in by other means. Tobacco seed often contains much chaff which may harbor the virus and may result in the introduction of virus into the seed-beds. On the other hand, plants may be infected in the seed-bed in more direct ways, such as by the use of tobacco refuse extract as an insecticide; the use of contaminated seed-bed frames, cloth, or sash; or the transfer of tobacco refuse by wind and animals or man to the seedbeds. According to our observations, the occurrence of heavy infections in the seed-bed is quite as likely to result from virus which has overwintered in the soil as from material transferred to the seed-bed in the spring of the year. Mosaic in the field, occurring as the result of infected seedlings, is usually readily recognized on account of the early development of symptoms. Mosaic infection developing from the soil in the field appears later and develops more or less gradually as the season progresses. The tendency of the plants transplanted to virus-infested soil is to remain healthy. The continuous exposure of the plants to infectious material, together with the only occasional occurrence of circumstances favoring infection, such as root-wounding, naturally results in the irregular or periodic development of the disease.

The virus overwintering in the soil usually comes from field refuse or roots of the preceding crop, and is consequently dependent on the preceding year's infection. That this is usually very abundant may be seen on the secondary growth in fields repeatedly grown to tobacco. Whether or not the virus overwinters in the soil and causes infection on the succeeding crop is apparently dependent upon a number of circumstances, some of which are indicated by our experimental work, such as the influence of moisture and aeration. There is plenty of field observational evidence that infection may or may not result from the field soil, and this is to be expected according to our own interpretation of the results. If overwintering of the mosaic virus should occur regularly and in quantity, the disease would be a much greater limiting factor to tobacco production than it now is.

According to our own interpretation of results, the control of tobacco mosaic will be dependent in a considerable measure on a certain

amount of rotation for the seed-beds and the field crop, together with some special effort to prevent seed-bed or field infection as a consequence of transferring crop refuse to such locations.

Summary

1. The mosaic disease of tobacco occurs to some extent in practically all tobacco fields. Fortunately, serious injury to the crop is less common; nevertheless the total loss from this disease is much higher than is generally recognized.

2. The early investigators of this disease considered soil infestation as an important source of infection. More recent investigators, however, have emphasized perennial wild hosts and aphid dissemination as the important factors in the occurrence of the disease.

3. The results presented in this paper point to the ability of the tobacco mosaic virus to exist for long periods of time in dead plant material as an important factor in the overwintering of the virus in the curing shed and in the soil.

4. A considerable amount of the mosaic occurring in the field is due to seed-bed infection. The seed-bed infection may result from tobacco or tobacco refuse transferred to the seed-beds in the spring or from the virus overwintering in the seed-bed soil. Field infections may also result from tobacco or tobacco refuse from curing barns or warehouses, but more commonly from field refuse, stubble and roots from the preceding infected crop.

5. The frequency and extent of overwintering of the mosaic virus in the soil is dependent upon a number of factors. Moist and well-aerated soils favor the inactivation of the virus as compared with dry, compact or waterlogged soils.

6. The tobacco mosaic virus has frequently been recovered in the spring of the year from soil and roots which have remained in the fields throughout the winter in Wisconsin.

7. Tobacco transplanted from the same seed-beds to fields known to be infested with mosaic and to fields known to be practically free from the disease has repeatedly shown very heavy infection in the former case and very light infection in the latter case.

8. Tobacco plants do not ordinarily become easily or rapidly infected from mosaic-infested soil. Infection is rather gradual throughout the season. Much of the late infection, however, is admittedly due to field cultural operations such as topping.

9. It is believed that future considerations of control measures must take into account more generally the bearing of the overwintering of the tobacco mosaic virus in the soil on epidemiology, and consequently the relation of rotation to the control of the disease.

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Inheritance of Fusarium Wilt Resistance in Canning Peas

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CONTENTS

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The Inheritance of Fusarium Wilt Resistance in Canning Peas[1]

THE FUSARIUM WILT of peas is a relatively new disease which has frequently been confused with Aphanomyces rootrot. Jones and Linford (6) mention it as an undescribed wilt disease occuring in fifty fields. In some cases destruction of the crop was almost complete.

Linford (8) named and described the causal organism (*Fusarium orthoceras* App. and Wr. var *pisi*) and made a thorough pathological study of the disease. In a later paper Linford (9) considers that this disease is second in importance only to rootrot. In some sections of the country, it is the most important pea disease. It is especially important in southern Wisconsin, western Maryland and southern Pennsylvania.

This disease is primarily vascular. The first and most characteristic symptom is a recurving of the margins of the younger leaves and stipules. An increase in turgor seems to be always associated with the disease. The affected plants do not become limp immediately but shrivel gradually from the top toward the base. Only in very late stages do the plants become entirely limp. Dwarfing, loss of color in the foliage, and vascular discoloration are more or less constantly associated with the disease.

Resistance to diseases caused by vascular Fusaria has been found in many different crops. Resistant strains of cotton, flax, cabbage and tomato are in use in some areas. In most cases resistant strains have been secured by mass selection from standard varieties grown on infested soil. In only two cases have genetic studies been carried to a place where a factorial explanation is possible, Walker (12) and Burnham (3).

Several commonly known but little used varieties of peas are known to be resistant to wilt, while most of the varieties used by canners are very susceptible (Linford 8.) The object of this work is to study the genetic relationship between susceptible and resistant varieties, in the hope that such a study may lead the way to a satisfactory breeding program in which resistance may be combined with commercially desirable characteristics. Occasional resistant plants occur in susceptible varieties but these are usually not type plants Linford (8).

Materials

Most of the seed used in this study was furnished by E. J. Renard, who has been working with peas for several years at the University of Wisconsin. The following compilation (Table I) shows the varieties, strains, degree of resistance and symbols used.

A culture of *F. orthoceras* var. *pisi* was obtained from Dr. Linford. This culture (180C), which could be traced to a single spore isolation,

[1] Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, No. 104. Published with the approval of the Director of the Station.

was increased to supply inoculum for all soil used in greenhouse studies throughout this experiment. The peas for the wilt study under field conditions were all grown on plots used by Linford in some of his earlier studies. These plots had been inoculated from many different cultures. The experiment of moving inoculated soil from the greenhouse to the field was also tried, but wilt occurred so late that it was impossible to take satisfactory notes on wilting.

The equipment for the greenhouse tests consisted of one greenhouse section containing four benches and a device for automatic temperature control. Recording thermometers and mercury thermometers were used to check the accuracy of the temperature control and the distribution of heat.

TABLE I.—*Strains and Varieties of Peas Used and Their Reaction to Wilt*

Variety or strain	Symbol	Reaction to wilt	Variety or strain	Symbol	Reaction to wilt
Horal	Ho	Resistant	Horsford	H	Susceptible
Green Admiral	Ad	Resistant	Susceptible Alaska	S	Susceptible
Resistant Alaska	R	Resistant	Surprise	Sp	Susceptible
Improved Surprise	Im	Resistant	Alaska Rogue	Ro	Susceptible
Fasciated Sweet	Sw	Resistant	Alaska Rogue	Rog	Susceptible
			Acme	Ac	Susceptible
			Perfection	P	Susceptible

Methods

Technique of the Disease Tests

The soil for the greenhouse tests was steam sterilized during the summer and inoculated with a single strain of the organism. The fungus was grown mostly in a nutrient solution (Tochinai, 11) composed of the following:

Peptone	10.00g
Mono-potasium phosphate	0.50
Magnesium sulphate	0.25
Maltose or sucrose	20.00
Water	1000.00cc

Some of the inoculum, however, was grown on barley, oat hulls, and corn meal sand. In these cases, however, the growth was not as great nor as rapid as in the nutrient solution.

The mycelial mat formed in the nutrient solution was ground to small pieces by means of a power driven, mechanical stirrer, and sprinkled on the sterilized soil with a sprinkling can with holes somewhat enlarged. After the inoculum was mixed with the soil, it was permitted to stand for two to eight weeks with an occasional stirring to insure a thorough distribution of the fungus. Furthermore, some of this finely divided mycelial mat was put in each row immediately before the seeds were planted in the greenhouse.

Since sufficient soil was required for four greenhouse benches averaging thirty feet long, three feet wide, and five inches deep, it was found necessary to inoculate the soil in several lots. These different lots were carefully mixed together over a rotary screen before distributing the soil

to the greenhouse benches. About 20 per cent of sand was included with the soil.

The first planting of genetic material was made September 15, 1928. Thirty seeds were planted in each 36-inch row and the rows were about eight inches apart. The depth of planting was approximately one inch. Seed from the parental stocks were planted as checks. Ten seeds from each check in each bench were considered sufficient. All the benches were alike in the material grown. The only difference was in the time of planting — one day later in each succeeding bench — and in the arrangement of the rows so as to secure a systematic distribution of the progenies. Soon after planting a trellis was built and threaded with soft twine to furnish support for the plants. Figure 1 shows the trellis and part of the equipment.

During the first run in the greenhouse, notes were taken on each bench at three day intervals, but during the second run the readings were made every fourth day, i. e. one bench each day, over a period of about 36 days after the beginning of the wilting. The note-taking was begun as soon as clear cut wilt symptoms could be noticed; usually about 20 days after planting. A plant was adjudged to be sufficiently wilted for removal from the wilt bed when it showed the positive stipular curl and increased turgor. In case of the taller types some were removed when they showed unilateral stipular curl and increased turgor. The same criteria were used under field conditions. In the field, however, unilateral wilting did not occur.

The difference between resistant and susceptible strains is very clearly defined. The resistant plants are entirely resistant while the susceptible plants are entirely susceptible when grown on disease infested soil. It was not found necessary to provide any classes for intermediate types of wilting. There is, however, a slight difference in the time of wilting of tall and of short plants which is considered later in this paper.

As a check on diagnosis, platings were made in duplicate, on acidified potato dextrose agar and read at the end of four or five days. Positive identifications were not made but the fungi isolated were compared with stock cultures of the fungus causing the disease. All contaminated plates were discarded except those in which the contaminant interfered in no way with the reading of the plate. Isolations were made during the first and second runs in the greenhouse but not under field conditions. During the first greenhouse run isolations were made at random from the wilted plants with some attempted isolations from healthy plants to serve as checks. During the second run practically all wilted plants were plated, and all the healthy survivors from one bench except those transplanted. A summation of plating results during the second run in the greenhouse is given in Table II.

These results may be interpreted to mean that there is a rather high correlation between the presence of what was considered *F. orthoceras* var. *pisi* and the occurrence of the disease. They also indicate that in some cases there may be an invasion of resistant plants but not sufficient to cause wilt symptoms.

It has been thought that perhaps the breaking of roots of wilt resistant varieties would permit the entrance of the fungus and cause the disease. To test the truth of this hypothesis a wilt resistant and a wilt susceptible variety were grown on healthy soil and transplanted to wilt infested soil with some breakage of roots. Only negative results were obtained as shown in Table III.

TABLE II.—*Results of Plating Plants on Potato Dextrose Agar to Determine the Presence of the Causal Organism*

Strains represented	Condition of plants when plated	Number plated	Number Positive	Per cent positive
Susceptible checks	Wilted	464	375	80.8
Resistant checks	Healthy	127	6	4.7
Segregating progenies	Wilted	621	533	85.8
Segregating progenies	Healthy	302	21	7.0
Total	Wilted	1085	908	83.7
Total	Healthy	429	27	6.3

TABLE III.—*An Experiment to Determine the Effect of Transplanting on Resistance*

Variety	Number seeds	Date planted in disease free soil	Date transplanted to infected soil	Number wilted	Jan. 5, 1929. Number plated	Number positive
Susceptible Horsford	6	Nov. 13, 1928.	Nov. 25, 1928.	5	5	5
Resistant Horal	6	Nov. 13, 1928.	Nov. 25, 1928.	0	4	0

Considering the possibility that the breaking of roots had not been sufficient injury a second experiment was conducted in which severe decortication of the roots was practiced. Transplantings were made at two different stages — about the second leaf stage, and at the fourth leaf stage — to test the effect of injury of plants of different ages. Table IV shows that the results are again negative. Perhaps a more extensive series covering a wider range of maturity of the plants might give different results but the present tests do not indicate this.

During the first run in the greenhouse practically all the plants in the test showed cortical lesions which are not typical of wilt. To avoid this lesioning special precautions were taken during the second run. The soil was slightly ridged at the row marks, the peas planted on top of this ridge, and covered with one-half inch of sand to a width of about two inches. Watering was done only between the ridges. Only slight cortex lesions occurred during the second run. Figure 2 shows how the peas were planted and watered during the second run in the greenhouse.

Plants with severe cortical lesions are weak, seldom produce any seed and under unfavorable conditions may die or become broken off. It was thought that perhaps organic mercury disinfectants might be of value in increasing germination and in preventing cortical lesioning. To test

TABLE IV.—*Effect of Severe Decortication and Transplanting on Resistance*

Variety	Number seeds	Date of planting	Number transplanted	Date first transplanting	Number plated and date Apr 3	Results	Date of transplanting 2nd lot of material	Number transplanted	Number plated and date April 10	Results
Horal(Ho 4-4)	35	Jan. 30, 1929.	11	Feb. 11	11	All negative	Feb. 25	12	12	All negative
Horsford(H_2-1)	12	Jan. 30, 1929.	3	Feb. 11	3	Two positive	Feb. 25	7	7	6 positive

the value of this hypothesis 60 seeds of a susceptible variety were shaken with organic mercury dust and compared with the same variety without treatment. Table V presents the details of this experiment. The organic mercury interfered with the entrance of the casual organism into the plants in no way since all the treated plants wilted. On the basis of this experiment all seeds planted in the field were treated with this mercury dust before sowing.

Throughout the tests in the greenhouse an effort was made to keep the temperature near the optimum (21°C) for wilt as found by Linford (8). Two recording thermometers (air and soil) gave records indicating that this effort was successful. As a check upon this three ordinary mercury thermometers were placed in the soil in each bench and read thrice daily. No temperature records were kept during the field tests.

Under field conditions elimination of the susceptible checks by the disease was almost complete. Only a few, mostly near the edges of the inoculated plot, escaped the infection. Figure 3 shows the resistant checks and the susceptible checks near the center of the plot about 50 days after planting.

In the greenhouse the parental strains were used as checks and under field conditions not only the parents but also a standard resistant check (Horal) and a susceptible check (Horsford). This was done so that different parts of the wilt bed might be readily compared with each other in case of incomplete elimination of susceptibles.

Sister strains of a pure line of Horal were used for the resistant check. In some cases a very small dwarf was produced which possessed a rosette appearance from the time of emergence until death; usually about four weeks later. In one check lot of ten plants there occurred seven normals and three rosette dwarfs. Figure 6 shows the dwarf in comparison with the normal. It could not be determined whether the dwarfs were resistant to disease or not.

Figure 5 shows segregation for wilt resistance and susceptibility in an F_2 progeny of a cross between a resistant and a susceptible variety.

Technique of the Genetic Studies

It seems to be generally conceded that natural crossing in peas is not of very frequent occurrence Bateson and Pellew (1), White (13), and

TABLE V.—*Effect of an Organic Mercury Compound upon Germination of Pea Seeds under Greenhouse Conditions*

Variety	Number seeds planted	Treatment	Number plants produced	Percent germination	Cortical lesioning
Horsford	60	Shaken in organic mercury dust	54	90	None
Horsford	150	No treatment	88	59	Slight

Sirks (10). However, Kappert (6) states that crossing is by no means rare, and Brotherton (2) found it necessary to protect his plants from natural crossing. Renard[2] working at Madison, Wisconsin, states that natural crossing occurs to a limited extent in canning peas. It is, of course, possible that climate and seasonal variation may have an effect upon natural crossing. If it rains for a few days during the blooming period, crossing by hymenopterous insects is almost precluded.

No precautions against natural crossing were considered necessary under greenhouse conditions. Under field conditions, however, most of the flowers were covered with glassine bags before the pollen was shed. In crosses of Horal x Horsford it was found more convenient to cover the entire plant with cheesecloth during the blooming period. The seed from the plants was harvested in separate lots as "selfed" and as "not selfed" material. The material was later labeled separately when planted. The results given in Table VI indicate that this precaution was probably unnecessary, since approximately the same ratios are obtained from protected as from unprotected material.

In Table VI and all other tables except tables of F_3 material, the unit used to express deviations and probable errors is the single plant. The unit used for the F_3 material is the single progeny. In designating the strain the first number given is the row number while the second number represents the number of the plant in the row.

Crossing was carried out according to the method of Giltay (5) except that pollen was transferred by means of the tweezers instead of using the de-petaled flower of the intended male parent. Most of the crosses were made in the greenhouse and no covering was used after crossing. All crosses made in the field were protected by glassine bags, and absorbent cotton.

The F_1 seeds used for growing further generations of plants were obtained by crossing the parents in the greenhouse during the winter of

2 Unpublished results.

TABLE VI.—*A Comparison of Wilt Ratios Obtained when the Flowers Producing the Seed Were Covered with Glassine Bags and Checked with Those Not Protected. (F_2 of Horsford x Resistant Alaska)*

Flowers not covered with bags: Strain	Total number plants healthy	Total number plants wilted	Deviation from 3:1 ratio	Probable error	D/P.E.	Flowers covered with bags: Strain	Total number plants healthy	Total number plants wilted	Deviation from a 3:1 ratio	Probable error	D/P.E.
200-4 N. S.	81*	20	5.25	2.94	1.79	200-4 ⊗⊗	142	56	6.50	4.11	1.58
204-4 N. S.	124	41	0.25	3.75	0.07	204-3 ⊗⊗	142	58	8.00	4.13	1.94
204-2 N. S.	147	38	8.25	3.97	2.08	204-3 ⊗⊗	140	37	7.25	3.89	1.86
204-1 N. S.	73	24	0.25	2.88	0.09	204-2 ⊗⊗	122	43	1.75	3.75	0.47
200-4 N. S.	74*	21	2.75	2.85	0.96	204-1 ⊗⊗	68	22	0.50	2.77	0.18
						203-1 ⊗	129	42	0.75	3.82	0.20

*These two lots of material were grown in places in the wilt bed at which wilting took place slowly.

1927-28. The F_1 seed used in wilt tests came from crosses made under field conditions. Backcrosses were all made in the field.

The F_2 seeds were obtained from plants spaced two feet by two feet in the field. These F_1 plants were heavily watered and fertilized. The response to spacing and watering was very good. Considering all the plants grown, the average number of seeds per plant was about 200. The highest producing plant had about 750 seeds.

A small part of the F_3 seeds used in this study was obtained from F_2 plants grown under field conditions very similar to those described for the F_2 seeds above. Most of the F_3 seed was obtained under greenhouse conditions and as a result the F_3 progenies are not very large. In one case F_3 seed was harvested from the survivors of a wilt test of F_2 plants.

The parents of the hybrids were grown near the hybrids under field conditions to obtain a check upon type and to increase the parental stocks.

During the summer of 1929 important P_1 and F_1 plants, from which seed was to be obtained, were enclosed in a large mosquito netting cage which excluded all hymenoterous insects that commonly visit the flowers of the pea.

Under field conditions germination was for the most part good but under green house conditions the germination was not very satisfactory. However, the tables show that variations in germination had no apparent affect upon the ratios obtained.

TABLE VII.—*Reaction of the Parental Strains when Subjected to Wilt Tests in the Greenhouse and in the Field*

Strain number	Susceptible strains				Strain number	Resistant strains			
	Greenhouse		Field			Greenhouse		Field	
	Total No. of plants healthy	Total no. of plants wilted	Total no. of plants healthy	Total no. of plants wilted		Total no. of plants healthy	Total no. of plants wilted	Total no. of plants healthy	Total no. of plants wilted
H_2	0	20	0	15	R11-12	13	0		
H_1	0	19	0	19	R11-14	25	0		
H_3	0	15	0	20	R6-1-1	24	0	17	
H_7	0	12	0	17	R11-19	20	0	20	
H_9	0	22	0	8	R7-3-2	18	0	17	
+H_2			0	36	R11- 9	13	0		
Ro 5	0	17	0	16	R11- 8	13	0		
Ro 7	0	14	0	17	R11-13			10	
Ro 3	0	15	0	18	+R6-1-3			31	
Ro 11	0	18	1	6	Ad 7	14	0	15	
Rog 5	0	16			Ad 5	16	0	15	
P_5	0	22			Ho 6	13	0		
P_6			0	9	Ho 4	12	0	17	
Sp 14	8	11			Ho 11	15	0	16	
Sp 11	3	9			Ho 9	19	0	8	
Sp 7	3	15			+ Ho 10			36	
Sp 1-1-1	0	9			Im 5	17	0	6	
Sp 8	0	14	0	8	Im 2	18	0		
Sp 6			0	9	Im 6			13	
+Sp 1-2-1			0	42	+ Im 5			36	1
S22	3	23			Sw 1	14	0		
S9-2-1	0	25	0	19	Sw 9	16	0	20	
S16	0	26	0	19	+ Sw 3			36	
S18	0	23	0	19	+ Sw 7			35	
S10-1-3	0	12			Resistant standard				
S10-3-3	0	10	0	20	checks**			598	
S7-5-2	0	23	0	9					
S15	5*	23							
S9-2-2	2	25							
+ S14			0	63					
+ S9-4-1			1	31					
Ac 3	0	19	2	14					
Ac 6	0	24	0	8					
Ac 11	0	19	0	19					
+ Ac 13			1	45					
Susceptible Standard Checks**			21	914					

* A progeny test showed two of these five to be susceptible. The other three died after transplanting.
\+ Sister strains were sometimes substituted in these progenies.
**The standard checks were planted at 75 different places in the field; a resistant check of ten seeds followed by ten or more from the susceptible check.

Experimental Results

Wilt Resistance in Parental Varieties

Before studying the inheritance of resistance in the crosses it was essential to know the wilt reaction of the parents. The parents were grown as near the segregating progenies as possible under both greenhouse and field conditions. The results presented in Table VII indicate that in most cases the parents gave the expected wilt reaction. In nine cases one or more plants did not wilt in progenies expected to be homozygous for susceptibility. Five of these cases occurred during the greenhouse tests. In each of three progenies under field conditions a single plant failed to wilt, and in another two plants failed. These plants should probably all be classified as susceptible plants which escaped, but there is, of course, the possibility that they are not. Unfortunately these progenies which gave aberrant results in the greenhouse did not yield sufficient seed for further tests.

One strain (Im 5) which is supposedly homozygous for resistance had one plant out of 37 wilted under field conditions. This may be ex-

TABLE VIII.—*Occurrence of Wilt in F_1 Progenies of Resisant Alaska x Susceptible Alaska and Reciprocal*

Cross	Row number	Number of seeds planted	Place tested	Total no. of plants healthy	Total no. of plants diseased
R6-2-2-11 x S10-3-3-1	700	7	Tanks	5	.....
S9-2-1-3 x R11-19-4	701	5	.....	4	.....
S9-2-1-4 x R11-19-4	702	3	.....	1	.....
S9-2-1-8 x R11-19-4	703	7	.....	5	.....
S7-5-2-1 x R1-1-1-2	704	1	.....	1	.....
R4-8-2-5 x S10-3-3-5	705	2	.....	1	.....
R6-2-2-10 x S10-3-3-1	706	3	.....	3	.....
R6-2-2-12 x S10-3-3-2	707	3	.....	1	.....
R6-2-1-2 x S9-4-2-2	710	4	.....	4	.....
R6-2-1-5 x S9-4-2-1	711	4	.....	1	.....
R6-1-3-2 x S10-1-2-3	712	3	.....	2	.....
R6-2-1-6 x S9-4-2-5	714	5	.....	1	.....
R6-2-1-7 x S10-1-3-5	716	2	.....	2	.....
R6-2-1-8 x S10-1-3-5	717	3	.....	1	.....
R6-2-1-8 x S9-4-2-5	718	6	.....	1	.....
			Subtotal	33	
R6-2-1-8 x S9-4-2-4	719	6	Field	6	.....
R6-2-1-9 x S10-1-3-5	720	3	.....	3	.....
R6-1-3-3 x S10-1-2-3	721	1	.....	1	.....
R6-1-3-3 x S10-1-3-5	722	1	.....	1	.....
R6-1-2-9 x S9-4-2-4	723	3	.....	3	.....
R6-1-2-9 x S9-4-2-1	724	6	.....	6	.....
R-1-1-2 x S7-5-2-4	728	5	.....	5	.....
R11-19-1 x S9-2-1-6	729	2	.....	2	.....
R11-19-2 x S9-2-1-6	730	2	.....	1	.....
R6-1-3-4 x S10-1-2-3	731	6	.....	6	.....
R6-2-1-10 x S10-1-3-5	732	1	.....	1	.....
S9-4-2-1 x R6-2-1-1	734	1	.....	1	.....
R6-1-3-5 x S10-1-2-3	735	1	.....	1	.....
			Subtotal	37	.....
	TOTAL			70	.....
Miscellaneous F_1 combinations					
Sp1-2-1-3xIm6-1	709	2	Tanks	1	.....
R6-1-3-4 x P_5-4	725	8	Field	7	.....
S9-4-2-4 x Sp 13-1	727	1	Field	0	1

plained in one of several ways — mis-classification, resistance breaking down or it may have been the result of seed mixture.

(1.) Wilt Resistance in Crosses

Crosses Between Resistant and Susceptible Strains of Alaska F_1 Generation

The Alaska is one of the most important early varieties of canning peas. The strain known as Alcross produces about fifty per cent resistant and about the same proportion of susceptible plants. The strains used in this test were single plant selections from Alcross and had been tested for wilt resistance by Mr. Renard, a few years before the beginning of this investigation.

The F_1 plants were tested under both greenhouse and under field conditions. In the greenhouse (in Wisconsin temperature tanks)[3] 33 plants were grown almost to maturity without showing any symptoms of the disease. The distribution of these 70 plants according to progenies is shown in Table VIII.

Table VIII also includes the results of a few miscellaneous F_1 combinations of other strains and varieties of peas. These results indicate that resistance is dominant.

[3] Wisconsin temperature tanks are devices in which the temperature is controlled automatically and very accurately. Dickson, J. G. *Making Weather to Order for the Study of Grain Diseases.* Wis. Agr. Exp. Sta. Bul. 379. 1926.

TABLE IX.—*Occurrence of Wilt in Backcross of F_1's to the Susceptible Parent Grown in Tanks*

Cross	Row number	Number of seeds planted	Place tested	Total no. of plants healthy	Total no. of plants diseased
S9-4-1-1 x (R6-1-3xS9-4-1)	600	2	Tank	1	1
S9-2-1-6 x (R6-1-1xS9-2-1)	601	1	.....	1	0
S10-3-3-7 x (S10-3-3xR6-2-2)	603	3	.....	1	2
S12-2 x (S12xR11-8)	604	11	.....	5	5
S9-2-2-1 x (S9-2-2xR4-2-2)	605	17	.....	8	9
S9-2-2-5 x (R6-1-1xS9-2-2)	606	3	.....	0	1
S9-2-2-4 x (R6-1-1xS9-2-2)	607	6	.....	3	2
S9-2-2-4 x (S9-2-2xR6-1-2)	608	3	.....	1	1
S9-2-2-3 x (S9-2-2xR6-1-2)	609	1	.....	0	1
S9-2-2-3 x (R6-1-1 x S9-2-2)	610	4	.....	2	2
S9-2-2-3 x (S9-2-2xR-2-4-2)	611	4	.....	2	1
S10-3-3-1 x (S10-3-3xR6-2-2)	612	4	.....	1	1
S10-3-3-3 x (R4-8-2xS10-3-3)	613	8	.....	3	1
S10-3-3-5 x (R4-8-2xS10-3-3)	614	5	.....	1	1
S10-3-2-1 x (S10-3-2 x R7-3-1)	615	4	.....	1	0
S10-3-2-3 x (S10-3-2 x R7-3-1)	616	4	.....	2	1
S10-1-1-1x(R6-2-1xS10-1-1)	617	3	.....	1	2
S10-1-1-2 x (R6-2-1xS10-1-1)	618	3	.....	2	1
S9-2-1-1 x (R6-1-1xS9-2-1)	619	6	.....	2	2
S9-4-1-3 x (R6-1-3xS9-4-1)	620	2	.....	1	1
S9-4-1-4 x (R6-1-3xS9-4-1)	621	5	.....	2	1
S9-4-1-2 x (R6-1-1xS9-2-1)	622	5	.....	2	3
(R11-16xS17) x S17-1	626	6	.....	2	3
(R11-11xS11) x S11-1	628	4	.....	1	2
(R4-8-1 x S10-3-3) x S10-3-3-2	629	4	.....	1	1
			Obs.	46	45
			Exp. (1:1 ratio)	45.5	45.5

$D = 0.50 \pm 3.22$ D/P.E. $= 0.16$

TABLE X.—*Occurrence of Wilt in Backcrosses of F_1's to the Susceptible Parent Grown in the Field*

Cross	Row number	Number of seeds planted	Place tested	Total no. of plants healthy	Total no. of plants diseased
(S10-3-2 x R7-3-1) x S10-3-2-3	632	6	Field	2	4
(S10-1-3 x R2-4-2) x S10-1-3-1	633	1	.....	0	1
(S10-1-3 x R2-4-2) x S10-1-3-4	634	7	.....	2	4
(R6-1-2 x S9-4-1) x S9-4-1-1	635	5	.....	5	0
(R6-1-1 x S9-2-2) x S9-2-2-1	636	5	.....	2	3
(R6-1-1 x S9-2-2) x S9-2-2-1	637	4	.....	3	0
(R6-1-1 x S9-2-2) x S9-2-2-1	638	5	.....	1	3
(R6-1-1 x S9-2-1) x S9-2-1-1	639	8	.....	3	4
(R6-1-1 x S9-2-1) x S9-2-1-3	640	12	.....	7	3
S16-1 x (S16 x R11-10)	643	5	.....	3	2
S16-2 x (S16 x R11-10)	644	7	.....	4	3
S16-3 x (S16 x R11-10)	645	5	.....	1	4
(R11-14 x Sp11) x Sp11-2	646	5	.....	2	2
S10-3-1-1 x (S10-3-1 x R2-4-3)	647	3	.....	3	0
S10-3-1-6 x (S10-3-1 x R2-4-3)	648	5	.....	0	4
S10-1-3-2 x (S10-1-3 x R2-4-2)	649	7	.....	1	5
S10-1-2-1 x (S10-1-2 x R6-1-3)	651	4	.....	2	2
S10-1-2-2 x (S10-1-2 x R6-1-3)	652	5	.....	2	3
S10-1-2-4 x (S10-1-2 x R6-1-3	653	10	.....	5	5
S9-4-2-1 x (S9-4-2 x R6-2-1)	654	11	.....	3	8
S9-4-2-2 x (S9-4-2 x R6-2-1)	656	6	.....	3	3
S9-4-2-4 x (S9-4-2 x R6-2-1)	657	5	.....	4	1
S17-2 x (R11-15 x S17)	658	5	.....	3	0
S17-5 x (R11-15 x S17)	659	5	.....	3	2
S17-4 x (R11-16 x S17)	660	1	.....	1	0
				65	66

	Healthy	Diseased
Observed	65	66
Expected (1:1 ratio)	65.5	65.5

D = 0.50 ± 3.86 D/P.E. = 0.13

Backcrosses to the Susceptible Parent

Backcrosses of the F_1 plants to the susceptible parent were likewise grown in tanks in the greenhouse, and also in the field. The individual progenies are very small, ranging in size from one to seventeen plants, but the results are consistent throughout the experiment. Of the 222 plants involved 111 were resistant to wilt and 111 were susceptible. This is a perfect fit to a 1:1 ratio. The details of the results are given in Tables IX and X.

TABLE XI.—*Occurrence of Wilt in Backcrosses of F_1's to Susceptible Parent A Miscellaneous Group*

Cross	Row number	Number of seeds planted	Place tested	Total no. of plants healthy	Total no. of plants diseased
Sp 1-2-1-1 x (Sp 1-2-1 x Im5)	624	6	Tank	2	1
Sp 1-1-1-7 x (Sp 1-1-1 x Im2)	625	2	.....	1	0
S7-5-2-2 x (Im5 x S7-5-1)	602	6	.....	3	3
(Sp6 x R11-13) x Sp6-1	627	3	.....	2	1
(Im6 x S10-3-3) x S10-3-3-2	641	6	.....	1	5
Sp6-4 x (Sp6 x R11-13)	642	1	.....	1	0
Sp6-5 x (Rog 1 x Sp6)	661	3	.....	1	1
Observed				11	11
Expected (1:1 ratio)					

D = 0.0 ± 1.58

TABLE XII.—*Occurrence of Wilt in Backcross of F_1's to the Resistant Parent, Resistant Alaska and Susceptible Alaska, and a Miscellaneous Group*

Cross	Row number	Number of seeds planted	Place tested	Total no. of plants healthy	Total no. of plants diseased
R4-8-1-4 x (R4-8-1 x S10-3-3)	751	2	Tanks	1	.....
R2-5-2-1 x (R2-5-2 x S3-5-2)	752	3	.....	1	.....
R2-5-2-6 x (R2-5-2 x S3-5-2	753	5	Field	5	.....
R6-2-2-7 x (S10-3-3 x R6-2-2)	754	5	.....	4	.....
R6-2-2-9 x (S10-3-3 x R6-2-2)	755	5	.....	4	.....
R2-4-3-1 x (S10-3-1 x R2-4-3)	756	20	.....	18	.....
R6-1-1-2 x (S10-1-3 x R6-1-1)	757	2	.....	2	.....
R6-1-1-5 x (S10-1-3 x R6-1-1)	758	2	.....	2	.....
R6-1-1-6 x (R6-1-1 x S9-2-2)	759	2	.....	2	.....
R6-1-1-7 x (R6-1-1 x S9-2-2)	760	8	.....	7	.....
R6-2-1-3 x (S9-4-2 x R6-2-1)	764	5	.....	4	.....
R6-2-1-4 x (S9-4-2 x R6-2-1)	765	7	.....	7	.....
R6-2-1-4 x (S9-4-2 x R6-2-1)	766	7	.....	7	.....
R6-1-3-1 x (S10-1-2 x R6-1-3)	767	3	.....	3	.....
R6-1-3-1 x (R6-1-3 x S9-4-1)	768	3	.....	3	.....
R6-2-1-5 x (S9-4-2 x R6-2-1)	769	5	.....	5	.....
R6-1-3-2 x (S10-1-2 x R6-1-3)	770	6	.....	5	.....
R6-1-3-3 x (S10-1-2 x R6-1-3)	771	1	.....	1	.....
(S11 x R11-8) x R11-8-1	774	3	.....	3	.....
(S12 x R11-8) x R11-8-2	775	1	.....	1	.....
(S9-2-2 x R2-4-2) x R2-4-2-1	776	3	.....	3	.....
(S10-3-1xR2-4-3) x R2-4-3-1	777	4	.....	3	.....
R11-15-1 x (R11-15 x S17)	778	6	.....	6	.....
R11-15-2 x (R11-15 x S17)	779	4	.....	4	.....
R11-11-3 x (R11-11 x S11)	780	4	.....	1	.....
R1-1-1-2 x (R1-1 x S9-4-3)	782	9	.....	9	.....
			Total	111	.....
Miscellaneous backcrosses					
Im5-3 x (Sp 1-2-1 x Im5)	761	5	Field	3	.....
Im2-1 x (Sp 1-1-1 x Im2)	762	2	.....	2	.....
Im2-2 x (Sp 1-1-1 x Im2)	763	1	.....	0	.....
Im5-8 x (Im5 x S7-5-1)	750	6	Tanks	2	.....
(Sp7xR11-10) x R11-10-1	772	5	Field	4	.....
(Ac11 x R11-19) x R11-19-1	773	3	.....	3	.....
Im 1-1 x (Im 1 x S10-1-3)	781	5	.....	5	.....

Table XI. gives the results of a few miscellaneous backcrosses to the susceptible parent involving other strains and varieties. The results are entirely in harmony with those given in Tables IX. and X. In this case, 22 plants were tested of which 11 were resistant and 11 susceptible.

Backcrosses to the Resistant Parent

None of the 111 plants considered in this backcross of the F_1 to the resistant parent showed any symptoms of the disease. Only two of these plants were tested under greenhouse conditions; the remainder were tested in the field wilt plot. Table XII. shows the distribution of the plants according to progenies.

The results of a few miscellaneous backcrosses to the resistant parent are also included in Table XI. These, likewise, show complete resistance.

F_2 Generation

A total of 335 plants were tested of which 264 remained healthy and 71 wilted. The deviation from a 3:1 ratio is 12.75 plants with a probable error of 5.35. This is a fairly close fit, i.e. the deviation is 2.38 times the probable error. The odds against the occurrence of a deviation as great or greater than the designated one due to chance alone are 8.48 to 1.

TABLE XIII.—*Occurrence of Wilt in F_2 Progenies from the Cross of Resistant Alaska x Susceptible Alaska.*

Original Crosses	Progeny numbers	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Deviation from a 3:1 ratio	Probable error	Dev. P.E.
R6-1-1 x S9-2-2	303-4	30	Green-house	20	2 *	3.50	1.37	2.55
R6-1-1 x S9-2-1	304-2	60	"	40	8	4.00	2.02	1.98
S15 x R11-8	262-(1 (2 (3	90	"	26	8 *	0.50	1.70	0.29
R6-1-3 x S9-4-1	300-1	70	Field I	47	18 *	1.75	2.35	0.74
R6-1-2 x S10-1-1	300-2	35	" II	23	9	1.00	1.65	0.61
R1-1-1 x S9-4-3	301-1	93	" III	69	16	5.25	2.69	1.95
	318-2	68	" IV	39	10	2.25	2.04	1.10
				264	71	12.75	5.35	2.38

*The susceptible parental check did not wilt completely.

It is of interest to note how the individual progenies deviate from a 3:1 ratio. The poorest fit among the seven progenies tested is that of progeny 303-4 which had twenty plants resistant and two susceptible. The best fit was that of the mixed progeny which had twenty-six resistant plants and eight susceptible. The other five progenies are well distributed between these two extreme cases. There is nothing to indicate that the progenies considered are significantly different from each other in their wilt reactions. The data are presented in Table XIII.

The F_3 Generation

Thirty-four F_3 progenies were tested for wilt resistance— 15 in the greenhouse and 19 in the field. A reasonably close fit to a 1:2:1 ratio was obtained —eight resistant, twenty-one segregating and five susceptible. *P* equals 0.32 in this case. Table XIV shows the reaction and classification of each progeny concerned.

However, the classification given in Table XIV cannot be considered as final, for according to the laws of chance it may happen that a progeny of say fourteen healthy plants (with none wilted) may in reality be segregating. In this case the deviation from a 3:1 ratio is 3.5 $\pm$ 1.09 or 3.2 times the probable error. The odds against it are 31.36:1. Figure 2 shows the sharply tri-modal effect obtained by plotting a curve for the amount of wilt occurring in F_3 progenies. The very sharp breaks between the central mode and the other two modes indicate that it is relatively improbable that very much misclassification of progenies occurred.

TABLE XIV.—*Occurrence of Wilt in F_3 Progenies from the Cross of Susceptible Alaska x Resistant Alaska and Reciprocal.*

Cross	Progeny number	Place tested	Number of seeds planted	Total number plants healthy	Total number plants diseased	Probable genotype*
R4xS1	553-1	Tanks	30	0	22	fw fw
	553-2	.....	34	22	8	Fw fw
	553-3	.....	18	14	0	Fw Fw
	553-4	.....	40	21	7	Fw fw
	554-1	.....	10	5	3	Fw fw
	554-2	.....	18	14	0	Fw Fw
	554-3	.....	17	15	0	Fw Fw
	555-1	.....	20	13	5	Fw fw
	555-2	.....	40	21	7	Fw fw
	555-3	.....	45	0	30	fw fw
	556-1	.....	22	13	5	Fw fw
	556-2	.....	46	30	10	Fw fw
	556-3	.....	14	8	3	Fw fw
	556-4	.....	27	21	5	Fw fw
	556-5	.....	12	5	3	Fw fw
	557-1	Field	29	19	9	Fw fw
	557-2	.....	22	17	6	Fw fw
	557-3	.....	25	18	4	Fw fw
	558-1	.....	20	12	0	Fw Fw
	558-2	.....	9	6	3	Fw fw
	558-3	.....	53	49	0	Fw Fw
	558-4	.....	29	29	0	Fw Fw
R1xS3	559-1	.....	44	38	0	Fw Fw
	559-2	.....	10	6	3	Fw fw
	560-1	.....	18	13	5	Fw fw
	560-2	.....	..	0	56	fw fw
	560-3	.....	42	30	11	Fw fw
	561-1	.....	43	30	12	Fw fw
	561-2	.....	32	18	14	Fw fw
	561-3	.....	..	0	16	fw fw
	562-1	.....	67	49	16	Fw fw
	562-2	.....	30	0	27	fw fw
	563-1	.....	12	5	0	Fw Fw
	563-2	.....	17	11	4	Fw fw

$X^2 = 2.41$ $P = 0.32$

	FwFw	Fwfw	fwfw
Observed	8	24	5
Expected (1:2:1 ratio)	8.50	17.00	8.50

*Fw is a factor for resistance; fw, its recessive allelomorph for susceptibility.

Crosses of Surprise and Improved Surprise

Surprise is a very early, wrinkled pea that is susceptible to wilt. Improved Surprise may be distinguished from Surprise by a slight difference in pod shape and by being entirely resistant to wilt.

In this cross so few F_1's and backcrosses were grown that the results from them are of little significance. Tables VIII, XI, and XII, show a few cases in which these two strains are involved. None from this particular cross were carried through to the F_3.

The F_2 results give a close fit to a 3:1 ratio. Of the 309 plants tested, 234 remained healthy and 75 wilted. This is a deviation of only 2.25 plants from the 3:1 ratio. This deviation is 0.44 times the probable error. The details are presented in Table XV. Of the eight F_2 progenies tested all gave very close fits to a 3:1 ratio. The poorest fit was thirteen healthy to two wilted, in progeny 305-1. This is a deviation of only 1.75 plants

TABLE XV.—*Occurrence of Wilt in F_2 Progenies From the Cross of Surprise x Improved Surprise and Reciprocal.*

Original Crosses	Progeny numbers	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Deviation from a 3:1 ratio	Probable error	Dev. P.E.
Sp 1-2-1 x Im5	305-1	20	Field Ia	13	2	1.75	1.13	1.55
Sp 1-2-1 x Im5	305-2	45						
Sp 1-2-1 x Im2	306-1	90	Field Ib	27 *	12	2.25	1.82	1.24
Sp 1-2-1 x Im2	306-2	60						
Sp 1-2-1 x Im2	306-3	60	" II	62	21	0.25	2.66	0.09
Im 2 x Sp 1-1-1	315-1	30	" III	22	9	1.25	1.63	0.77
Im 2 x Sp 1-1-1	315-2	68	" IV	10	3	0.25	1.05	0.24
Im 2 x Sp 1-2-1	316-1	46	" VI	22	7	0.25	1.57	0.16
			" VII	51	14	2.25	2.35	0.96
			" VIII	27	7	1.50	1.70	0.88
				234	75	2.25	5.13	0.44

*The resistant parent had one plant out of 37 wilted.

TABLE XVI.—*Occurrence of Wilt in F_2 Progenies from the Cross Horsford x Resistant Alaska.*

Original Crosses	Progeny numbers	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Deviation from a 3:1 ratio	Probable error	Dev. P.E.
H_2 x R11-12	200-4	90	Greenhouse	45	23	6.00	2.41	2.49
H_2 x R11-9	201-1	90	"	55	22	2.75	2.56	1.07
H_2 x R11-12	200-2	171	Tanks	104	40	4.00	3.50	1.14
H_2 x R11-12	200-1	240	"	128	46	2.50	3.85	0.65
H_2 x R11-12	200-4	100	Field	81	20	5.25	2.94	1.79
H_2 x R11-9	201-1	50	"	32	17	4.75	2.04	2.33
H_3 x R11-12	204-4	200	Field I	142	56	6.50	4.11	1.58
H_3 x R11-12	204-4	200	Field II	124	41	0.25	3.75	0.07
H_3 x R11-12	204-3	200	" III	142	58	8.00	4.13	1.94
H_3 x R11-12	204-3	200	" IV	140	37	7.25	3.89	1.86
H_3 x R11-12	204-2	200	" V	147	38	8.25	3.97	2.08
H_3 x R11-12	204-2	200	" VI	122	43	1.75	3.75	0.47
H_3 x R11-12	204-1	100	" VII	141	46	0.75	3.99	0.19
H_3 x R11-12	203-1	200	" VIII	129	42	0 75	3.82	0.20
H_3 x R11-12	203-1	200	" IX	152	47	2.75	4.12	0.67
H_3 x R11-12	200-4	100	"	74	21	2.75	2.85	0.96
H_3 x R11-12	201-1	50	"	36	10	1.50	1.98	0.76
			Total	1794	607	6.75	14.31	0.47

TABLE XVII.—*Occurrence of Wilt in F_3 Progenies of the Survivors of an F_2 Wilt Test of the Cross Horsford x Resistant Alaska.*

	Progeny no.	No. of seeds planted	Place tested	Total no. plants healthy	Total no. plants diseased	Probable genotype	% Wilt
H_2 x R11-12 (200-2)	1165	6	Field	6	0	Fw Fw	0.
	1166	6	.....	2	4	Fw fw	66.66
	1167	7	.....	4	2	Fw fw	33.33
	1168	8	.....	7	0	Fw Fw	0.
	1169	6	.....	5	0	Fw Fw	0.
	1170	9	.....	9	0	Fw Fw	0.
	1171	6	.....	6	0	Fw Fw	0.
	1173	6	.....	6	0	Fw Fw	0.
	1174	14	.....	12	0	Fw Fw	0.
	1177	7	.....	7	0	Fw Fw	0.
	1178	6	.....	4	1	Fw fw	20.00
	1179	16	.....	10	4	Fw fw	28.57
	1181	11	.....	5	5	Fw fw	50.00
	1182	6	.....	4	1	Fw fw	20.00
	1183	17	.....	16	0	Fw Fw	0.
	1185	6	.....	4	2	Fw fw	33.30
	1188	13	.....	9	3	Fw fw	25.00
	1189	11	.....	5	1	Fw fw	16.66
	1191	8	.....	8	1	Fw fw	11.11
	1192	8	.....	6	2	Fw fw	25.00
	1193	5	.....	5	0	Fw Fw	0.
	1194	7	.....	5	2	Fw fw	28.57
	1195	5	.....	5	0	Fw Fw	0.
	1197	8	.....	8	0	Fw Fw	0.
	1199	7	.....	6	1	Fw fw	14.28
	1202	4	.....	4	1	Fw fw	20.00
	1208	8	.....	5	1	Fw fw	16.66
	1209	7	.....	3	2	Fw fw	40.00
	1210	9	.....	3	2	Fw fw	40.00
	1212	5	.....	4	1	Fw fw	20.00
	1215	10	.....	9	0	Fw Fw	0.
	1217	6	.....	5	1	Fw fw	16.66
	1218	6	.....	5	1	Fw fw	16.66
	1220	11	.....	8	2	Fw fw	20.00
	1221	7	.....	4	1	Fw fw	20.00
	1224	9	.....	3	2	Fw fw	40.00
	1227	8	.....	3	4	Fw fw	57.14
	1228	6	.....	5	1	Fw fw	16.66
	1230	7	.....	4	2	Fw fw	33.33
	1231	9	.....	8	3	Fw fw	27.27
	1232	11	.....	8	2	Fw fw	20.00
	1233	7	.....	5	3	Fw fw	37.50
	1234	8	.....	4	4	Fw fw	50.00
	1235	9	.....	6	0	Fw Fw	0.
	1236	10	.....	2	3	Fw fw	60.00
	1238	6	.....	6	0	Fw Fw	0.
	1239	5	.....	5	0	Fw Fw	0.
	1240	8	.....	8	0	Fw Fw	0.
	1241	5	.....	3	3	Fw fw	50.00
	1242	4	.....	4	1	Fw fw	20.00
	1243	9	.....	8	1	Fw fw	11.11
	1244	5	.....	5	0	Fw Fw	0.
	1356	8	.....	6	0	Fw Fw	0.
	1357	6	.....	5	0	Fw Fw	0.
	1358	13	.....	7	2	Fw fw	22.22
	1359	8	.....	5	0	Fw Fw	0.
	1361	17	.....	8	0	Fw Fw	0.
	1363	6	.....	3	3	Fw fw	50.00
	1364	8	.....	5	1	Fw fw	16.66
	1365	5	.....	5	0	Fw Fw	0.
	1366	6	.....	3	2	Fw fw	40.00
	1367	6	.....	5	1	Fw fw	16.66
	1369	7	.....	4	1	Fw fw	20.00
	1370	9	.....	9	0	Fw Fw	0.
	1371	10	.....	8	2	Fw fw	20.00

	Fw Fw	fw fw
Observed	24 : 41	: 0
Expected (1:2:0 ratio)	21.67 : 43.33	:0
Dev.	2.33 ± 2.35	

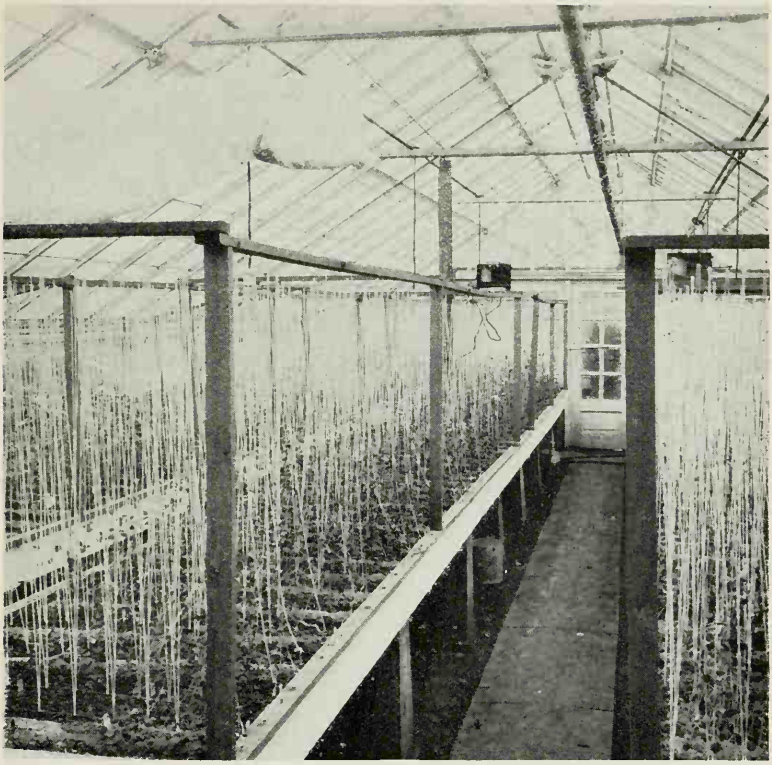


Fig. 1.—A view of the greenhouse during a wilt test. This shows recording thermometers, trellis, and part of the air distributing system.

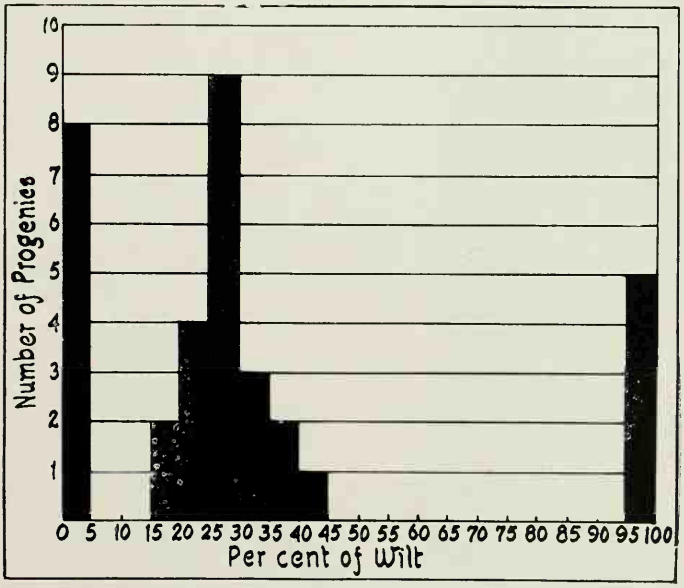


Fig. 2.—Relation between number of progenies and percentage of wilt.

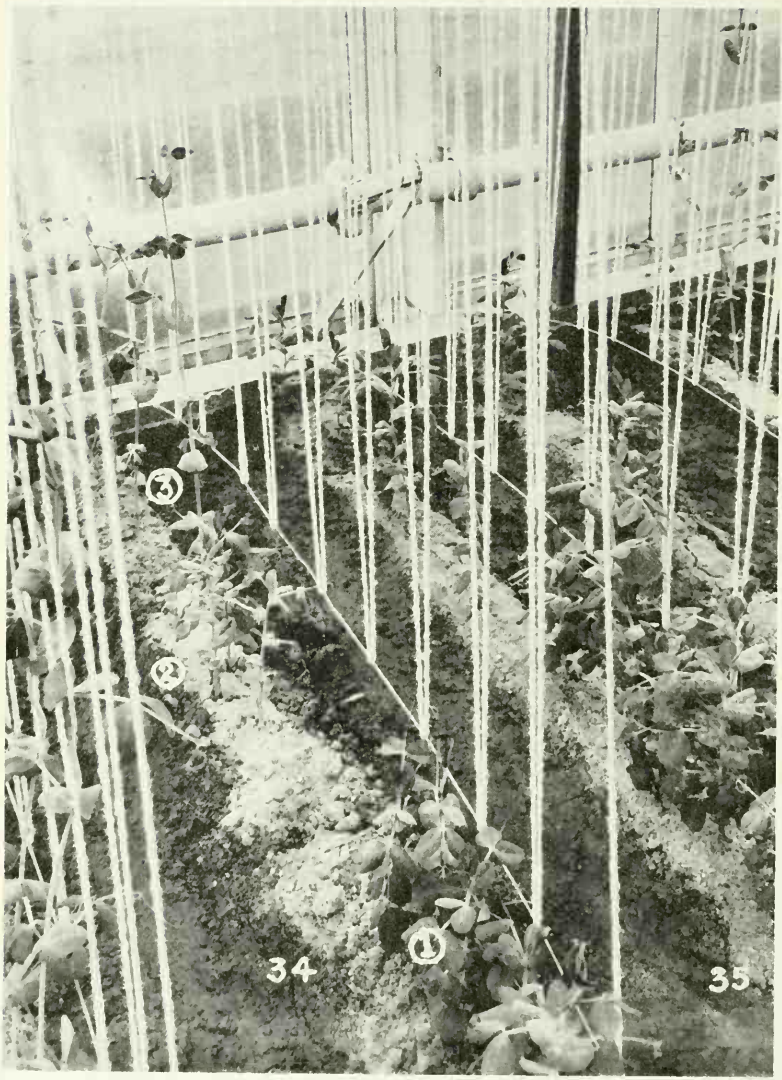


Fig. 3.—The use of sand to prevent cortex lesioning. Row 34 shows (1) resistant Horal (2) susceptible Horsford and (3) another parental strain 34. Row 35 contains F_2 segregating progenies from the cross of Horsford with Horal.

Fig. 4.—Resistant checks (left) and susceptible checks (right) 50 days after planting in the disease plot. The resistant checks show no symptoms of the disease, while the susceptible plants are almost dead. The small plants in the resistant check are due to delayed germination.

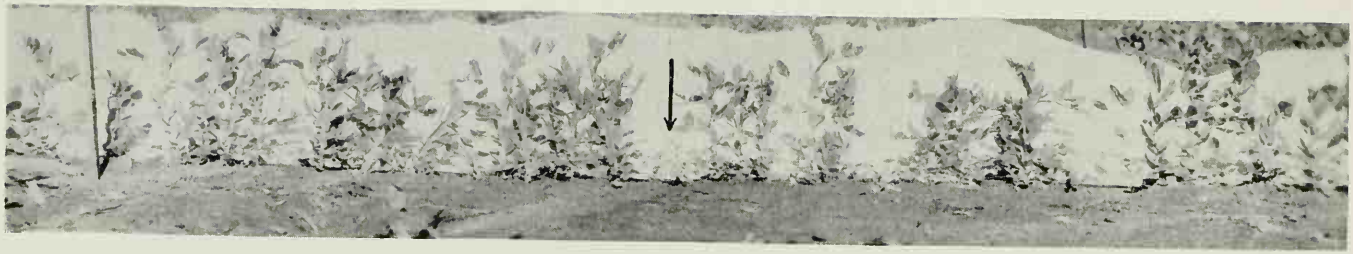


Fig. 5.—Wilting of F_2 segregates in the cross of Horal x Horsford 50 days after planting. Note the healthy appearance of the resistant segregates and the shrivelled appearance of the susceptible segregates. (Arrow points to susceptible plant).

Fig. 6.—A normal Horal plant at the left and a rosette dwarf at the right, fifty days after planting.

The dwarf is beginning to shrivel slightly and will probably be dead within 60 days after planting.

TABLE XVIII.—*Occurrence of Wilt in F_3 Progenies of the Survivors of an F_2 Wilt test of the Cross Horsford x Resistant Alaska.*

	Progeny number	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Probable genotype
H_2xR11-12 (200-1)	1245	6	Field	6	0	Fw Fw
	1248	6	.....	5	0	Fw Fw
	1249	7	.....	5	0	Fw Fw
	1251	8	.....	6	0	Fw Fw
	1254	6	.....	4	2	Fw fw
	1256	7	.....	4	1	Fw fw
	1259	7	.....	6	1	Fw fw
	1274	8	.....	5	0	Fw Fw
	1275	9	.....	5	1	Fw fw
	1276	14	.....	8	3	Fw fw
	1279	7	.....	6	0	Fw Fw
	1280	9	.....	5	2	Fw fw
	1284	10	.....	7	3	Fw fw
	1286	7	.....	7	0	Fw Fw
	1291	6	.....	2	5	Fw fw
	1293	6	.....	5	0	Fw Fw
	1296	9	.....	4	5	Fw fw
	1297	10	.....	8	2	Fw fw
	1298	9	.....	5	0	Fw Fw
	1299	11	.....	4	3	Fw fw
	1300	6	.....	5	1	Fw fw
	1301	7	.....	6	0	Fw Fw
	1302	6	.....	3	2	Fw fw
	1305	8	.....	6	1	Fw fw
	1306	9	.....	6	2	Fw fw
	1307	7	.....	5	0	Fw Fw
	1309	..	.....	7	1	Fw fw
	1310	7	.....	4	1	Fw fw
	1311	5	.....	4	1	Fw fw
	1312	8	.....	5	2	Fw fw
	1317	5	.....	5	0	Fw Fw
	1320	13	.....	11	2	Fw fw
	1321	9	.....	5	1	Fw fw
	1322	12	.....	6	0	Fw Fw
	1323	12	.....	7	2	Fw fw
	1325	8	.....	7	0	Fw Fw
	1328	8	.....	8	0	Fw Fw
	1336	7	.....	5	0	Fw Fw
	1338	10	.....	4	1	Fw fw
	1339	9	.....	8	1	Fw fw
	1340	9	.....	7	0	Fw Fw
	1343	8	.....	6	0	Fw Fw
	1344	11	.....	8	0	Fw Fw
	1346	12	.....	5	3	Fw fw
	1347	6	.....	4	2	Fw fw
	1349	9	.....	3	4	Fw fw
	1351	13	.....	10	0	Fw Fw
	1352	6	.....	6	0	Fw Fw
	1353	9	.....	7	0	Fw Fw
	1354	12	.....	6	6	Fw fw

			FwFw : Fwfw
	Observed		22 : 28
	Expected (1:2 ratio)		16.7 : 33.3
		D = 5.33 ± 2.06	
F_3's from 200-1	22	28	
F_3's from 200-2	24	41	
Observed	46	69	
Expected (1:2 ratio)	38.33	: 76.67	
	D = 7.67 ± 3.13		

and the probable error is 1.13 plants. The best fit occurred in progeny 306-1 in which 62 plants remained healthy while 21 wilted. This is a deviation of only 0.25 plants and the probable error is 2.66. These results indicate that none of the F_2 progenies tested differ from each other significantly in their reaction to wilt.

(3) *Inheritance of Resistance in Horsford and Resistant Alaska*

The progeny from this cross was given as extensive a test as possible, since in this cross two of the most widely divergent of the commonly cultivated canning peas are involved; and it was thought that if more than one factor were concerned in wilt resistance, that they should be revealed in this cross. Horsford is a late, dwarf wrinkled pea, with dark green foliage while Alaska is a very early, semi-dwarf, smooth pea with yellowish green foliage. Horsford is entirely susceptible to wilt while the Alaska strain considered in this case is completely resistant.

Greenhouse, temperature tanks, and field results, as shown in Table XVI, all indicate that only a single factor difference is involved. The deviation from a 3:1 ratio in F_2 in this case is very small since out of a total of 2,401 plants, 1,794 remained healthy and 607 wilted. The actual deviation is 6.75, which is 0.47 times the probable error. The nine progenies involved were tested in a total of 17 different places in the field and greenhouse. The greatest deviation from a 3:1 ratio occurs in progeny 200-4 when this progeny was grown in a greenhouse. Of a total of 68 plants 45 remained healthy and 23 wilted. This is a deviation of 6 plants with a probable error of 2.41. When other plants from this same progeny were tested under field conditions a better fit was obtained. In one case, a total of 95 plants gave 74 healthy to 21 wilted. This is a deviation of 2.75 plants with a probable error of 2.85. The best fit obtained was with a test of progeny 204-4 in which 124 plants remained healthy and 41 wilted. This is a deviation of 0.25 and the probable error of 3.75.

Progeny 200-1 was also segregating for white seedlings. These, however did not seem to interfere with the ratio of resistant to susceptible.

The survivors of the F_2 wilt test carried on in tanks (Table XVI, progenies 200-1 and 200-2) were permitted to mature in the greenhouse and the seed planted under field conditions. In view of the fact that one group was segregating in F_2 for white seedlings while the other was not, the two classes of F_3 progenies are listed separately in Tables XVIII. and XVII, respectively.

In this case, a 1:2:0 ratio is expected since all the susceptible F_2 plants should have been eliminated in the tanks. The F_3 test also serves as a check on the accuracy of the F_2 test. Since no homozygous susceptible progenies were found in the F_3 generation, the results indicate that all susceptible segregates were eliminated during the test in the tanks.

In the case of progeny 200-2, shown in Table XVII in which no white seedlings occurred, the deviation is approximately the same size as the probable error, $D = 2.33 \pm 2.35$. This deviation from a 1:2 ratio is based upon a total of 65 progenies of which 24 were classified as resistant and 41

as segregating. Progeny 200-1 does not give quite such a close fit: D = 5.33 ± 2.06 or approximately two and one-half times the probable error as may be seen in the Table XVIII. In this case 50 progenies were tested of which 22 were found to be resistant and 28 segregating. Combining the two sets of data, however, there is still a very fair fit: D = 7.67 ± 3.13. The total number of F_3 progenies concerned is 115 of which 46 are resistant and 69 segregating for resistance. The classification of F_3 progenies as resistant or segregating is, of course, not to be regarded as final since it is based upon very small numbers. Misclassified progenies would all go in the resistant class, so, that some excess of resistant progenies might be expected when the small number of plants per progeny is considered.

Inheritance of Resistance in Horal and Horsford

In this case two similar sweet varieties are considered. Horal is a a resistant strain extracted from a cross of Horsford with Alaska. This variety was developed by E. J. Delwiche of the University of Wisconsin. The most outstanding differences are a slightly smaller seed in the case of Horal and a somewhat shorter and earlier plant.

Under greenhouse conditions the deviation from a 3:1 ratio in the F_2 is rather large, 11.25, with a probable error of 4.10. This deviation is 2.74 times the probable error. However, this result is based on only 197 individuals of which 161 remained healthy and 36 wilted. Considering the total population dealt with from both greenhouse and field there is a fair fit since 1,496 remained healthy and 461 wilted. This gives a deviation from a 3:1 ratio of 28.25 plants, which is 2.19 times its probable error. The odds against the occurence of a deviation as great or greater than the designated one due to chance alone is 5.38 to 1. Of the 20 progenies considered in a total of 21 tests the largest deviation from a 3:1 ratio was in progeny 278-4 which had 57 healthy and 10 wilted plants. This is deviation of 6.75 plants with a probable error of 2.39. The odds against the occurrence of such a deviation due to chance alone is 13.58 to 1. The details are given in Table XIX.

The F_2 plants from which the F_3 seeds for a progeny test were harvested, were grown on non-infested soil under greenhouse conditions so that the number of plants per F_3 family is small ranging from five to fifteen. The results are in very close agreement with a 1:2:1 ratio. The total number of F_3 families considered is 114 of which 33 are classified as resistant, 55 as segregating, and 26 as susceptible. Considering the cross, Horal x Horsford, in which 18 resistant, 30 segregating, and 12 susceptible progenies occur, X^2* has a value of 1.20 with a corresponding value for P of 0.56. This indicates that a fit as bad or worse could be expected in 56 out of 100 trials, due to random sampling alone. For the reciprocal cross in which 15 resistant, 25 segregating, and 14 susceptible progenies occur, X^2 has a value of 0.34 and P equals 0.87.

In this case the F_3 results indicate a monofactorial difference between the parental types with a greater degree of certainty than do the F_2 results.

*X^2 = Chi Squared.

TABLE XIX.—*Occurrence of Wilt in F_2 Progenies..From the Cross Horal x Horsford and Reciprocal.*

Original Crosses	Progeny number	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Deviation from 3:1 ratio	Probable error	Dev. P. E.
Ho 11 x H7	278-4	90	Greenhouse	57	10	6.75	2.39	2.82
H7 x Ho 11	287-2	90	"	44	10	3.50	2.15	1.63
H9 x Ho 9	282-2	90	"	60	16	3.00	2.55	1.18
			Subtotal	161	36	11.25	4.10	2.74
Ho 11 x H 7	278-2	50	Field	32	12	1.00	1.94	0.52
H7 x Ho 11	287-2	20	"	15	4	0.75	1.27	0.59
H9 x Ho 9	282-1	23	"	15	6	0.75	1.34	0.56
Ho 10 x H2	279-1	100	" I a	68	21	1.25	2.76	0.45
Ho 10 x H2	279-3	100	" I b	69	31	6.00	2.92	2.05
Ho 10 x H2	279-4	100	" IIa	78	19	5.25	2.88	1.82
Ho 10 x H2	279-5	100	" II b	72	24	0.00	2.86	0.00
H 11 x Ho 9	280-2	200	" III	149	48	1.25	4.10	0.30
H 11 x Ho 11	281-1	200	" IV	143	54	4.75	4.10	1.16
H 8 x Ho 11	284-2	200	" V	118	35	3.25	3.61	0.90
H 9 x Ho 11	283-1	100	" VIa	71	28	3.25	2.91	1.12
H 7 x Ho 11	285-1	100	" VIb	80	17	7.25	2.88	2.52
H 8 x Ho 11	284-1	75	" VIIa	54	17	0.75	2.46	0.30
H 7 x Ho 10	286-4	125	" VIIb	90	28	1.50	3.17	0.47
H 7 x Ho 10	286-3	110	" VIIIa	73	18	4.75	2.79	1.70
H 7 x Ho 10	286-1	90	" VIIIb	33	8	2.25	1.87	1.20
H 7 x Ho 10	286-2	200	" IX	143	43	3.50	3.98	0.88
Ho 11 x H7	278-2	50	"	32	12	1.00	1.94	0.52
			Sub Total	1335	425	15.00		
			Total	1496	461	28.25	±12.92	2.19

TABLE XX.—*Occurrence of Wilt in F_2 Progenies From the Cross Surprise x Resistant Alaska and Reciprocal.*

Original Crosses	Progeny number	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Deviation from 3:1 ratio	Probable error	Dev. P. E.
Sp 7 x R 11-10	240-2	90	Greenhouse	59 *	11	6.50	2.44	2.66
R 11-14 x Sp 11	224-1	77	"	54 *	13	3.75	2.39	1.57
	224-3							
Sp 6 x R 11-13	239-2	64	Field I	42	16	1.50	2.22	0.68
			Total	155	40	8.75	4.08	2.14

*The susceptible parent did not wilt entirely in these cases.

Inheritance of Resistance in Surprise x Resistant Alaska Crosses

Surprise differs from the Alaska strain used in being wrinkled and susceptible to wilt.

The total number of F_2 plants considered in this case is 195 of which 155 remained healthy and 40 wilted. This is a very fair fit to a 3:1 ratio since the deviation of 8.75 plants is 2.14 times the probable error. The odds against the occurrence of a deviation as great or greater due to chance alone is 5.38 to 1. The small number of F_2 plants considered is due to the relative unproductiveness of the F_1 plants. Of the three progenies tested, 240-2 gave the largest deviation from a 3:1 ratio, with 59 healthy and 11 diseased plants, while 239-2 gave the smallest deviation, having 42 healthy and 16 diseased plants. The deviations with probable errors are: 6.50 ± 2.44 and 1.50 ± 2.22, respectively. The data are presented in Table XX.

Although the number of F_3 progenies is small, an excellent fit to a 1:2:1 ratio is obtained. Twenty progenies were considered of which six were found to be resistant, nine segregating, and five susceptible. The value of P in this case is 0.88. The number of plants per progeny is again small, ranging from five to nine. The possibility should be borne in mind, therefore, that some of the families classified as resistant or susceptible may belong in reality to the segregating class.

Inheritance of Resistance in Susceptible Alaska and Horal

Unfortunately this cross did not furnish sufficient seed for both greenhouse and field tests. A greenhouse test was conducted with a total of 74 plants. Of these 50 were classified as resistant and 24 as susceptible. This is a deviation from a 3:1 ratio of 5.50 plants which is 2.19 times the probable error. Ordinarily this would be considered a very fair fit but in view of the fact that there is an excess of susceptible segregates when the checks in the adjoining rows showed less than complete elimination, this may in reality indicate a poor fit. Table XXI shows the results in detail.

Fifty-nine F_3 progenies containing from five to twenty-five plants each, were tested. Eighteen families were classified as resistant, 33 as segregating, and 8 as susceptible. These results indicate only a fair fit to a 1:2:1 ratio. The value of X^2 is 4.23 and P equals 0.12. There is a large deficiency in the recessive class and an excess of segregating progenies, suggesting perhaps, that elimination of the susceptible plants in F_3 was not complete enough to satisfactorily differentiate these two classes. Weight is given to this suggestion by the fact that these F_3 progenies were grown next to the Perfection x Horal F_3 progenies in which a close check of the soil revealed that inoculation was not complete.

Inheritance of Resistance in Perfection and Horal Crosses

Perfection is one of the most important of the wrinkled canning peas. It is a slightly taller plant than Horal and is completely susceptible to wilt.

TABLE XXI.—*Occurrence of Wilt in F_2 Progenies From the Cross of Susceptible Alaska x Horal.*

Original Crosses	Progeny number	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Deviation from 3:1 ratio	Probable error	Dev. P. E.
S 22 x Ho 4	223-2	90	Greenhouse	50	24 *	5.50	2.51	2.19

*In this case the susceptible parent did not wilt entirely.

TABLE XXII.—*Occurrence of Wilt in F_2 Progenies From the Cross Perfection x Horal.*

Original Crosses	Progeny number	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Deviation from 3:1 ratio	Probable error	Dev. P. E.
P11 x Ho 4	212-2	90	Greenhouse	47	15	0.50	2.30	0.22
P11 x Ho 4	212-2	15	Field	10	4	0.50	1.09	0.46
P11 x Ho 4	212-2	15	"	12	3	0.75	1.13	0.66
P6 x Ho 6	209-1	200	"	148	48	1.00	4.09	0.24
			Total	217	70	1.75	5.71	0.31

TABLE XXIII.—*Occurrence of Wilt in F_2 Progenies from the Cross of Fasciated Sweet x Acme and Reciprocal.*

Original Crosses	Progeny number	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Deviation from 3:1 ratio	Probable error	Dev. P. E.
Sw4 x Ac 3	234-2	90	Greenhouse	42	21	5.25	2.32	2.26
Ac6 x Sw 1	233-1	30	"	12	4	0.00	1.17	0.00
Sw4 x Ac 3	234-4	50	Field	24	19	8.25	1.92	4.30
Sw4 x Ac 3	234-3	50	"	34	12 **	0.50	1.98	0.25
Sw7 x Ac13	268-1	200	" I	129	46 *	2.25	3.86	0.58
Sw8 x Ac 9	269-1	200	" II	149	42	5.75	4.04	1.42
Sw8 x Ac 9	269-1	200	" III	102	33	0.75	3.39	0.22
Sw8 x Ac 9	269-3	200	" IV	127	33	7.00	3.69	1.90
Sw8 x Ac 9	269-3	200	" V	115	32	4.75	3.54	1.34
Sw8 x Ac 9	269-2	200	" VI	123	42	0.75	3.75	0.20
			Total	857	284	1.25	9.87	0.13

*In this case one susceptible parent plant out of 46 failed to wilt.
**In this case two susceptible parent plants out of 16 failed to wilt.

Under both greenhouse and field conditions a very close fit to a 3:1 ratio in F_2 was obtained. The total number of plants tested was 287 of which 217 were classified as healthy and 70 as wilted. This represents a deviation of 1.75 plants and it is only 0.31 times the probable error. Only two progenies are considered of which 212-2 was tested in three different places. The details are given in Table XXII.

Fifty-nine progenies from this cross, comprising from five to twenty-two plants each, were tested in F_3. Of these 26 were classified as resistant, 25 as segregating, and 8 as susceptible. The expectation on a 1:2:1 basis is 14.75:29.50:14.75, respectively. The value of X^2 is 12.35 and P is equal to 0.0021. In only one trial in about 500 should a fit as poor or worse than this occur due to random sampling alone.

It was noticed during the wilt test that a part of the F_3 progenies from this cross were very much retarded in development of wilt symptoms. To test the distribution of the inoculum, check plants were planted approximately one to the foot throughout the rows upon which these progenies were planted. Of the 122 special check plants considered, only 72 wilted. The other fifty plants remained healthy. To make sure that it was not the effect of season on the wilting of these special checks, a similar lot of twenty seeds was planted at a place in which complete elimination had occurred. In this case, all the plants wilted promptly. The results with these special checks indicate that the inoculum was distributed in a rather irregular manner in this part of the field.

Inheritance of Resistance in Fasciated Sweet and Acme

Fasciated Sweet is a taller pea than Acme, and is resistant to wilt. Fasciated Sweet is a wrinkled segregate from a cross of Arthur field pea with Perfection. Acme is a wrinkled segregate from a cross of Horsford with a segregate from a previous cross of Horsford with French June.

Considering the total number of plants worked with (1141) a very good fit to a 3:1 ratio is obtained. Eight hundred fifty-seven (857) plants remained healthy and 284 wilted. The deviation of 1.25 plants is only 0.13 times the probable error. In one case, however, the deviation is 4.30 times the probable error based on a total of 43 plants. This result may be due to the accidental destruction of some of the healthy survivors since this progeny was damaged in the cultivation of nearby plots. On the other hand, it may be due to random sampling, but the odds against this occurrence due to chance alone are 267.2 to 1. In the case of progeny 233-1 a perfect fit to a 3:1 ratio was obtained—12 resistant and 4 susceptible. The data are presented in Table XXIII.

Inheritance of Resistance in Fasciated Sweet and Susceptible Alaska Crosses

In this case there occurred segregates which seemed to mature somewhat earlier than Alaska. Since maturity masks wilt symptoms to a certain extent it is possible that a part of the deficiency of recessives shown here may be due to mistakes in classification as a result of this condition. On the other hand, it is possible that modifying factors affected the result.

TABLE XXIV.—*Occurrence of Wilt in F_2 Progenies From the Cross Fasciated Sweet x Susceptible Alaska.*

Original Crosses	Progeny number	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Deviation from 3:1 ratio	Probable error	Dev. P. E.
Sw 9 x S 18	270-2	90	Greenhouse	40	16	+ 2.00	2.19	0.91
Sw 9 x S 18	270-2	83	Field	60	21	+ 0.75	2.63	0.29
Sw 9 x S 18	270-2	83	"	67	13	— 7.00	2.61	2.68
Sw 3 x S 14	236-3	125	" Ia	101	24	— 7.25	3.27	2.22
	236-2	75	" Ib	35	9	— 2.00	1.94	1.03
	237-3	121	" IIa	89	28	+ 1.25	3.16	0.40
	237-2	79	" IIb	65	12	— 7.25	2.56	2.83
	237-2	200	" III	131	30	— 10.25	3.71	2.76
	237-1	200	" IV	122	38	— 2.00	3.69	0.54
	237-1	200	" V	137	37	— 6.50	3.85	1.69
	237-1	200	" VI	76	22	— 2.50	2.89	0.87
	236-4	200	" VII	142	46	— 1.00	4.00	0.25
	236-4	200	" VIII	124	44	+ 2.00	3.79	0.53
			Total	1189	340	— 42.25	11.42	3.70

TABLE XXV.—*Occurrence of Resistance in F_2 Progenies from Miscellaneous Crosses.*

Original Crosses	Progeny number	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased	Deviation from 3:1 ratio	Probable error	Dev. P. E.
H 1 x Ad 7	202-1	90	Greenhouse	41	16	1.75	2.20	0.80
Ad 5 x H 3	219-2	90	"	55	19	0.50	2.51	0.20
H 1 x Ad 7	202-1	50	Field H I	28	13	2.75	1.87	1.47
Ad 5 x H 3	219-1	43	" H I	32	9	1.25	1.87	0.67
H 1 x Ad 7	202-1	50	Field H I	34	12	0.50	1.98	0.25
Ad 5 x H 3	219-1	42	" H II	27	13	3.00	1.85	1.62
Ac 11 x R 11-19	257-2	90	Greenhouse	51	16	0.75	2.39	0.31
	257-4	67	Field H I	39	18	3.75	2.20	1.70
	257-3	67	" H II	49	14	1.75	2.32	0.75
Ro 11 x R7-3-2	271-1	90	Greenhouse	34	10	1.00	1.94	0.52
Ro 11 x R7-3-2	271-2	42	Field H I	31	8	1.75	1.82	0.96
Ro 11 x R7-3-2	271-2	42	" H II	28	5 *	3.25	1.68	1.93
Im 5 x S7-5-1	309-1	90	Greenhouse	51	15	1.50	2.37	0.63
Im 5 x S7-5-1	309-1	26	Field H I	17	7	1.00	1.43	0.70

*In this case the susceptible checks did not wilt completely, 1 out of 7 remaining healthy.

In no case does the deviation go as high as 3.2 times the probable error when the progenies are considered separately. However, nearly all the deviations are in the same direction so that in the total population the deviation becomes significant and is actually 3.70 times the probable error, as shown in Table XXIV. The total number of plants considered is 1,529 of which 1,189 were classified as resistant and 340 as susceptible. The deviation from a 3:1 ratio is 42.25 plants.

A Miscellaneous Group of Crosses

In this group, the number of individuals is rather small in each F_2 progeny, but there is no indication of any mode of inheritance other than the simple monohybrid relationship found in other strains and varieties. In Table XXV may be found the data covering the wilt reactions of this group of progenies. These crosses produced such a small number of seeds that they were not deemed worthy of separate consideration. The greatest significance of this group of progenies lies in the fact that they extend the range of the investigation to additional types of peas.

Results of Crossing Susceptible Strains and Varieties of Peas

The crosses of Susceptible Rogue Alaska x Perfection and of Susceptible Rogue Alaska x Susceptible Alaska gave F_2 progenies which were entirely susceptible, under both greenhouse and field conditions.

Under field conditions the F_2's of Horsford x Susceptible Rogue Alaska and of Susceptible Rogue Alaska x Susceptible Alaska were entirely susceptible. Nevertheless, under greenhouse conditions some apparently resistant plants occurred. However, these plants all occurred in the same bench and a progeny test showed three of the twelve (205-1) to be homozygous for susceptibility. The other nine plants failed to produce seed. In progeny 251-4 no seed was produced.

Susceptible Rogue Alaska x Surprise gave four plants out of 70 with no symptoms of wilt when this F_2 progeny was tested in the greenhouse.

TABLE XXVI.—*Occurrences of Wilt in F2 Progenies From the Crosses of Susceptible Varieties*

Original crosses	Progeny number	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased
Ro 7 x P 7	230-3	90	Greenhouse	0	71
Ro 7 x P 7	230-2	51	Field	0	41
Ro 7 x P 7	230-1	35	"	0	36
Rog 6 x S 16	245-4	90	Greenhouse	0	71
Rog 6 x S 16	245-5	50	Field	0	48
Rog 6 x S 16	245-3	50	"	0	47
H 3 x Ro 5	205-1	90	Greenhouse	12 *	47
H 3 x Ro 5	205-1	50	Field	0	48
H 3 x Ro 5	205-1	50	"	0	46
Ro 3 x S 16	251-4	90	Greenhouse	8 **	65
Ro 3 x S 16	251-5	33	Field	0	30
Ro 3 x S 16	251-5	33	"	0	32
Rog 6 x Sp 8	244-2	90	Greenhouse	4 ***	66

*These 12 plants all occurred in one bench. A progeny test of three plants showed all three to be susceptible.
**These all occurred in one bench.
***These all occurred in one bench. Causal organism was isolated from two.

As in the other two cases, these plants all occurred in one bench. Unfortunately, no seed was available for a field test.

It seems probable that the resistant plants found in these crosses are not genetically resistant but are escapes. The data are given in detail in Table XXVI.

Results of Crossing Resistant Strains and Varieties of Peas

Out of 96 F_2 plants from the cross of Horal x Admiral, raised almost to maturity on wilt infested soil, none showed any indications of wilt. This material was tested in the greenhouse on soil selected from places in the benches at which very rapid elimination of susceptible plants had occurred. The susceptible checks for this material wilted completely.

The results of the cross of Resistant Alaska x Horal are entirely in harmony with those of the first mentioned cross. The number of plants involved is again small (107) but the tests were made under very severe conditions so that it is quite probable that if any susceptible plants were present they could not have escaped the disease. The results are shown in Table XXVII.

TABLE XXVII.—*Occurrence of Wilt in F2 Progenies from Crosses of Resistant Varieties*

Original crosses	Progeny number	Number of seeds planted	Place tested	Total number plants healthy	Total number plants diseased
Ho 4 x Ad 6	319-1	103	Greenhouse	96	0
R 11-15 x Ho 6	320-1	116	"	107	0

Linkage Relationships

Relationship Between Sugary and Resistance

Two F_2 progenies were tested to determine if any linkage existed between the gene for sugary (a simple recessive to non-sugary) and the gene for wilt resistance. These two progenies were from the cross of Horsford x Resistant Alaska. The first (200-1) showed white seedlings but the presence of these apparently does not influence in any way the segregation for wilt resistance. The X^2 value of 1.88 with a value for P of 0.60 indicates that there is no linkage to disturb the 9:3:3:1 ratio, as shown in Table XXVIII.

TABLE XXVIII.—*Linkage Studies of Sugary and Resistance in F2 Progenies of the Cross Horsford x Resistant Alaska*

Original cross	Progeny numbers	Type of seed	Number of seeds planted	Number of plants healthy	Number of plants wilted
H 2 x R 11-12	200-1	sugary	75	31	8
	200-1	non-sugary	152	97	38
	200-2	sugary	43	31	10
		non-sugary	103	73	30

X^2 (9 : 3 : 3 : 1 ratio) = 1.88 P = 0.60

It is possible that the gene for sugary may modify the expression of resistance but the results given here indicate that it does not. A comparison of rate of wilting for sugary and non-sugary plants would give a better opportunity to study the effect of the sugary gene on resistance.

Relationship Between Height and Resistance

After making a few preliminary observations under greenhouse conditions, it seemed evident that there existed some linkage between tallness and resistance. F_2 progenies from the cross of medium-tall Fasciated Sweet x dwarf Acme were tested under field conditions to determine the amount of this linkage. The results of this study are shown in Table XXIX.

TABLE XXIX.—*Linkage Studies of Height and Resistance in F_2 Progenies of the Cross Fasciated Sweet x Acme*

Original cross	Progeny numbers	Type of plant	Number of plants healthy	Number of plants wilted	Type of plant	Number of plants healthy	Number of plants wilted
Sw4 x Ac3	234-4	Tall	22	12	Short	2	7
Sw4 x Ac3	234-3	.....	30	4	.....	4	8
Sw7 x Ac13	268-1	.....	108	32	.....	21	14
Sw8 x Ac9	269-1	.....	129	18	.....	20	24
	269-1	.....	78	22	.....	24	11
	269-3	.....	106	17	.....	21	16
	269-3	.....	98	17	.....	17	15
	269-2	.....	100	24	.....	23	18
Totals		.....	671	146	.....	132	113

X^2 (9:3:3:1 ratio) = 78.62 P = .000000+

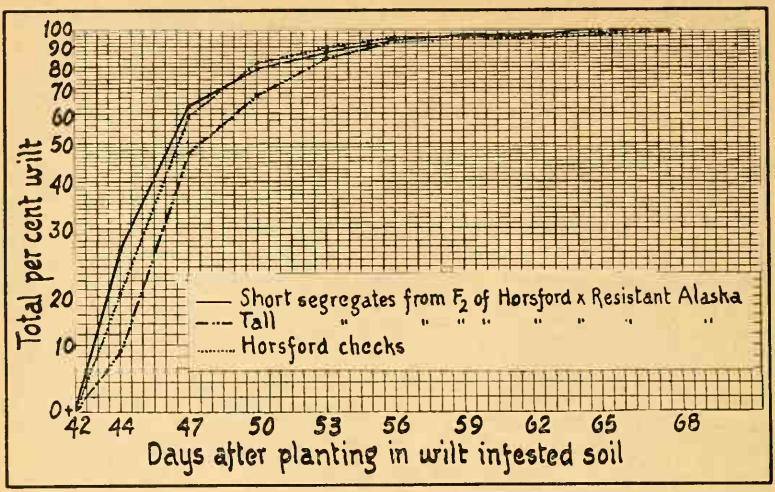
In using the X^2 test, the deviations are so large that P has no positive value to at least six decimal places, and hence the data support the idea of a linkage relation existing between the characters involved.

These figures, nevertheless, should be regarded as only approximate since the distinction between tall and short is rather arbitrary under some conditions.

The calculated gametic ratio based on F_2 results (Emerson, 4) is 2.23:1.00:1.00:2.23. This is equivalent to a crossover value of about 31 per cent between the factor for tall and the factor for resistance. The actual number of plants considered was 817 tall of which 671 remained healthy and 146 wilted, and 245 short of which 132 remained healthy and 113 wilted. On the basis of the calculated gametic ratio the zygotic expectation is 657.5:139:139:126.5, respectively. This is a close fit with X^2 equal to 2.42 and P equal to 0.47.

Rate of Wilting

It has been observed that tall plants wilt more slowly than short plants and some varieties more quickly than others. However, there are no grades of resistance and when the total number of plants wilted is considered at the end of a test it is found that elimination of a susceptible variety or strain is always complete, provided that the plants have been



fairly exposed to infection. That differences in rate of wilting may be more apparent than real is evidenced by the fact that most plants that are going to wilt during a test can be picked out a few days before wilting actually begins, and that these preliminary symptoms seem to occur in all varieties and strains at almost the same time. The preliminary symptoms referred to are a slight loss of color, with a very slight shrivelling of the basal leaves. It also requires approximately the same time for complete elimination in one variety as in another.

The wilt notes under field conditions were taken in such a manner that rate of wilting can be studied for most of the progenies under observation. The cross selected for consideration of this matter is Horsford x Resistant Alaska. This cross is considered for two reasons. First, the number of plants involved is relatively large, and secondly, a very good fit to the expected 3:1 ratio of resistant to susceptible plants was obtained.

The chart above shows the curves plotted for the data at hand. The curves have the same general form with the most rapid wilting from the 44th to 47th day after planting. The short segregates wilt much more rapidly from the 42nd to the 44th day after planting than do the tall segregates, and slightly faster than the Horsford checks. The greater initial rate of the short segregates is about three times that of the tall i.e. 26.14 per cent and 8.52 per cent respectively from the 42nd to 44th day after planting. Since all three types are completely wilted at about the same time — 68 days after planting — it is necessary that at sometime during the wilting season that the rate of wilting be reversed with the tall segregates wilting at a more rapid rate than the shorter. This change is shown to occur from the 44th to the 47th day after planting. The percentages are based on 159 short susceptible segregates, 223 tall susceptible segregates, and 122 Horsford checks.

It is possible, of course, that the period selected (every third day, except first reading) may be too gross a measure for an accurate study of

rate of wilting. The plants were examined in detail on the 42nd day after planting and it was decided that none showed sufficiently advanced symptoms to justify removal from the wilt bed.

Since the expression of wilt in a tall plant is somewhat different from that in a short plant, it is possible that the two kinds of plants may not really have been in comparable stages of wilt when removal occurred. On a wilt bed in which elimination of susceptibles does not take place so quickly during the first few days of wilting, there may be a better opportunity to compare rate of wilting in the tall and in the short plants.

Nevertheless, from the data at hand, it appears probable that the tall plants do really have a somewhat lower initial rate of wilting than do the short plants. This may be attributed to a modifying factor or factors or to the modifying effect of the tall gene itself. Although each variety of peas may differ from other varieties of peas in its expression of wilt, the final results are the same. A study of F_3 progenies homozygous for the characters under consideration would give much more reliable information than that obtained from F_2 results.

Discussion and Conclusions

The results of the wilt tests cover a wide range of material, including some of the peas most commonly grown by canners and other strains of importance on account of their wilt resistance. The results clearly indicate that, in the varieties concerned, only a single factor difference is involved in resistance to *F. orthoceras* var. *pisi*. Most of the material considered is F_2 material from crosses of resistant x susceptible strains or reciprocal. It made no difference from the standpoint of ratios obtained whether the material was closely or distantly related.

In six crosses some of the material was carried to the F_3. These results give further support to the monofactorial explanation.

Crosses of susceptible x susceptible, resistant x resistant, backcrosses of F_1 to the susceptible parent, backcrosses to the resistant parent and F_1 produced results indicative of a monohybrid difference, with resistance dominant to susceptibility.

It is of interest to note that of the three Fusarium vascular diseases which have been studied genetically two give monofactorial segregation for resistance (cabbage wilt and pea wilt) while one (flax wilt) shows a more complicated relation. In the case of cabbage the situation is very similar to that in peas, with resistance dominant (Walker, 12) but in flax (Burnham, 3) more than one factor is concerned and susceptibility is dominant.

Linkage studies of resistance with sugary and of resistance with height have been conducted. The data indicate that there is no linkage between sugary and resistance. The linkage data for height and resistance show that these two genes are rather loosely linked, with a crossover value of above 30 per cent. This crossover value is probably only an approximation since it is frequently difficult to distinguish between genetic short and environmental short plants.

The linkage of height with resistance indicates a linkage between the factors "Le" (White, 13) and "Fw". The factor for height is considered to be "Le" since the two varieties concerned have approximately the same number of internodes but with a difference in length. Due to the destructive nature of the wilt disease this factor may be of but little value in linkage studies. Since linkage is so loose it should not be difficult to incorporate resistance in plants of any height.

A rate of wilting test showed that in the case of Horsford x Resistant Alaska the shorter type of peas wilted at a slightly more rapid initial rate than did the tall segregates. This result may be due to modifying factors or to the modifying effect of the gene for tallness itself. Further tests of rate of wilting are desirable.

While not stated, it is implied that there is in reality but one strain of the fungus or that the hosts react to all the strains in the same manner. Under greenhouse conditions a single strain was used, but the field plot had been inoculated with a mixture of many strains, and in both cases approximately the same type of reaction occurred. However, since no effort was made to canvass all sources of the fungus, it is possible that other strains of the fungus may be encountered.

In a few cases the expression of the disease has indicated that there may be modifying factors concerned. However, modifying factors must necessarily remain speculative until more satisfactory methods of controlling some of the pathological phases have been worked out. Since the environment is so variable under field conditions, it seems that further work should preferably be done in the greenhouse. Under field conditions, we have to consider that confusion may arise from sudden changes in temperature, heavy rains causing erosion and elimination of some plants, and complications arising from other diseases. Under greenhouse conditions there are also many difficulties but temperature variations and losses of plants from causes other than wilt can probably be better controlled than in the field.

From the results obtained in this study there are indications that resistance to Fusarium wilt can be readily combined with other desirable characteristics and that it should be possible in the course of a few years to incorporate resistance in the valuable commercial varieties without sacrificing either yield or quality. It is, of course, possible that in other cases different factors for resistance may be involved; but since such a wide range of varieties and strains was tested for genetic relationships, it seems highly probable that the information gained in a study of the peas mentioned in this paper should be readily applicable to other varieties of peas.

Since resistance to wilt has proved to be a simple Mendelian dominant the method of obtaining wilt resistant peas of a desirable type is correspondingly simplified. The first step in breeding disease resistant peas should, of course, be to ascertain if the desired resistance does not already exist in the chosen variety. This can be quickly determined by planting the variety on severely infested soil and examining the survivors for type. In case no resistant type plants are secured, it will be necessary

to resort to crossing with a resistant variety. In hybridizing, it is desirable to make crosses between varieties as nearly alike as possible in order to simplify selection. The F_1 plants should be well spaced so as to secure a large yield of seed. The F_1 may be grown on either infested or disease free soil. The F_2 plants should be grown on infested soil where the susceptible plants will be eliminated. The seed should be harvested separately from each seemingly desirable F_2 plant. This seed should be planted separately according to parent plant, on infested soil where the F_3 families that are homozygous for resistance may be readily detected. If desirable plants are found in any homozygous resistant progenies, the segregating progenies may be discarded. It is usually advisable to bulk the seed from the segregating progenies and hold for a few years. In case it should be necessary to start over again, this seed should be at least equivalent to F_2 seed.

After securing the desired homozygosity for resistance the next step is to fix the type of the new strain. Since type is a much more complex thing than resistance, it will probably require many more generations to secure exactly the type desired. This may be done by making single plant selections from each generation or by bulking the seed and making selections after several generations. The peas by this time should tend to be homozygous for most characters since they are a self-fertilized crop.

Probably the most important thing to observe about the foregoing plan is the fact that it is not necessary to consider wilt resistance after the third generation if the work has been carried along properly. After this time, the attention should be given to other characters.

This plan is, of course, subject to many modifications. For instance, the F_1 plants may be backcrossed to the type parent, and the process carried through as above, considering the backcross generation as equivalent to an F_1. This procedure may hasten somewhat the attainment of the desired type.

Summary

Data have been presented to show that resistance to Fusarium wilt in the peas studied is due to a single dominant factor.

For the most part, the data strongly support the single factor hypothesis. There are some exceptions which are explained in the text as possible masking of wilt symptoms by early maturity and escape of susceptible plants due to an irregular distribution of inoculum.

The new factor for resistance is designated "Fw" and its recessive allelomorph for susceptibility as "fw."

Linkage studies of resistance with two other factor pairs were carried out. These indicated no linkage between resistance and sugary, but a loose linkage of about 31 per cent between resistance and tallness.

A rate of wilting study indicated that tall F_2 segregates from the cross of Horsford x Resistant Alaska wilted at a somewhat lower rate than did the short segregates. The greatest difference was found in the initial stages.

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Resistance to Fusarium Wilt

in garden, canning and field peas

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Contents

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THE FIRST RECORD of the Fusarium wilt of peas was made from material collected in Wisconsin by Jones and Linford (2) in 1924. A full description of the disease was later published by Linford (3) in which the causal organism was established as *Fusarium orthoceras* App. and Wr. var. *pisi*. A comprehensive survey by Linford (4) in 1928 definitely established the disease as present not only in Wisconsin but also in Maryland, Pennsylvania, Ohio, Michigan, Indiana, Illinois, Montana, and in the upper Snake River Valley of Idaho. It has recently (1930) been found by B. L. Wade[1] and by L. K. Jones[1] to be a serious disease in the Palouse section of eastern Washington and northwestern Idaho.

Linford (3) pointed out that a number of the standard varieties of canning peas were highly resistant to the disease while others were very susceptible. Wade (6) studied the inheritance of wilt resistance in a number of pea varieties and concluded that it is due to a single dominant factor. Thus he found that pea plants may be classified into two discontinuous classes, resistant and susceptible, and that intermediate grades of resistance to wilt did not occur in the materials he studied. He pointed out the important possibility of combining wilt resistance with desirable type through hybridization and selection.

Since Fusarium wilt is increasing in distribution and severity in the pea growing areas of Wisconsin its control through disease resistance is becoming more important each year. Certain of the canning varieties now in use have been tested as to their resistance or susceptibility. Some have not been tested while in others the observations have been confined to a relatively few stocks. Furthermore, many varieties used commonly for fresh peas have never been studied as to their reaction to this disease. The purpose of the present investigation has been to secure a wider knowledge of the proportion of susceptible and resistant plants in a larger number of standard varieties. The inquiry has been directed with the three following objects in mind: (1) To test a large number of stocks of the canning varieties most widely used in Wisconsin, especially Alaska and Perfection, both of which have been listed as susceptible, and to ascertain whether or not certain stocks contain resistant desirable-type individuals in percentages sufficient to warrant improvement through selection. (2) To determine which, if any, of the standard garden varieties are resistant, since it may be reasonably expected that this disease may soon become of importance to the home and market gardener. (3) To inquire into the possibility of the existence of resistant varieties which may prove of value to the canning industry either in their present form or as parents for hybridization with other more desirable susceptible plants.

[1]Correspondence

Sources of Materials

The materials used in the trials reported herewith were from three general sources. (1) Several seed growers submitted samples of the numerous varieties listed by each. This list, therefore, represents a cross section of the major varieties grown at the present time for the canner and for the retail seedsman. (2) Some five hundred samples were secured through Dr. E. J. Renard from various canning companies of Wisconsin and from seedsmen supplying the canning trade. The majority of these was about equally divided between the two varieties, Alaska and Perfection. This list gave a comprehensive representation of the various lots of seed being used by the canners of the state and gave an opportunity to determine the variation in resistance among stocks of canning varieties. (3) Several hundred samples were submitted by Dr. D. N. Shoemaker of the United States Department of Agriculture. This group contained a much wider range of varieties than the first two groups and included many which are little used in America or are entirely foreign stocks.

Method of Experimentation

In order to carry out reliable comparative tests with such a large number of samples it was necessary to use a field which was uniformly and thoroughly infested with the wilt organism and relatively free from other pea diseases which might complicate the readings as to the wilt disease. The field selected was one on which peas were a complete failure because of wilt in 1928 according to the observations of Linford. A preliminary study of the extent and severity of infestation was made in 1929. One acre was selected and planted with the Perfection variety. Four bushels of seed were drilled in, as for the canning crop, which yielded aprpoximately 500,000 plants. Wilt appeared with equal promptness throughout the entire plot and slightly less than 500 widely scattered plants remained healthy. Each of these was more or less off-type. The progenies from thirty of them, when tested upon wilt soil in 1930, all showed complete or relatively high resistance to wilt, indicating that most of the survivors were probably resistant individuals and not escapes. In any case the number of survivors was so low in the 1929 plantings that the value of this field as a testing ground for wilt resistance was clearly established.

In the 1930 trials duplicated plantings were made of each sample in groups 1 and 3 noted above, and single plantings of those in group 2. A double four foot row was made in each planting, approximately fifty seeds being used in each test. Check plots of Perfection were scattered throughout the plot and, as in 1929, there was complete elimination of this variety by wilt except for the survival of an occasional off-type plant.

In both 1929 and 1930 root rot (*Aphanomyces euteiches* Drechsler) was rarely encountered and then only in very mild form so that the readings for wilt were not impaired. Other pea diseases were negligible in amount.

Experimental Results

The 1930 plot was planted on May 6 and 7. On May 29 a few very slight indications of wilt were apparent, but the symptoms of the disease increased rapidly after that date. On June 19 a large percentage of the susceptible plants were dead. As the season progressed the clear differentiation of plants into two discontinuous classes, resistant and susceptible, was strikingly evident throughout this wide range of stocks and varieties and coincided with the observations of Wade (6) in a more limited group. A comparison of the duplicate plantings of given samples in different positions in the plot showed very uniform reactions as might have been expected from the general infestation found in 1929. In order to secure definite ratings of the various samples, the actual number of diseased and healthy plants in one plot of each lot was recorded, with certain exceptions noted later. It would have added to the value of the data if both plots of each sample had been counted where duplicated plantings were made. Since time did not permit of this, the second planting of each sample was examined but not actually counted. In no case was any appreciable difference in the reaction of the counted sample and its duplicate noted.

The first disease count was made on June 19 and the days immediately following. A second and final count was made on July 1 and 2. On the basis of these counts the percentage of plants remaining healthy (resistant) was calculated.

In the tabulation of results (Tables II, III, IV, and V) the varieties are grouped according to the scheme of classification used by Hedrick (1). To simplify their examination they are arbitrarily divided into two classes, resistant and susceptible. Those showing 50 per cent or more survivors are placed in the resistant column, those showing less than 50 per cent survivors are placed in the susceptible column. The rating, i.e., the percentage of resistant plants of each sample, is placed in parentheses following the name of the variety. In many varieties more than one sample was tested; this is indicated by the number of rating figures following each variety. There were a few instances where the various samples received under a given name varied so widely in percentage of resistant plants, that it became necessary to place a variety in both the resistant and the susceptible classes. In such cases the ratings are placed at opposite points in the two columns.

Studies in the Alaska Variety

Of the early varieties Alaska is the most widely used for canning in Wisconsin and at present comprises about forty per cent of the total acreage. Linford (3) classed Alaska as a susceptible variety although he did find some resistant individuals in the lots he tested. All progeny tests from survivors showed complete resistance on infested soil. With one exception, however, these selections differed distinctly in type from the parental variety. On the other hand he noted that Alcross, a very uniform variety developed by Delwiche from a cross between two individual Alaska plants contained about 50 per cent resistant plants. One sample trial of this

variety furnished by a seedsman showed 44 per cent resistant plants in the 1930 trials. The practicability of developing resistant pure lines from Alcross has been demonstrated by Renard whose resistant selections from this variety served as parental stocks for a considerable portion of Wade's (6) genetic studies. Linford (3) found a strain of Alaska selected by Prof. C. E. Temple of the Maryland Agricultural Experiment Station to be resistant. What is probably a continuation of this same strain, submitted to us by a seedsman as Maryland Alaska, was included in the 1930 trials and was found to contain 97 per cent resistant individuals (Table II). Insofar as could be judged from gross morphological characters it is a good uniform Alaska.

In a variety as popular as Alaska with the canning industry which requires uniform stock of desirable quality, considerable effort has been directed by seed growers in the improvement and maintenance of stocks. Even though such improvement work may not have been done with particular reference to resistance to wilt it seemed not at all unlikely that much variation might occur in the percentage of resistant individuals in various stocks.

In order to secure a cross section of the Alaska stocks now in use in Wisconsin, samples of seed lots delivered to about fifty canners by various seedsmen for 1930 planting were secured through Dr. Renard. This list included 217 samples. It may be assumed, of course, that many of the samples came originally from one stock, but no attempt was made to trace the history of the stock represented by each sample. In addition to these, 26 samples were secured from other sources, chiefly seedsmen. Each sample was given a four-foot plot test in the 1930 trials.

There was not sufficient time to make complete counts of every sample in this group. Seventeen representative samples were counted and the data secured are to be found in Table II under the Alaska group. It is to be seen that the proportion of resistant plants ranged from none in some samples to 100 per cent in another sample. All of the samples were examined and, without actual counting of individual plants, they were divided into five classes according to the percentage of resistant survivors. The classes and the number of samples which fell in each are given in Table I. Even though this is a rough classification it is important in showing that an appreciable number of the samples showed relatively high percentages of resistant plants. In only one sample were all plants resistant and unfortunately it contained a number of distinctly off-type plants. In a large percentage of the samples in classes 3 and 4 the survivors appeared to be good type Alaskas. The samples which fell into these two classes were quite generally distributed throughout the various sources.

At the end of the 1930 season the seed from eleven of the Alaska stocks containing relatively high percentages of survivors in the Waupun plot was collected and mixed. Some of the soil from the plot was removed to the greenhouse and seed from this lot planted in it along with a known homozygous susceptible stock and a known homozygous resistant stock of the

same variety. After a period of six weeks, 100 per cent of the 49 plants in the susceptible lot had wilted and died. In the known resistant lot all of the 49 plants were healthy. From the seed saved from the survivors on the Waupun plot 203 plants were grown and none of them became affected with wilt. This shows that, if not all, at least a high percentage of the survivors in the lots tested were homozygous for resistance since no segregation into resistant and susceptible classes took place.

Table I—Relative Resistance to Wilt in Various Samples of Alaska Variety Tested

Class number	Approximate percentage of resistant plants	Number of samples	Percentage of samples
1	0	40	16
2	1—25%	112	46
3	26—50%	58	24
4	above 50%	32	13
5	100%	1	1—
	Total	243	100

The present situation with regard to the Alaska variety may thus be briefly summarized as follows. A great variation prevails among various stocks as to the proportion of plants resistant to Fusarium wilt. Certain stocks contain practically 100 per cent resistant plants, as the sample of Maryland Alaska tested, for instance (Table II). The sample of Delwiche's Alaska No. 19 contained about 76 per cent resistant plants while the sample of Alcross contained about 44 per cent, (Table II). Other stocks vary from 0 to 75 per cent or more. With the basis of inheritance of resistance established by Wade's (6) investigations, the opportunity and method are clearly indicated whereby seedsmen may and should bring their stocks up to contain a high percentage of resistant individuals. This may be done by selection from survivors of good type on thoroughly and uniformly wilt-infested soil. Single-plant selection from such survivors is, of course, advisable but it naturally involves a protracted period of increase. On the other hand where a stock is already very uniform in type and shows fifty per cent or more resistant individuals the procedure may be shortened by mass selection. This should be done by planting the seed stock in larger quantity on thoroughly and uniformly infested soil for one or more generations until most or all of the susceptible individuals have been eliminated. The practicability of this latter method is strengthened by the fact that the partially resistant stocks appear to consist largely of homozygous resistant and homozygous susceptible individuals and very few hetero-

zygous individuals. In this connection the limitations of mass selection in a rogue-infested stock should be emphasized. The work of Renard (5) shows that after the rogue content of a stock reaches a certain point elimination of off-type becomes almost impossible because of the fact that the "rogue germplasm" is being constantly transferred to type plants by cross-pollination before the rogues are removed.

Where mass selection is used it should not be looked upon as a substitution for the improvement of stocks through the more refined methods of the developing pure lines through single-plant selection, but merely as a temporary expedient to serve during the interval required to bring up stocks by the latter method, improvement by which requires five to ten years to secure sufficient seed for commercial use. When it is used, certain precautions are advised. The stock should be of high grade, i.e. of desirable type, uniform, and free from off-types. The fact that it has a relatively high percentage of homozygous resistant individuals should be determined by a preliminary test on wilt-infested soil. The field to be used should be known by previous observation or testing to be thoroughly and uniformly infested with the wilt organism, and located in a region where the climate and soil are such as to favor the prompt and severe development of wilt. These conditions are absolutely essential in order to secure the development of wilt in most or all of the susceptible individuals with sufficient rapidity to insure their elimination before any seed is set. Complications due to the prevalence of root rot and other diseases of pea in the soil should be avoided. Obviously this plan will best succeed with the help and advice of someone sufficiently familiar with pea diseases to give reliable diagnosis of wilt and differentiation from other diseases.

Studies in the Perfection Variety

Perfection at present is the major wrinkle-seeded variety used by Wisconsin canners. It has already been stated that the entire trial field comprising one acre in 1929 was planted with this variety. The seed in this case did not come from a single stock since it consisted of remnants of a large number of samples submitted by canners and seedsmen to Dr. Renard for type studies. Less than one-tenth of one per cent of the plants were resistant and none of the survivors were true Perfection plants. In 1930, 199 samples from canners and seedsmen were included in the trials on the wilt plot. All proved to be very susceptible. Out of a total population of around 10,000 plants, only 23 survived and none of these were of true Perfection type.

It thus appears that all the stocks of Perfection represented in this wide range of samples are practically homozygous for susceptibility to wilt. The possibilities for selection of resistant lines within the variety are small. A few of the survivors of the 1929 plot were preserved for progeny tests. Most of those tested so far are completely resistant but on the other hand most of them are also sufficiently different from Perfection to be of doubtful value for the canner. Certain of these lines are being increased

for further study as to their adaptability to canning. At the present time it appears that the development of a resistant Perfection type suitable for the present needs must apparently depend in a large measure upon hybridization of standard Perfection with other resistant varieties and reselection for a combination of Perfection characters and wilt resistance. Progress in the development of a new variety by this method is necessarily slow and requires a period of several years.

Studies in Other Garden and Canning Varieties

As already stated, one of the major purposes of this investigation was to gain more information as to the occurrence of resistant plants in a wider range of varieties than has hitherto been tested. The results of the trials with garden and canning varieties are compiled in Tables II, III, and IV.

As these varieties are grouped together according to the Hedrick system (1) it may be seen at a glance that with the exception of the Advancer and Champion of England groups the samples in every group range from those in which all plants were susceptible to those in which all were resistant. One of the limitations of these results, of course, is the fact that in many varieties the tests are confined to a single sample. This is particularly true of relatively new or little used varieties. Their ratings are included because of their possible interest in the future to seedsmen and growers and to those searching for resistant parent stocks for hybridization. The number of samples of a given variety tested is a fair index to its present popularity, since several specialists in pea seed were asked to submit all samples of varieties which they were listing for sale.

From what has already been shown from tests with Alaska it is to be expected that different stocks listed under a given name may vary in percentage of resistant individuals. These differences are further increased by the variation in the conceptions of seedsmen as to the ideal of a given variety. Furthermore it is not uncommon for seedsmen to substitute under one name stock of another closely similar variety.

In certain varieties variation from a high percentage of susceptible plants to high percentage of resistant individuals was found. This is to be seen in addition to Alaska, already mentioned, in Extra Early, Early Bird, and Ameer (Table II), in Hundredfold, English Wonder, and World's Record (Table III) and in Sherwood, Admiral Beatty, Quite Content, and Prizewinner (Table IV). In contrast to these, certain widely used varieties consistently showed high percentages of susceptible plants such as: Large White Marrowfat (Table II); Gradus, Duchess of York, American Wonder, Premium Gem, Notts' Excelsior, Surprise, Little Marvel, World's Record, Laxtonian, Laxton's Progress, Onward, Horsford, Sutton's Excelsior, Advancer, and Perfection (Table III); Thomas Laxton, and Rice's 13 (Table IV). Among the most noteworthy of the varieties consistently high in percentage of resistant plants are Black eyed Marrowfat, Rice's 330, Horal, Harrison's Glory (Table II); Allan's Canner, Green Admiral, Rogers K, Improved Surprise, Prince of Wales, Everbearing, Dwarf Tele-

phone, Yorkshire Hero, and Dwarf Champion (Table III); Stratagem, Dwarf Defiance, Champion of England, Telephone, Alderman, Prince Edward, and most of the edible-podded varieties (Table IV).

From this list the varieties now used by Wisconsin canners, in addition to Alaska and Perfection discussed earlier, may be classified as follows:

Susceptible	Resistant
Canners' Gem	Green Admiral
Horsford	Yellow Admiral
Ashford	Roger's K
Surprise	Prince of Wales
Badger	Senator
Rice's 13	Bruce
Winner	Horal
Laxtonian	Rice's 330
Onward	Improved Surprise
Thomas Laxton	

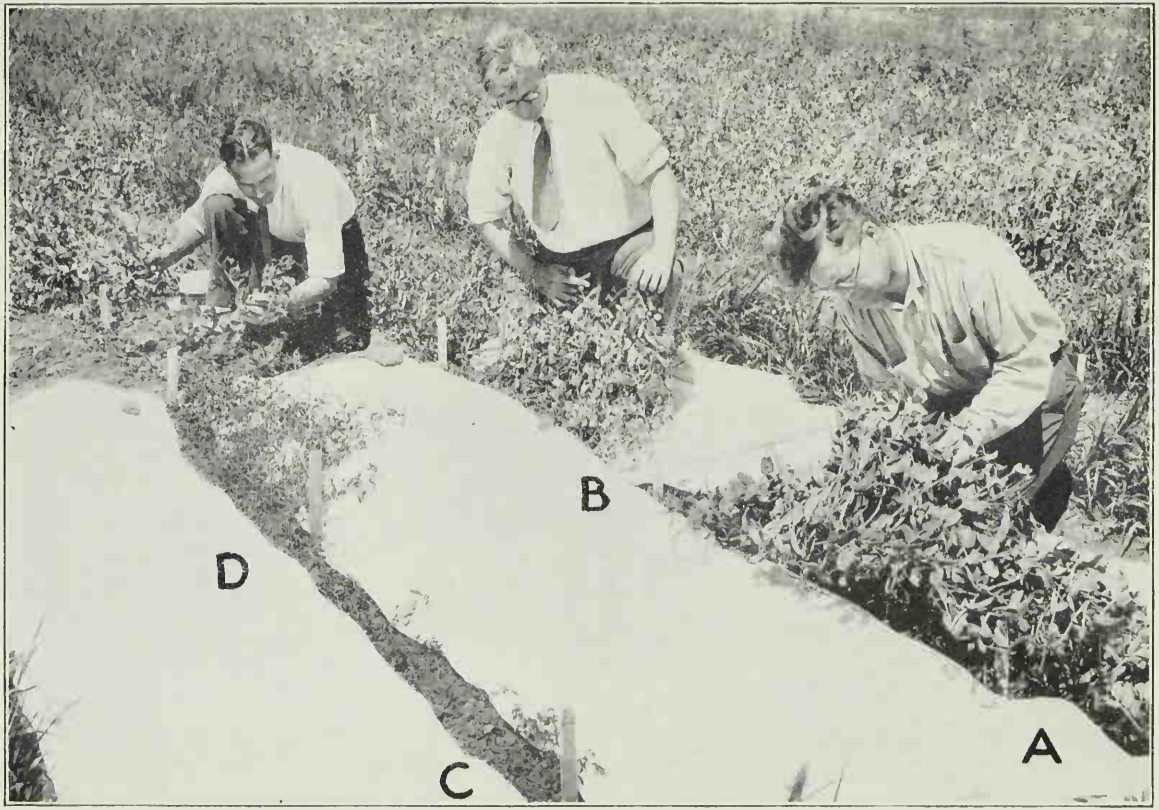

Plate 1.—VIEW OF THE 1930 WAUPUN PLOT SHOWING REACTION OF FOUR PEA VARIETIES TO FUSARIUM WILT. A. DISCOVERY, 100 PER CENT RESISTANT PLANTS; B. MARYLAND ALASKA, 97 PER CENT RESISTANT PLANTS; C. PYRIMIDAL, 100 PER CENT SUSCEPTIBLE PLANTS; D. A CHINESE FIELD PEA, 100 PER CENT SUSCEPTIBLE PLANTS.

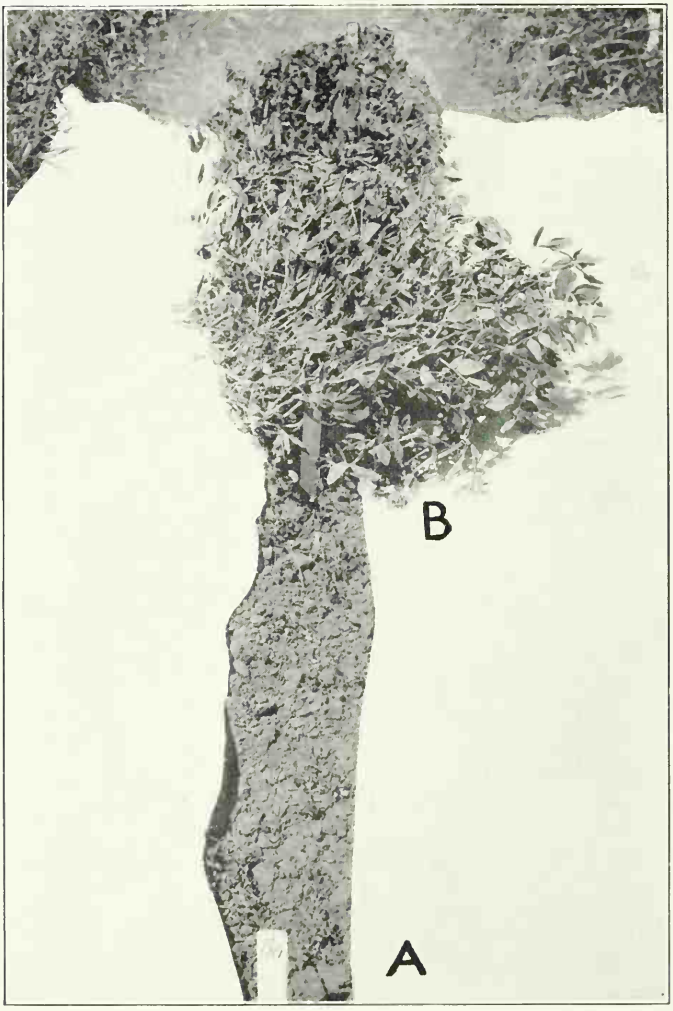

Plate II.—TWO CANNING VARIETIES ON THE WAUPUN WILT PLOT, 1930.

A. BADGER, ALL PLANTS KILLED WITH WILT; B. ROGERS K, ALL PLANTS RESISTANT TO WILT.

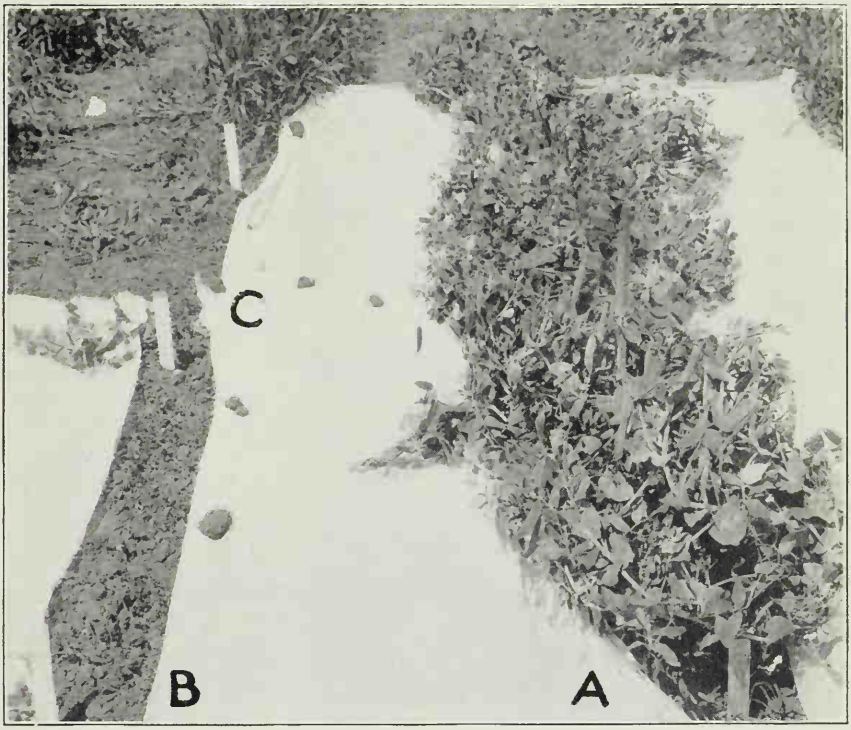

Plate III.—A. SENATOR, A CANNING VARIETY, ALL PLANTS RESISTANT TO WILT; B. PIONEER, A GARDEN VARIETY, ALL PLANTS KILLED WITH WILT; C. SURPRISE, A CANNING VARIETY, 96 PER CENT KILLED WITH WILT. WAUPUN WILT PLOT, 1930.

Plate IV.—COMPARISON OF TWO GARDEN VARIETIES UPON THE WAUPUN WILT PLOT, 1930. Left, DAFFODIL, AN ENGLISH GARDEN VARIETY; ALL PLANTS DEAD BECAUSE OF WILT. Right, EVERBEARING, A GARDEN VARIETY USED COMMONLY IN AMERICA. ALL PLANTS IN THIS SAMPLE WERE RESISTANT TO WILT.

Table II.—Resistance and Susceptibility to Fusarium Wilt in the Extra Early, Tom Thumb, Alaska and Dimple-Seeded Groups

Susceptible	Resistant
Extra Early Group	
Extra Early (0)[a] (0)	Extra Early (98) (100)
Mammoth Pod Extra Early (0)	Prince Albert (100) (90)
Sutton's Early Champion (2)	First of All (96)
Ringleader (0)	
Caractacus (29)	
Giant Lightning (0)	
First on Market (27)	
First and Best (0) (24)	
Saxonia (0)	
Early Dwarf or Prince Arthur (0)	
Tom Thumb Group	
Bishop Long-pod (0)	Tom Thumb (100)
Marrowfat Group	
Large White Marrowfat (7) (29) (0) (2) (0)	Improved Sugar Marrowfat (100)
Kinver Marrowfat (0)	Very Dwarf White Marrowfat (100)
Melting Marrowfat (0)	Early Green-Seeded Marrowfat (100)
Springtide (0)	Webb's Advancer Marrowfat (100)
	Webb's Stourbridge Marrowfat (97)
	Daniel's Matchless Marrowfat (93)
	Favorite Marrowfat (100)
	Wonder Marrowfat (83)
	Blackeyed Marrowfat (100) (100)
Alaska Group	
Alaska (0) (0) (0) (0) (2) (7) (29) (42) (47) (48)	Alaska (55) (57) (64) (65) (67) (71) (100)
Kentish Invicta (0) (0)	Alcross (44)
Earliest of All (12)	Maryland Alaska (97)
Eclipse (2) (4)	Alaska No. 19 (76)
Winner (0) (0) (0)	Rice's 330 (100)
Kentish (0)	Earliest Blue (68)
Express (0)	Rapide (52)
Velocity (0)	
Dimple-Seeded Group	
Early Bird (0) (0)	Early Bird (55)
Ameer (0) (0) (0) (3)	Ameer (100)
Radio (0) (0)	Charlton's Radio (88)
Primo Pilot (0)	Harrison's Glory (100) (100) (100)
Pilot (0) (0)	Johnson's New Glory (100)
Eight Weeks (0) (0)	Old England (94)
Leader (0) (0)	Britisher (100)
Blue Peter (0)	Councillor (100)
Fillbasket (0)	
Pride of the Market (0)	
Bountiful (0)	
Claudit (0)	
Acquisition (5) (0)	
Superb (0)	
Eldorado (0)	
Talisman (0)	
Benefactor (0)	

[a]The numbers in parentheses after each variety represent the percentage of resistant plants in the individual samples tested on the Waupun wilt plot.

Table III.—Resistance and Susceptibility to Fusarium Wilt in the Wrinkle Cream-Seeded, Gem, Large-Podded Dwarf, and Advancer Groups

Susceptible	Resistant
Wrinkled Cream-Seeded Group	
Hundredfold (0)[a] (0) (0)	Hundredfold (96)
Reading Wonder (6)	Sutton's Harbinger (100)
Marvellous (0)	Lincoln (92)
Daisy (0)	Allan's Canner (100)
Gradus (0) (0) (0) (0) (26) (32)	Green Admiral (100) (100) (100) (100) (98)
Duchess of York (0) (0)	Yellow Admiral (98) (100) (100)
Prestige (0) (11)	Rogers K (100) (100)
Edward VII (0)	Horal (88) (100) (100)
	Profusion (100)
	Champion of Scotland (100)
	Prince of Wales (100) (100) (98) (100)
	Langport (100)
	Early Giant (100)
	Sweet Market (100)
Gem Group	
English Wonder (yellow seed) (7)	English Wonder (green seed) (95)
Surprise (0) (0) (3) (4) (4)	Surprise (Improved) (100)
McLean's Little Gem (0)	Everbearing (100) (100) (100) (100) (100)
American Wonder (0) (0) (0) (0) (0) (29)	(100) (97) (97)
Premium Gem (0) (0) (0) (0) (4) (12)	William Hurst (97)
Nott's Excelsior (0) (0) (0) (2) (2) (2) (41)	
Little Marvel (0) (0) (0) (0) (4) (23) (26)	
Canner's Gem (0)	
Delicious (0) (0) (0)	
Prince Arthur (0)	
Melbourne Market (0)	
Large-Podded Dwarf Group	
World's Record (0) (0) (2)	World's Record (58) (70)
Laxtonian (0) (0) (0)	Discovery (100)
Pioneer (0) (0)	Dwarf Telephone (100) (100) (97) (96) (86)
Peter Pan (0) (0)	Early Morn (73)
Blue Bantam (0)	Yorkshire Hero (100) (97)
Marchioness (0) (0)	Dwarf Champion (100) (100) (98)
Laxton's Progress (0) (0) (0) (0) (0)	British Wonder (100)
Onward (0) (0)	Matchless (100)
Horsford (0) (0) (0) (0) (0) (0)	Sutton's Perfection (100)
Ashford (0)	Giant Stride (100)
Sutton's Excelsior (0) (0) (0) (0) (0) (0) (2) (6)	Arcadian (100)
	Dwarf Prolific (90)
Reading Gem (0)	Ideal (61) (69) (96)
Buttercup (0)	Victor (100) (93)
Sutton's Supreme (0)	Lancashire Lad (74)
Sensation (0)	Bruce (100)
Renown (0) (31)	Commander (100)
Top O' Th' Morn (0) (0)	
Mayflower (0)	
Daffodil (0)	
Green Gem (0)	
Early Duke (16)	
Advancer Group	
Advancer (0) (5) (13)	
Perfection (0) (0) (0) (2)	
Abundance (0)	
Veitch's Exonian (2)	
Delicacy (0)	

[a]The numbers in parentheses after each variety represent the percentage of resistant plants in the individual samples tested on the Waupun wilt plot.

Table IV.—Resistance and Susceptibility to Fusarium Wilt in the Stratagem, Champion of England, Ne Plus Ultra, Telephone, Senator, and Edible-podded Groups

Susceptible	Resistant
Stratagem Group	
Improved Stratagem (0)[a] Sherwood (0)	Stratagem (100) (100) Sherwood (100) (100) Dwarf Defiance (100) (100) (87) Potlatch (100) Battleship (76) Majestic (81)
Champion of England Group	
	Champion of England (100) (100) (97) (96) (95) (93)
Ne Plus Ultra Group	
Thomas Laxton (0) (0) (0) (0) (0) (0) (0) (0) Magnum Bonum (0) G. F. Wilson (4) Snowdrop (0) Captain Cuttle (0) Prince of Peas (9) Dreadnought (0)	Ne Plus Ultra (92) (89) Juno (100) (100) King Edward (92) Liberty (83) Omega (98)
Telephone Group	
Admiral Beatty (0) Carter's Quite Content (0) Sutton's Prizewinner (0) Up to Date (0) Market Gardener (34) Good Indeed (5) Lord Kitchener (7) Premier (0) Exhibition (26) Centenary (12) Victory (14)	Admiral Beatty (100) Quite Content (100) (100) Prizewinner (95) (100) Telephone (100) (100) (95) Duke of Albany (100) (89) Admiral Dewey (100) Alderman (100) (100) (100) (100) (100) (100) (98) (95) Duke of York (77) Duchess (100) American Champion (100) Harvestman (94) Wm. Richardson (100) Lord Leicester (97) Amateur Pride (93) Maincrop (100) Bell (100) Prince Edward (100) (100) (100) (94) Light-Podded Telephone (100) (97) Standard (100)
Senator Group	
Gladstone (42) Delicatesse (0) Unique (0) Rearguard (0) Union Jack (0) (0) (0) (30) Rice's 13 (0) (0) (23) (3)	Senator (100) (100) (100) 100) Shropshire Hero (100) Heroine (55) Eureka (smooth seeds) (100) Eureka (wrinkled seeds) (53) Maincrop (100) Glory of Devon (100) Advance Guard (100) Sutton's Best of All (68)
Edible-Podded Group	
New Giant Butter (0)	Giant Luscious Sugar (100) Melting Sugar (100) (100) (97) (93) Dwarf Grey Sugar (100) (100) (100) (100) Giant Butter (100) French Sugar (100)

[a]The numbers in parentheses after each variety represent the percentage of resistant plants in the individual samples tested on the Waupun wilt plot.

Studies in Field Peas

A large number of field pea samples were included in the Waupun trials of 1930. The results with a portion of the better known varieties are listed in Table V. The range in percentage of resistant plants is quite as wide as in garden varieties. In Canada Field two samples showed 25 and 95 per cent resistant individuals, respectively, while Victoria varied from all susceptible in one sample to all resistant in two other samples. It therefore should not be assumed that field peas are generally resistant. The possibilities for improvement in resistance are as great here as in garden varieties when and where the needs justify the attention of the plant breeder.

Table V.—Resistance and Susceptibility to Fusarium Wilt in Field Peas

Susceptible	Resistant
Cream (White)—Seeded Section	
Canada Field (25)[a]	Canada Field (95)
Canada Beauty (0)	White Canada (100)
	Gregory (90)
	Arthur (98)
	Golden Marrow (100)
	Colorado Field (76)
Victoria (0) (22)	Victoria (100) (100)
Cream-seeded, Black-eyed Section	
	Canada (97)
Green-Seeded Section	
	Dwarf Blue Imperial (100)
	Bangalia (100)
	Blue Prussian (100)
Dark-Seeded Section	
Kaiser (0)	Delano (100)
Carleton (15)	Solo (98) (100)
Peluschka (12)	French Grey (100)
Killarney (30)	Large White Capucijner (100) (98)
Austrian Winter (21)	
Maple Partridge Brittany (7)	

[a] The numbers in parentheses after each variety represent the percentage of resistant plants in the individual samples tested on the Waupun wilt plot.

Discussion

Because of the increasing importance of Fusarium wilt as a limiting factor in pea production in Wisconsin the matter of wilt resistance must be considered in any well-balanced program of pea improvement. The primary purpose of this paper has been to bring together, for the use of the plant breeder as well as the seedsman and the canner, such information as has been obtained regarding the proportion of plants resistant to this disease in a wide range of stocks and varieties.

The writer wishes to make it clear that the data presented should be properly interpreted as indicating percentages of resistant plants only in those samples tested. It is hardly possible to give a final rating of any variety as a whole. The wide variation in the stocks of such a variety as Alaska, where a large number of samples were studied, naturally raises the question as to whether in such a consistently resistant variety as Alderman, for example, further sampling from a larger number of sources would not show a greater range in the proportion of resistant and susceptible plants. For this reason stocks of varieties to be used in improvement work should be carefully tested beforehand on wilt-infested soil.

The data presented refer to resistance to a single disease of pea, the Fusarium wilt, as found in a representative field of southern Wisconsin. Similar studies may well be extended to the disease as found in other regions. Resistance to wilt in a given variety does not imply similar resistance to any of the other numerous diseases of pea.

In the discussion already given with regard to Alaska and varieties derived from it, such as Alcross and Alaska No. 19, the opportunity to build up highly resistant satisfactory stocks through selection has been pointed out. The survey of Perfection stocks shows that resistant plants true to type in this variety are very rare. The possibility of developing a resistant Perfection by selection alone is therefore quite remote and improvement in resistance in this variety will therefore naturally depend in a large measure upon hybridization with other resistant varieties and reselection along the lines already outlined by Wade (6) and others. The same may be said for those garden and canning varieties which are shown to be consistently susceptible since in these as well as in Perfection the occasional resistant survivor usually has been found to be an off-type plant.

Summary

The proportion of plants resistant or susceptible to Fusarium wilt was studied with a large number of varieties of garden, canning, and field peas by means of field trials on thoroughly infested soil.

Variation from 100 per cent susceptible plants to 100 per cent resistant plants was found in a survey of 243 samples of Alaska variety.

Trials of 199 samples of Perfection showed all to contain very high percentages of susceptible plants.

In addition to these two popular canning varieties numerous garden, canning, and field peas were studied. Many varieties were consistently resistant while others were quite uniformly susceptible. A few varieties showed wide variation in the reaction of individual samples.

The facts presented are of importance to the plant breeder and seedsman in connection with pea improvement work, and to the canner whose choice of stocks and varieties may be influenced by the occurrence of Fusarium wilt in his territory.

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Acknowledgments

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Wisconsin Studies on Aster Diseases and Their Control

Agricultural Experiment Station of the University of Wisconsin, Madison

CONTENTS

Wisconsin Studies on Aster Diseases and Their Control

L. R. Jones and Regina S. Riker

TWO DISEASES in particular threaten the culture of the China aster in the United States. These are the yellows, due to a virus, and the wilt or stem rot, caused by a Fusarium. Most aster plantings are troubled to a greater or less degree by one or both of these diseases. The two diseases are sometimes confused. They are, however, maladies caused by different agencies, and each requires specific precautions for control. This bulletin points out certain well established facts as to the occurrence, symptoms, and causes of these diseases, and discusses especially results with specific control measures.

The China aster is one of the choicest annual flowers of late summer and early autumn, both for the home gardener and for the commercial florist. When the two diseases are clearly distinguished, and the mode of introduction and spread of each is understood, ordinary garden culture of the China aster may, with proper attention to seed, soil, and rotation, be carried on with satisfaction. For the commercial florist, however, the situation is more difficult. Therefore, the present studies upon control of aster yellows and aster wilt have been concerned especially with the conditions and problems of the commercial florists, particularly of Wisconsin and the adjacent territory. Brief reports upon progress in this work have already appeared (Jones and Riker 1928, 1929, 1930, Weiss 1929, Ball 1930).

IMPORTANCE OF ASTER DISEASES

BOTH ASTER yellows and aster wilt are widespread in the United States. A generation or even a decade ago the China aster was considered as one of the most popular and reliable of the annuals. Year by year these two plagues have spread and increased until few entirely healthy aster plantings can be found. In many instances both diseases are present. Figure 1 indicates the distribution of the two maladies in this country, as far as it has been reported.

The severity of the two diseases varies with different localities. Either may be present to a sufficient degree to practically destroy an aster plantation. The two occurring together, as they commonly do, are therefore doubly discouraging. This is evidenced in Table I, which analyzes the damage from these two maladies in the horticultural[1] garden at Madison, Wis., in 1927. It will be noted that although

[1] The authors are indebted to their colleagues in the Department of Horticulture for cooperation in this and other ways during the progress of these investigations.

there were four types of China aster, including 15 varieties, the loss with most ranged from 95 to 100 per cent and the best of them yielded only 10 per cent of healthy plants at the end of the season. Figure 2B illustrates the destructiveness of wilt in a susceptible variety. Moreover, in the strain relatively resistant to wilt (Figure 2A), many of the plants were infected with yellows, although this does not show

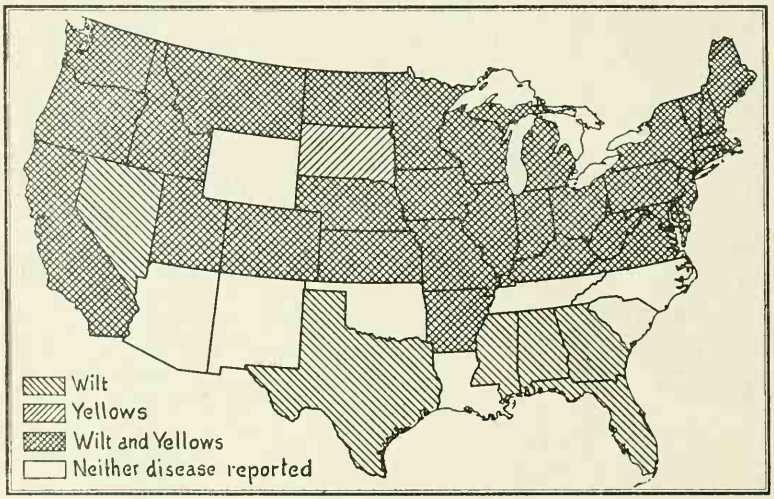

FIG. 1.—WIDESPREAD DISTRIBUTION OF ASTER YELLOWS AND ASTER WILT
The distribution shown is recorded mainly in the *Yearbook of the United States Department of Agriculture* and in the *Plant Disease Bulletin and Reporter.*

in the photograph. As a result, this commercial aster field at Randolph, Wis.,[2] which had been planted for both cut flower and seed purposes, was considered by the grower a total loss commercially. Such conditions are typical of many aster plantings throughout the country whether in the home garden or in the commercial cut flower or seed field. Indeed, the aster seed industry in places in the eastern United States has been practically destroyed by the combination of yellows and wilt. It is especially significant, therefore, that both diseases have appeared in the chief aster seed growing centers of the Pacific regions (Figure 1) and seem destined to increase unless control measures are taken.

These two diseases, aster yellows and aster wilt, are thus widespread, and seriously menace aster culture in the United States.

[2] In this connection the authors wish to acknowledge the generous cooperation during the last three years of the J. W. Jung Seed Co. of Randolph, Wis., in granting the free use of 'aster-sick' soil and in other ways. During this period courteous advice or assistance with seed has been given by other commercial aster growers, including G. J. Ball, West Chicago, Ill.; Bodger Seeds, Ltd., El Monte, Cal.; H. L. Cady, Fox Lake, Wis.; Vaughan's Seed Store, Chicago, Ill.; and H. B. Williams, Baldwinsville, N. Y.

Table I.—Aster Diseases in the Horticultural Garden at Madison, Wisconsin, in 1927

Variety	Total number of plants	Per cent of healthy plants	Per cent of wilted plants	Per cent of yellowed plants
OSTRICH FEATHER				
soft pink	59	7	22	71
vermilion carmine	51	2	71	27
light blue	31	0	48	52
Average		*3*	*47*	*50*
AMERICAN BRANCHING				
white	31	0	100	....
flesh pink	26	4	42	54
crimson	28	4	39	57
lavender	112	1	56	43
dark violet	32	3	47	50
Average		*2*	*57*	*41*
CREGO				
rose pink	21	10	38	52
crimson	53	2	79	19
lavender	46	3	15	82
purple	19	0	47	53
Average		*4*	*45*	*51*
KING				
rose	27	3	41	56
lavender	62	5	55	40
violet	41	0	51	49
Average		*3*	*49*	*48*

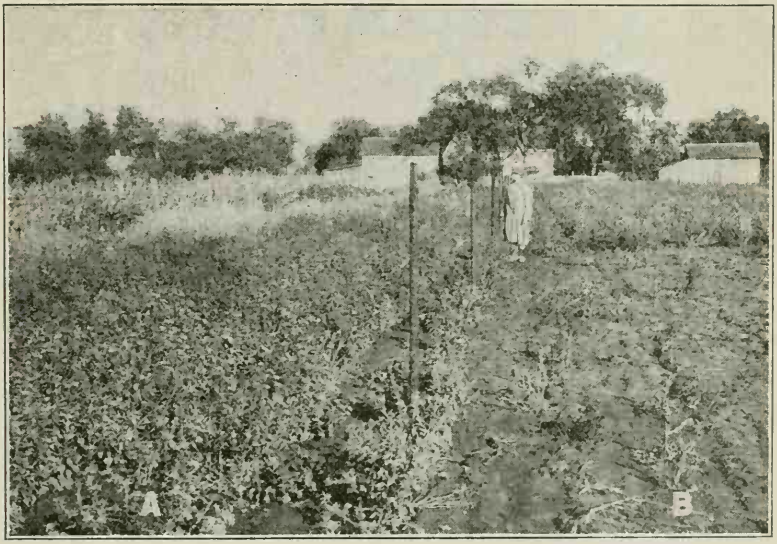

FIG. 2.—COMMERCIAL ASTER PLANTING AT RANDOLPH, WISCONSIN, OCTOBER 1927.

The soil is heavily infested with the aster wilt Fusarium, i.e., it is aster-sick. The destructiveness of wilt with a susceptible variety is shown in the Royal lavender pink in the right foreground (B). Individual plant resistance to wilt appears within this variety. Differences in resistance to the wilt in aster strains is apparent especially between the susceptible one mentioned above and the relatively wilt-resistant Comet-like selection from Peerless shell pink in the left foreground (A). However, many plants in the latter selection which escaped the wilt were attacked by the yellows so that the field was considered a total loss commercially by the grower. From this relatively wilt-resistant strain, individual plants were saved whose progeny, by repeated selection, have attained considerable wilt resistance. (See later details and Figures 12 and 13.)

SYMPTOMS OF ASTER YELLOWS AND OF ASTER WILT

ASTER YELLOWS and aster wilt which, as mentioned earlier, are so widespread as to occur either singly or together in almost every large aster plantation, are often confusedly regarded as a single malady. Yet they differ in cause, in dissemination, and in response to control measures. Therefore, it is essential to recognize the characteristic symptoms of each.

ASTER YELLOWS

Aster yellows has been described in American literature from the early writings of Smith (1902) to the recent publications of Kunkel (1926b, 1927) and others. Therefore, only a summary of the essential points is given in this bulletin.

The symptoms of aster yellows are in part illustrated in Figures 3 and 4. The most important changes in the diseased plants are in habit and color. Often the changes are more or less one-sided because of the location of the infection from which the disease started. There may be little change in the older mature leaves or other parts of an infected plant but the subsequent growth shows disease symptoms. In general habit the infected plant becomes more constricted and straight (Figure 3D), the upper leaves especially tending toward an upright position, and remains dwarfed (Figure 4C). In the young leaves a slight yellowing or "clearing" appears along the veins. In all the new growths there is a tendency to abnormal increase in the number of branches, giving a "bunchy" or "rosetted" appearance (Figure 4C). These later shoots are yellowish, with the leaf petioles elongated and leaf blades narrowed. Following infection the flowers do not develop normal color or size. The flower heads tend to be dwarfed, greenish in color, and often show one-sided deformity. Although the diseased plants never recover, most of them live throughout the season, unless the disgusted grower pulls and destroys them. This should, indeed, be done at the first appearance of disease since each such sick plant is a center of continued disease dissemination.

ASTER WILT

Aster wilt in America was probably first recorded by Galloway (1896). However, within the last decade it has received especial attention, particularly from Beach (1918), Jackson (1927), and Weiss (1925, 1929) who have included descriptions of this disease. It, therefore, again suffices to give a brief summary of the distinguishing characters.

The symptoms of aster wilt are illustrated in Figures 3, 4, 5, and 6. Plants may be destroyed by the wilt disease at any period from the seedling stage to full bloom. In very young seedlings the symptoms are similar to damping off. In plants with only a few leaves the

entire seedling will wither quickly and die. With plants which are attacked by the wilt at a somewhat older age one finds two types of symptoms. The one, which seems associated with a one-sided and slow invasion of the parasite, gives a stunted growth with a one-sided development of the plant and leaves, and a decided yellowing of the leaves or parts of leaves most stunted (Figures 3B, and 5). These symptoms suggest those of the closely related cabbage yellows disease. When the stem of such an aster plant is cut the vascular ring is found to be brown, particularly on the side most affected. Eventually such plants wither. The other type of aster wilt seems associated with a more complete invasion of the fungus. In this type, the lower leaves first show signs of wilting. This is followed more or less rapidly by the collapse of the entire plant which then withers and dries. The stem in these plants is usually externally blackened at the base and for some distance up, thus giving rise to the name stem rot (Figures 3C, and 6). If a cross section of the stem is taken a general browning of the vascular elements is evident. In some cases, wilt may not become apparent until the plants are coming into full bloom when they suddenly collapse and wither due to the demand at that time for a larger supply of moisture (Figure 4B). The contrast between the wilt and the yellows disease thus becomes striking at blooming time (Figure 4B and C). With wilt there may be a sudden collapse and death of plants even when full of normal blossoms. With yellows, although the symptoms of one-sided or complete discoloration of flowers as well as foliage may be serious, the plant remains stiff and unright and often lives to the end of the season.

The above discussions and illustrations give the essential characteristics of aster yellows and aster wilt and enable one to distinguish between the two diseases.

ASTER YELLOWS: ITS CAUSE AND CONTROL

THE CAUSE OF ASTER YELLOWS

THE CAUSE of aster yellows must be understood in order to insure intelligent control. Aster yellows was first described by Smith (1902) who, in discussing its nature says, "Caused by no fungus, insect or other organism, not due to any apparent effect of treatment or environment, it is notwithstanding a sharply defined, widespread and destructive disease of this plant." Smith noted similar symptoms on marguerite, Calendula, African marigold, and ragweed or Roman wormwood. He also suggested the similarity of this disease to peach yellows and to "calico" of tobacco. It remained, however, for the recent painstaking researches of Kunkel (1924, 1925, 1926a, 1926b) to demonstrate certain virus characteristics of the aster malady. Kunkel found that, although asters are attacked in nature by various insects such as aphids, tarnished plant bugs, and several species of leaf hoppers, the dissemination of this virus from sick to

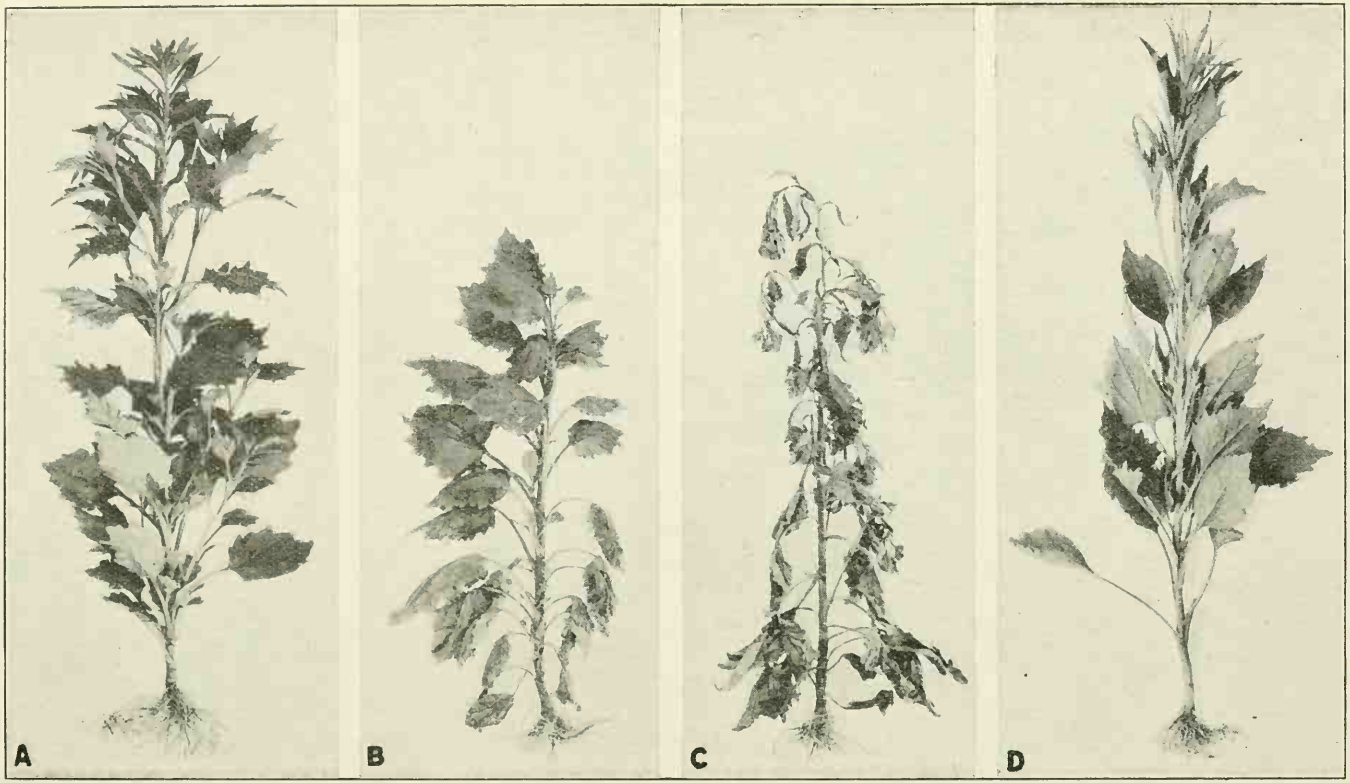

FIG. 3.—HEALTHY AND DISEASED ASTER PLANTS FROM EXPERIMENTAL PLOTS AT MADISON, WISCONSIN, JULY, 1928.

(A.) Healthy aster plant.
(B.) Aster showing wilt symptoms. "One-sided" type.
(C.) Aster showing wilt symptoms. "Wilt" or "stem-rot" type.
(D.) Aster showing yellows symptoms.

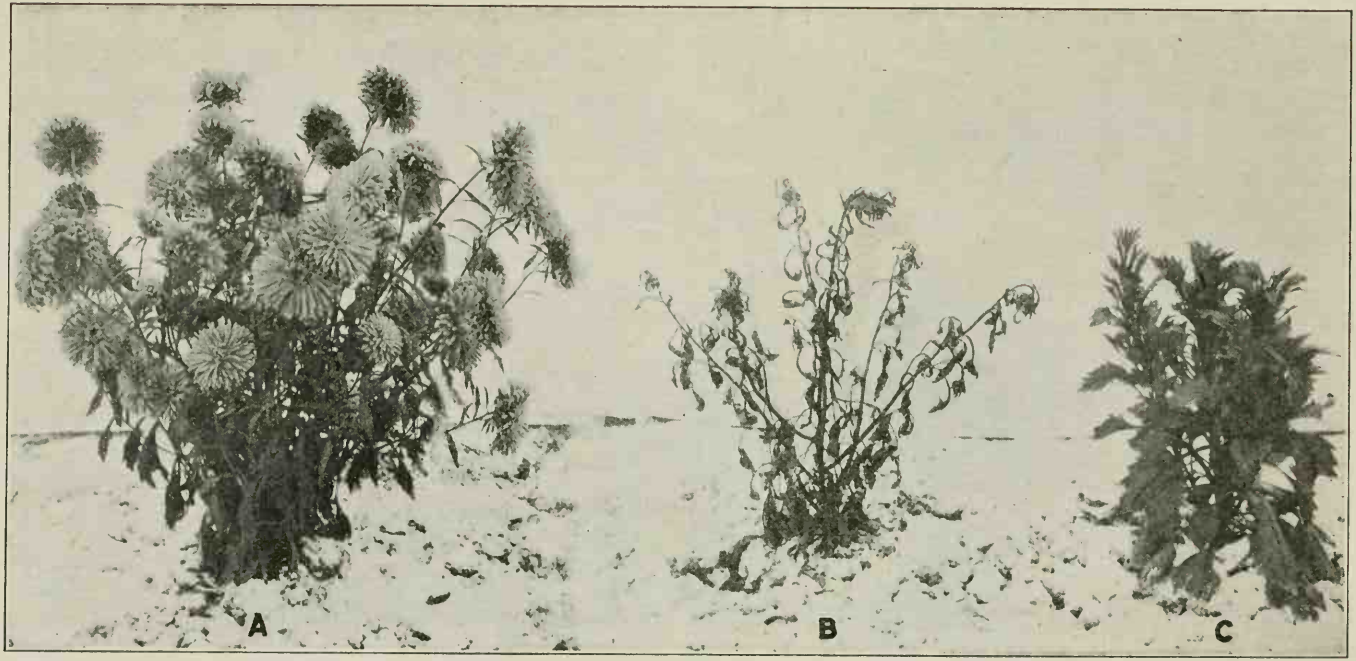

FIG. 4 HEALTHY AND DISEASED ASTER PLANTS FROM THE HORTICULTURAL GARDEN AT MADISON, WISCONSIN, SEPTEMBER, 1927.
(A.) Healthy aster plant.
(B.) Aster showing wilt symptoms.
(C.) Aster showing yellows symptoms.

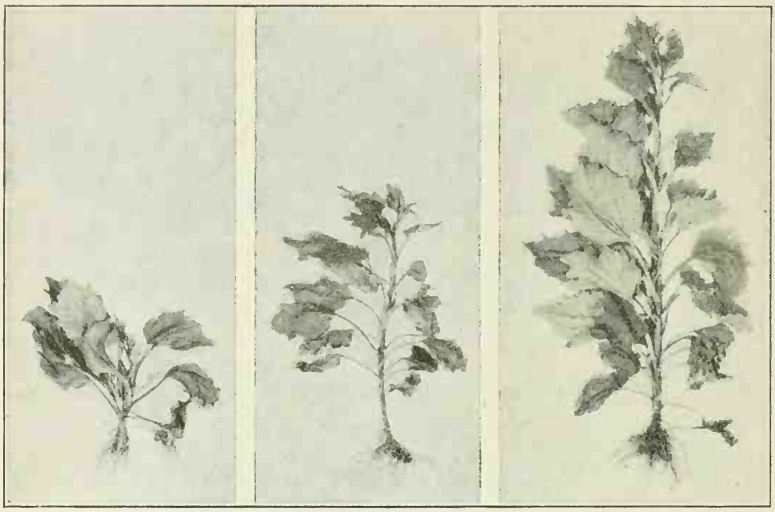

FIG. 5.—ASTERS SHOWING WILT SYMPTOMS, "ONE-SIDED" TYPE, FROM EXPERIMENTAL PLOTS AT MADISON, WISCONSIN, JULY, 1928.

Three plants are illustrated which were arrested at slightly different stages of normal development by the invasion of the aster wilt Fusarium. The type of symptoms shown here seems to be associated with an unequal and slow invasion of the fungus. Eventually such plants wither and resemble those in Figure 6.

healthy plants was accomplished experimentally only by a single species of leaf hopper, *Cicadula sexnotata* Fall., of the insects tried. Apparently this virus is not carried over winter in the aster seed nor in the eggs of the infectious leaf hoppers. Probably all aster seedlings early in the season are free from yellows infection, and all leaf hoppers when hatched in the spring are free from the virus. The yellows virus may, however, infect any one of many plants besides aster, including Roman wormwood, horseweed, lettuce, plantain, and dandelion. Some of the many hosts are biennials or perennials common about fields or garden borders and in these the disease may persist from year to year. By feeding upon a diseased biennial or perennial the leaf hopper may become infected in early summer and then, after an incubation period of at least ten days, if such an insect feeds upon an aster plant, the disease may be transmitted. To keep aster plants free from the yellows it is necessary to keep them free from the attacks of such leaf hoppers.

THE CONTROL OF ASTER YELLOWS

The essential factor in the control of aster yellows is, therefore, to keep the viruliferous leaf hoppers from the aster plants. Theoretically, at least, several methods might be considered. (1) Plants capable of carrying the virus over winter might be eliminated from the vicinity of aster plantings. (2) Repulsion or destruction of the

leaf hoppers might be accomplished. (3) The aster plants themselves might be shielded from the attacks of the viruliferous leaf hoppers. The first two methods have proven difficult in practice (Weiss 1925, 1929, White 1931). The third method has been found successful both by Kunkel and by the present writers.

Kunkel (1929a) protected the aster plants by the use of fences. He found that a screen of 18 wires to the inch prevented the passage of the leaf hoppers. He, therefore, surrounded his experimental aster plantations (10 by 25 feet) with wire screen fences of this mesh but of various heights (4, 5, 6, and 8 feet). When careful and persistent roguing of all yellowed plants was practiced, loss from the disease within the enclosures was 20 per cent, whereas 80 per cent of the unfenced check plants was diseased. Weiss (1929) suggests a fence five or six feet high as most desirable. Williams (1930) reports a reduction of yellows, by the use of 18 mesh wire screens five feet high, from 60 per cent outside to 16.9 per cent inside the enclosures.

In the Wisconsin studies, control of aster yellows was undertaken simultaneously with that of aster wilt in 1925. In view of the successful control of various Fusarium diseases, particularly cabbage yellows,

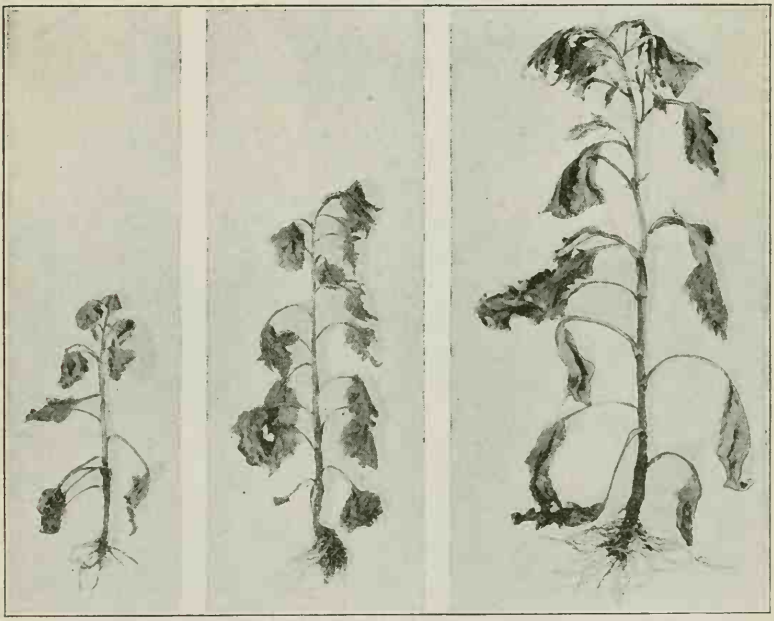

FIG. 6.—ASTERS SHOWING WILT SYMPTOMS, "WILT" OR "STEM-ROT" TYPE, FROM EXPERIMENTAL PLOTS AT MADISON, WISCONSIN, JULY, 1928.

Three plants are illustrated which were arrested at slightly different stages of normal development by the invasion of the aster wilt Fusarium. The type of symptoms shown here seems to be associated with a more complete invasion of the fungus than that producing the symptoms in Figure 5. No unequal development of parts appears but a more or less sudden wilting of the plant occurs, frequently accompanied by a blackening of the lower portions of the stem.

by wilt-resistant varieties, this method of approach was taken with the aster, as will be discussed later. However, the losses in out-door plots in the horticultural garden in previous years because of the yellows were so disturbing that, when the control of the wilt was attempted, the control of the yellows was also tried.

Control of aster yellows by small cages

Aster yellows was controlled successfully by enclosing the experimental plants in small cheesecloth-covered cages. These were of the simple type commonly used for insect exclusions in virus studies, about two and one-half feet high by four by six feet on the ground (Figure 7). Such cages proved fully effective in preventing the yellows in 1925 and the two succeeding seasons. Every year twelve cages were prepared, each covering 42 plants. In addition an uncovered adjacent plot was planted with 200 plants. Not a plant was lost from yellows under such cages in the three years' trials, although the viruliferous leaf hoppers were so abundant outside that a large majority of the unprotected aster plants were stricken by the yellows each year.

By the summer of 1928, the Fusarium-resistant strains discussed later had reached such numbers that the use of the smaller cages for their trials was no longer practicable and a new means of protecting the plants from the viruliferous leaf hoppers was needed. Two general methods were tried. The one was the use of fencing as tried by

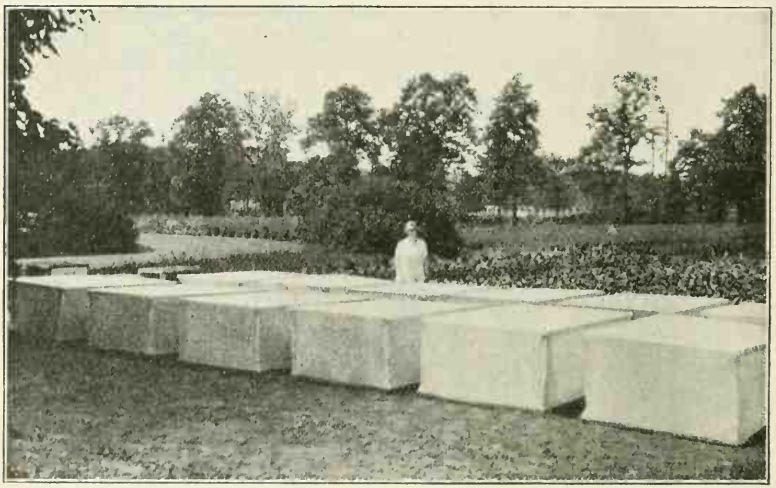

FIG. 7.—TYPE OF SMALL CAGE USED TO EXCLUDE THE VIRULIFEROUS LEAF HOPPERS IN EXPERIMENTS ON THE CONTROL OF ASTER YELLOWS FROM 1925 TO 1929.

During the first three years coverings of cheesecloth (38 x 44 threads per inch) were employed, and perfect control of yellows was obtained. In 1928 and 1929 like cages were constructed with coverings of cloth of various meshes. Any mesh coarser than 22 x 22 threads per inch proved ineffective as a control for yellows (Tables 2 and 3). Madison, Wisconsin, 1927.

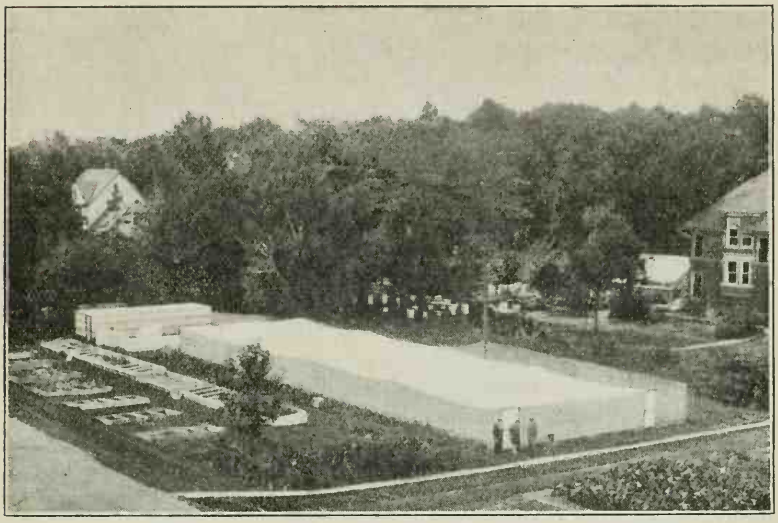

FIG. 8.—EXPERIMENTAL ASTER PLANTATION AT MADISON, WISCONSIN, 1928. The prominent feature is the large cage or house (6+ by 30 by 98 feet) completely covered with cloth of 22 x 22 threads per inch. To the left and rear are the small cages (2½ by 4 by 6 feet) covered with cloths of different mesh, and adjacent open plots for controls. To the side and right of the large cage is the enclosure (6+ by 10 by 49 feet) with open top, and sides covered with cloth of 22 x 22 threads per inch. Around the latter, asters were also planted in the open for controls.

Kunkel (1929a). The other was the employment of the caging method on a large scale.

Partial control of aster yellows by fencing

The fencing method was tested in 1928. Rectangular enclosures were constructed with cloth-covered side walls somewhat over six feet high, and open tops. These were adjacent to the large completely cloth-covered cages which will be discussed later. In choosing the cloth the advice of tobacco-shade cloth experts was sought and a trial use of a cloth having 22 x 22 threads per inch was decided upon partly because of its availability and partly because of the assurance courteously given by Kunkel that in his experience a wire screen of 18 meshes to the inch served to exclude the hoppers although they passed through coarser screens. In an enclosure 10 by 49 feet in size (Figure 8) with open top and sidewalls of cloth of 22 x 22 threads per inch at Madison, Wis., yellows appeared in 44 per cent of the plants which survived the wilt as against 90 per cent of yellows in an unfenced control plot. In a similar enclosure 45 by 90 feet, at Randolph, Wis., 82 per cent of the non-wilt plants showed evidence of yellows while 95 per cent of the non-wilt unfenced plants developed yellows. In these enclosures roguing was practiced every week. It may be noted that there was a considerably greater percentage of yellows in the larger enclosure. However, too much significance must not be attached to the comparative figures since the enclosures were not in

the same locality. Kunkel's (1929a) greater success with the fencing method may be in part attributed to more prompt roguing of infected plants and possibly in part to his use of smaller enclosures. Allowance must also be made in any such comparisons for differences in the relative prevalence of leaf hoppers in a locality and possibly in the character of the adjacent vegetation.

Control of aster yellows by large houses

The use of the caging method on a large scale in 1928 (Figure 8)[3] proved more successful under experimental conditions in Wisconsin than the fencing method. The effectiveness of these enclosures was proven by the results in two large cages or houses, one at Madison and the other at Randolph (6+ by 30 by 98 feet and 6+ by 16 by 98 feet respectively), each completely covered with cloth 22 x 22 threads per inch. Not a single case of yellows developed within them until after midsummer. In August a night storm tore openings of considerable size in the tops of both cages. Although the damage was repaired within a few hours, early symptoms of yellows showed about two weeks later on three out of 2,160 plants in one of the houses and on two out of 1,080 plants in the other house. These were promptly removed and no other yellowed plants appeared. Evidently a few viruliferous hoppers entered through the torn top during the brief exposure. The side walls, some six feet high, had not been torn by this storm. Hence, if the above interpretation is correct, the hoppers found their way very quickly over the tops of such side walls but were eliminated by prompt roguing.

In 1929 similar results were obtained with cloth 22 x 22 threads per inch in two large cages (6+ by 30 by 98 feet at Madison, and 6+ by 60 by 98 feet at Randolph).

Cages of like size were used in 1930. In that year no yellows developed within the enclosure at Randolph. At Madison two plants showed early symptoms of yellows within three weeks after planting. They were promptly removed but in spite of roguing, 4 per cent of the plants showed some symptoms of yellows by the end of the season. The plantings in previous years and at Randolph that year were made some days after the cloth house was constructed. At Madison, however, in 1930 the planting immediately followed the completion of the cloth house. Whether or not a time interval after construction serves to starve out any viruliferous leaf hoppers which might be enclosed during construction remains to be seen. In this connection it should be noted that in several smaller houses at Madison, even with coarser top coverings, where planting was not done for two weeks after construction, no cases of yellows developed until relatively late in the season.

The above experiments indicate that large houses covered com-

[3] A brief description of the construction of these first large cages is given by Weiss (1929). Modifications in detail, based on experience, may be found in an article by Ball (1930). John E. Luddy, The Windsor Company, Windsor, Connecticut, who has had much experience in the construction of similar cloth shades for tobacco culture, will gladly furnish suggestions or specifications for aster houses of desired sizes.

pletely with cloth of 22 x 22 threads per inch are generally effective in excluding the viruliferous leaf hoppers and thus in controlling aster yellows. In practice it seems safest to erect and cover the house several days in advance of the transplanting.

Mesh of cloth in control of aster yellows

In order to secure information as to the effectiveness in excluding the leaf hoppers of the various other grades of screening cloths in the market an experimental series of the smaller cages (2½ by 4 by 6 feet, Figure 7) was set up in 1928, each cage covered with a different cloth. Asters were grown under these on Fusarium-free soil alongside the larger cloth-covered house at Madison (Figure 8). Table II shows the results with these smaller cages, each completely enclosed with the cloth of mesh indicated. Cheesecloth, as in the preceding three years, and cloth of 22 x 22 threads per inch, as in the large houses, were effective in excluding the leaf hoppers. Cloth of the next degree of fineness tried, 12 x 12 threads per inch, and all coarser cloths admitted the viruliferous leaf hoppers. In 1929 a similar trial was made using cloths of grades intermediate between 22 x 22 and 12 x 12 threads per inch, since these were the grades respectively effective and ineffective in the 1928 trials (Table II). The results are given in Table III. Results were similar to those of the 1928 trials with the same grades of cloth, and the new intermediate grades proved ineffective in fully excluding the leaf hoppers.

Simultaneously one of these intermediate grades, 22 x 16 threads per inch, was used for top and sides in a house 6+ by 10 by 49 feet. Within this enclosure 22 per cent of yellows appeared in the plants

Table II.—Yellows Control in Small Cloth-Covered Cages, 1928

Mesh of cloth	Plants with yellows
Threads per square inch	Per cent
38 x 44*	0
22 x 22	0
12 x 12	30
10 x 10	53
10 x 8	67
8 x 8	47
8 x 8	67
Not caged	73

*Cheesecloth

Table III.—Yellows Control in Small Cloth-Covered Cages, 1929

Mesh of cloth	Plants with yellows
Threads per square inch	Per cent
22 x 22	0
22 x 16	30
16 x 12	44
12 x 12	54
Not caged	71

escaping the wilt. The entrance of the viruliferous leaf hoppers into this enclosure corroborates the results in the small cages with cloth of the same mesh. It would seem that any coarser cloth than 22 x 22 threads per inch is relatively ineffective when used for both top and sides.

The possibility was also tried of employing a coarser cloth for the top while using cloth 22 x 22 threads per inch for the sides. In 1929 a cage 6+ by 10 by 49 feet was constructed with top of cloth 16 x 12 threads per inch and sides 22 x 22 threads per inch. Within this enclosure, 6 per cent of yellows developed in the plants not affected by wilt.

Further study of the modification of top covering, coupled with roguing, was made in 1930. Four cages 6+ by 10 by 24½ feet were constructed at Madison with sides of cloth 22 x 22 threads per inch and tops of cloth of the following meshes: 8 x 8, 12 x 12, 12 x 16, and 16 x 22 threads per inch. Within these enclosures the per cent of yellows developing in the plants not affected by wilt were 14, 8, 6, and 3.6 per cent respectively. In the open, 96 per cent of plants not affected by wilt showed symptoms of yellows. Thus some of the coarser top coverings, while permitting the entrance of leaf hoppers, gave relatively satisfactory control.

Commercial control of aster yellows by fencing and caging

Concerning the practical aspects of the control of aster yellows, the experience of one commercial firm, the J. W. Jung Seed Co., Randolph, Wis., may be cited. This firm was interested in trying out the use of screening cloths on a practical scale in 1928, 1929, and 1930. The first year, 1928, all the cloth used was 22 x 22 threads per

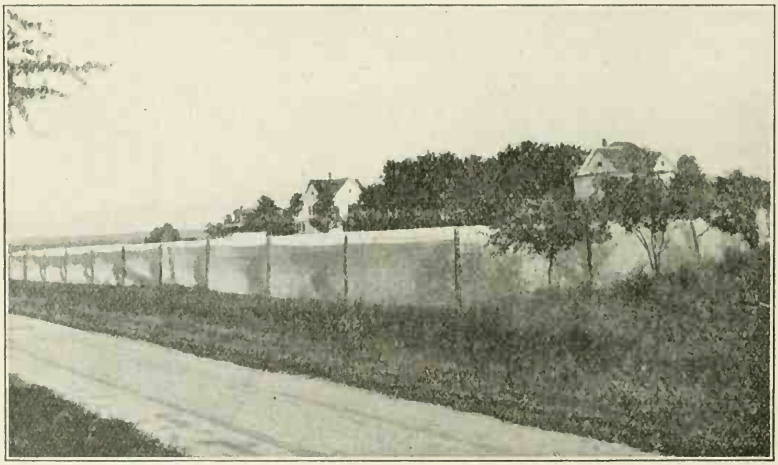

FIG. 9.—COMMERCIAL ASTER CAGE OR HOUSE SUCCESSFUL IN CONTROLLING THE YELLOWS

This house was completely covered with cloth of 22 x 22 threads per inch. (J. W. Jung Seed Co., Randolph, Wisconsin, 1929.) A profitable commercial crop was obtained.

inch. Part of their plantation was enclosed by six foot side walls only, i.e., without top cover; part was covered completely like the experimental houses. Where the top was open the yellows disease appeared so abundantly that they despaired of roguing it out, and very little benefit resulted. In the plants which were grown under the complete covering of cloth no yellows occurred. Almost equally significant was the fact that the flowers were of superior quality. This same company in 1929 grew a considerably larger proportion of their asters under cloth (Figure 9). Again they lost practically all those unprotected, whereas those under full protection showed practically no yellows. Again the flowers grown under cloth were of superior quality for cutting purposes—topping the price in the Chicago cut flower market toward the end of the season. In 1930 no yellows developed in a large house completely covered with cloth 22 x 22 threads per inch up to August first. At that time a severe storm removed the top. This was not replaced and subsequently a considerable percentage of yellows appeared. This was late enough not seriously to affect the cutting of flowers except in the very late varieties. In two other cages with sides of 22 x 22 threads per inch and tops of 8 x 8 and 12 x 16 threads per inch there developed, respectively, 27 and 16 per cent of yellows.

Thus the results secured commercially by the J. W. Jung Seed Co., corroborate those obtained experimentally at Madison and Randolph. Corroborative evidence is also found in the experiences of another commercial firm near Chicago (Ball 1930).

Discussion and conclusions on the control of aster yellows

The conclusions to date as to the control of aster yellows may be briefly summarized. The control of the disease is dependent upon shielding the plants from viruliferous leaf hoppers. Effective shielding materials are cloth not coarser than 22 x 22 threads per inch, as shown in Wisconsin trials, and wire screens of 18 meshes to the inch, as shown by Kunkel (1929a). The difference in mesh in the two materials may be easily explained by the fact that with cloth the threads often spread irregularly, thus evidently requiring a closer original mesh than suffices with the more stable wire screen.

Two types of shields have been tried, i.e., fences and cages. Kunkel (1929a) reports encouraging results with the use of open top and screened sidewalls coupled with careful and persistant roguing. Williams (1930) likewise records fair success with fences. Under conditions of Wisconsin experimental and commercial trials the degree of control by this method was not commercially satisfactory.

However, in Wisconsin experimental and commercial trials aster yellows was practically controlled by the use of cages or houses covered, top as well as sides, with cloth not coarser than 22 x 22 threads per inch. This was the most satisfactory control tried. However, the use of a cloth top as coarse as 12 x 12 or 16 x 12 threads per inch, with cloth walls of 22 x 22 threads per inch has shown promise. Such

modification of the top covering, with roguing if disease appears, seems worthy of further attention.

Based on the data above the following recommendation for commercial culture of asters in the north central section of the United States is made:

1. The construction of complete cloth-covered enclosures rather than open top ones.

2. The employment of cloth not coarser than 22 x 22 threads per inch for the side walls of such enclosures.

3. The use of the above mesh cloth for the top cover unless further tests show somewhat coarser cloths to be effective and more desirable.

ASTER WILT: ITS CAUSE AND CONTROL

THE CAUSE OF ASTER WILT

ASTER WILT, as has been stated earlier in this paper, is due to an entirely different cause than aster yellows. As previously noted, aster wilt was probably first mentioned in America by Galloway (1896). He recognized that it was produced by a fungus similar to the one attacking cotton, watermelon, cabbage, and other plants. Woods (1899) and others ascribed the disease to a Fusarium. It was left for Beach (1918) to make and report the first detailed study of the causal organism which he named *Fusarium conglutinans* Woll. var. *callistephi* because he found it so closely related to the cabbage yellows organism *F. conglutinans* Woll. Jackson (1927) agreed with Beach as to the pathogenicity of *F. conglutinans* v. *callistephi* but also listed four other Fusarium strains pathogenic to asters. One of these he identified as *F. angustum* Sherb. while the other three were not named.

In the Wisconsin studies on aster wilt the original experimental plots at Madison were inoculated partly with Beach's Michigan strain of *F. conglutinans* v. *callistephi*, and partly with Jackson's Ontario strain of the same fungus. In recent years other experimental plots have been located in an aster-sick field at Randolph, Wis. Fusaria have been isolated from wilting plants both from this field and from the horticultural garden at Madison which in culture (on potato dextrose agar and steamed rice) and in pathogenicity resemble Beach's original strain.

The senior author has also seen the aster wilt disease as it occurs in commercial aster plantings in various localities from New England and New York to California. All the observations made indicate that wilt or stem rot of the China aster in the United States is in general one and the same disease.

In this connection it should be noted that there are many references in foreign literature to a wilt or stem rot disease of aster which seems from descriptions to resemble the American disease. The causal agent has frequently been considered a Fusarium whose identity has not been definitely determined. Several species have been listed as

associated with diseased asters but the pathogenicity of these species in general has not been clearly established.[4] Because of the difficulty in comparing the evidence it has seemed best to restrict the scope of the present bulletin to the aster wilt disease as it occurs in the United States. It is, however, probable that this same Fusarium wilt may occur in foreign countries wherever the China aster has been long cultivated.

The fungus of aster wilt may easily be introduced into new localities, since it may be seed-borne (Beach 1918, "Marketman" 1921, Gloyer 1924, 1931a, 1931b, Rose 1925, Weiss 1925, 1929, Jackson 1927, Gregory 1929, White 1931, Williams 1931). This probably accounts for the widespread distribution of the disease in the United States (Figure 1).[5]

The Fusarium of aster, following its introduction, seems to persist indefinitely in the soil much like its close relative the cabbage Fusarium. Consequently, where asters are grown in short rotation, the disease rapidly increases until it is calamitous.

Control of aster wilt must, therefore, be directed against a seed-borne, soil-inhabiting Fusarium of wide distribution.

THE CONTROL OF ASTER WILT

The control of aster wilt depends upon the clear recognition of a few simple facts which have already been suggested. These are (1) that it is a specific disease caused by a parasitic fungus, a vascular Fusarium; (2) that this parasite may be seed-borne, therefore it is widely distributed and liable to be introduced into virgin soils; (3) that once introduced it persists indefinitely and may show rapidly increasing severity if asters are replanted on old soils; and (4) that, as with the closely related vascular Fusaria of cabbage and other plants, there may be a natural variation in susceptibility or relative disease resistance as between host individuals or varieties.[6]

[4] The writers are studying the pathogenicity of a number of the organisms isolated from diseased asters in Europe and furnished through the courtesy of Dr. H. W. Wollenweber. These include several strains of Fusarium, Verticillium, and Cephalosporium. Tests on seed germination and very young transplants indicate that several strains besides *F. conglutinans* v. *callistephi* are pathogenic to asters at the above stages. Further trials are under way.

[5] Since the aster wilt Fusarium may be carried on the seed and commercial seedsmen are distributing seed promiscuously from country to country, this disease may doubtless soon occur wherever the China aster is grown, subject only to the limitations imposed by environment. In this connection the junior author has shown that high temperatures favor the disease and that continued low temperatures inhibit the disease even though the fungus is present. It is probable, therefore, that in certain regions this wilt may never become serious. Such a limitation apparently occurs for the similar Fusarium disease of cabbage. Aster wilt, however, the junior author has found, is a somewhat lower temperature disease than that of the cabbage.

[6] The literature on aster wilt has numerous discussions of control measures including: (1) seed disinfection (Beach 1918, "Marketman" 1921, Gloyer 1924, 1931a, 1931b, Rose 1925, Weiss 1925, 1929, Jackson 1927, Gregory 1929, Rager 1930, White 1931, Williams 1931); (2) rotation (Paddock 1902, Findlay 1917, Weaver 1917, 1924, Beach 1918, Beal 1919, "Marketman" 1921, Muller 1922, Rose 1925, Weiss 1925, 1929, Mumford 1926, White 1931); soil disinfection (Weaver 1917, Beach 1918, "Marketman" 1921, Mumford 1925, 1926, 1928, Rose 1925, Weiss 1925, Jackson 1927, Gregory 1929, White 1931); (4) direct planting (Beach 1918, "Marketman" 1921, Mumford 1925, 1926, White 1931); (5) hardening or outdoor sowing (Smith 1902, Stone and Smith 1902, 1903, Beal 1919, Weiss 1925, Newton 1929, Ball 1930); and (6) sanitation (Paddock 1902, Findlay 1917, Beach 1918, Beal 1919, Weiss 1925, Jackson 1927, Rager 1930).

It is evident, therefore, that the simplest way to escape the disease is by the use of clean seed and clean soil. Home gardeners may continue to succeed in cultivating the aster by careful attention to these matters. They may well save their own seed from strictly healthy plants and then by rotation keep their asters on clean soil. Where asters are started in special seed beds and later transplanted as is so frequently done it is especially important to use clean soil for seed bed purposes. Since commercial seed may carry more or less of the wilt fungus, seed disinfection is a wise precautionary measure.[7] Whenever direct planting of the seed in its permanent site is practicable it is preferable, since it lessens the danger of seed bed infection, avoids root mutilation and growth check associated with transplanting, and insures early deep rooting in cool soil. Where the seed bed method is preferred, then, in addition to attention to clean soil, or soil disinfection,[8] care should be taken so to harden off the plants as to insure full vigor and consequently quick deep rooting following transplanting. Finally, if the disease appears, soil contamination and further spread may be reduced by attention to sanitary measures, i.e., the prompt pulling and burning of diseased plants as they appear.

Attention to these methods may suffice to give the home gardener or small grower reasonable freedom from aster wilt. With the large scale commercial grower, however, the hazards remain great, and even with the small grower the difficulties are such as increasingly to discourage aster culture.

Obviously the one way to avoid all these troublesome expedients and restore confidence in aster culture is to seek to obtain and to use wilt-resistant strains. The results of the Wisconsin work with the closely related Fusarium disease of cabbage indicated the probability that this would prove successful. This possibility is especially significant for the florists' use since the avoidance of wilt by rotation is more difficult with florists operating on large areas than with small gardeners. Moreover, this difficulty will be further increased if the practice is adopted of culture under cloth to avoid the yellows disease, as advocated earlier in this bulletin. It is to be noted that the problem of securing disease resistant strains for the florists' trade is simplified because but few types and colors are needed, as will be discussed in detail later.

To obtain wilt-resistant strains of China aster has therefore been

[7] The specific measures for seed disinfection which have been recommended are: (1) mercuric chloride, one-tenth per cent, for one-half hour (Jackson 1927, Weiss 1929, White 1931); (2) same for ten minutes or one-half hour depending upon the presoaking (Weiss 1925, 1929, Gregory 1929, Gloyer 1931a, 1931b); (3) Uspulun or Semesan, one-fourth per cent, for one-half hour (Jackson 1927, Weiss 1929, Williams 1931); (4) hydrogen peroxide, three per cent, for one and one-half hours (Beach 1918, Rose 1925, Weiss 1925, Gregory 1929); and (5) Dipdust, or Semesan Bel, applied as a dry coating (Weiss 1929).

[8] The specific measures for soil disinfection which have been recommended are: (1) steaming (Weaver 1917, Beach 1918, "Marketman" 1921, Rose 1925, Jackson 1927, Gregory 1929); (2) treatment with mercuric chloride, one-tenth per cent (Jackson 1927); (3) treatment with formaldehyde, the details of application varying slightly (Beach 1918, "Marketman" 1921, Mumford 1925, 1926, 1928, Rose 1925, Weiss 1925, Jackson 1927, Gregory 1929, White 1931).

the chief aim of the Wisconsin experiments on the control of aster wilt. Experience with other vascular Fusarium diseases indicated two ways of approach as hopeful. These were (1) the search for outstandingly resistant varieties of asters among the established commercial types, (2) the search for more or less resistant individuals in commercial strains that were in general susceptible, followed by repeated reselections from the progeny of these in the hope of developing and establishing wilt-resistant strains. Both of these methods have been in mind and in use simultaneously. For clarity of discussion, however, they will be considered separately so far as practicable. Since in the early years no outstandingly resistant strains of asters were found, studies were begun with the second method, and the historical developments of the work will therefore be made clearer by discussing first the progress year by year through repeated selections starting with relatively susceptible strains.

The development of wilt-resistant strains of China asters by selection from susceptible commercial varieties

Although few commercial strains of asters are uniformly resistant, resistance to the wilt disease among individuals is met with in most of them (Figure 2B). That by conserving the seed from such plants and by selecting from the progeny, wilt-resistant strains of asters might eventually be developed, has been suggested by several writers.[9]

In the Wisconsin studies attempts have been made to develop such resistant strains by the selection of individually resistant plants in most part from susceptible commercial strains. In doing so the emphasis has been placed upon what was most desirable to the commercial florists. Fortunately, the greater part of the needs in the florists' field may be met by a relatively limited range of types and of colors. The general reliance at present in the Northern Mississippi Valley seems to center about the American Branching type, although the American Beauty with its chrysanthemum-like flower and non-lateral habit is meriting increasing attention. In color the white is the favorite, with pink, rose, lavender and purple following in succession. The first selections in the Wisconsin trials were made in the American Branching type and the Heart of France. Later attention was paid to the American Beauty. In the East the Royal, in the same range of colors, is in great demand. Therefore the 1930 trials gave increased attention to this type.

[9] Beach in 1918 said "The selection of resistant plants is probably to be the ultimate means of controlling the disease." Arnold (1919) also suggested selection for resistance. Curtiss (1926) reported that he was saving seed from healthy plants. Jackson (1927) said "doubtless this means must finally be depended upon to control wilt." He secured seed from some resistant plants and found that it stood up better than commercial seed when planted in inoculated and in aster-sick soil. Adams (1928) believes the solution of the problem to be the development of wilt-resistant varieties. Kunkel (1929b) reported high resistance in four strains grown from seed selected by Milbrath and also the selection of seed from the most resistant plants in these promising strains in the hope of still further improving them. Gregory (1929), Ball (1930), and White (1931) advise sowing seed from resistant individuals. Skinner (1930) records that, whereas one plant of several hundred Queen of the Market proved resistant in 1927, the progeny of this plant showed 88 per cent resistance in 1928.

Results in 1925

In 1925 in Wisconsin trials at Madison in artificially inoculated soil the commercial aster strains used proved very susceptible to the wilt. American Branching mixed showed 16 per cent resistance to the disease and Heart of France 29 per cent resistance. From twenty of the individual plants which resisted the wilt, enough seed was obtained for experimental purposes in 1926. Fifteen of these were of the American Branching type in a variety of colors and five of the Heart of France type.

Results in 1926

In 1926 certain selections showed greater resistance to wilt than did the parent stock planted in 1925. With the American Branching selections, grown in sick soil, stands ranging from 7 to 60 per cent were obtained in 1926. From ten of these fifteen strains, twenty-three reselections were made for trial in 1927. With the Heart of France selections stands ranging from 28 to 50 per cent were obtained in 1926. From three of these five strains, five reselections were made for trial in 1927.

Results in 1927

The behavior on sick soil of the selections mentioned above as compared with that of commercial varieties during the season of 1927 suggested that progress was being made in the development of wilt-resistant strains. With twenty-three American Barnching selections, stands were 0 to 100 per cent when flowering began and of these, twelve selections showed stands of 50 per cent or more. For convenience 50 per cent has been chosen as an arbitrary division between undesirable and desirable wilt-resistant strains. By the end of the flowering season, following a period of high temperature and excessive drought, more of the plants had succumbed so that only eight selections had stands of 50 per cent or greater at this time. It was observed in this and following years that many of the selections would produce a fine crop of blossoms suitable for cut flower purposes but before the end of the season would show some symptoms of wilt. The latter plants were considered as undesirables for experimental or commercial seed purposes. From eleven of the twenty-three selections tried in 1927, twenty-eight reselections were made for trial in 1928. Three plantings of commercial American Branching gave 3, 11, and 22 per cent stands, respectively, at the end of the season.

With five Heart of France selections stands ranged from 14 to 94 per cent when flowering began, four of the five being over 50 per cent. At the end of the blooming season two selections had stands over 50 per cent. Twelve reselections for trial in 1928 were made from four of these five strains. In sharp contrast with the above selections, a planting of commercial Heart of France gave a 7 per cent stand at the end of the season.

Results in 1928

In 1928 the work was much extended, as explained earlier, and the small cages were supplanted by large cloth-covered enclosures. Plantings were made not only in the artificially inoculated soil at Madison but in naturally infested soil at Randolph, Wis., both in 1928, 1929, and 1930. It was possible in these houses to use much larger numbers of plants. In 1927 the largest number of plants of a strain tested was 17. In a majority of cases seven or less were used. Chance therefore played a large part in the results obtained. In 1928, 1929, and 1930 the smallest number used of any one strain was 30 plants. When possible 50 or 60, and with the most promising strains, 100 or 120 plants were tested. As a consequence the results in these years are considered of much greater significance than those of previous years. The seed from the most promising strains in 1928 was collected for the first time in bulk as well as by individual plants, thus allowing more extensive trials of these strains in 1929. Bulk selections were also made in 1929 for trials in 1930. Since the results at Madison and at Randolph always showed close agreement the data given is an average for the two plantings.

The results obtained with certain of the selections were even more promising in 1928 than in preceding years. In this year with twenty-eight selections of American Branching, stands were 29 to 100 per cent when flowering began, with twenty-five selections 50 per cent or over. Late in the season, again after a period of high temperatures and drought, ten selections had stands of 50 per cent or over. Reselections for trial in 1929 were made from these ten strains either by individual plants, bulk, or both. Four plantings of commercial American Branching gave 3, 6, 8, and 8 per cent stands, respectively, at the end of the flowering season. New individual plant selections were made from the commercial strains in the experimental plots in this year.

In Heart of France, with twelve selections, stands were 48 to 98 per cent when flowering began with eleven selections over 50 per cent. At the end of the blooming season, nine selections had stands over 50 per cent. Five of these strains were collected in bulk for trial in 1929 and an individual plant selection made from the best strain. A planting of commercial Heart of France gave a 7 per cent stand at this time.

The plantings in 1928 included not only the first series of strains but also certain selections made in 1927 from commercial plantings on naturally infested aster-sick soil. Of those collected at Randolph, stands of the Comet-like selection from Peerless pink (Figure 2A), and of Royal purple were promising. Of those collected at Fox Lake, a strain of white American Branching did well.

In 1928 a beginning was made with the American Beauty variety. Commercial seed of this variety gave the following results at the end of the season: white 3 per cent, pink 9 per cent, rose 1 per cent, lavender 15 per cent, and purple 5 per cent resistance to wilt. The

seed from the plants resisting the disease was collected for trial in 1929.

Results in 1929

In 1929 the larger cloth-covered enclosures both at Madison and Randolph were again employed. Figure 10 shows the interior of the aster cage or house at Randolph. For the first time certain of the wilt-resistant strains which had been collected in bulk were tested, not only in Wisconsin but also in other localities, notably California. The results obtained in this year and 1930 will be given in greater detail than those of the preceding years in order to show clearly the present status of the work. The data from Wisconsin will be considered first.

In the case of the American Branching type (Table IV) twenty-two out of twenty-seven selections gave at least a 50 per cent stand when flowering began and sixteen maintained this degree of resistance at the end of the blooming season. Of these sixteen strains two were of white. Of the pink there were two shades in which selections showed 76 per cent resistance. A commercial strain comparable to one of them showed a 25 per cent resistance to wilt. Of rose, the next most desirable color from the florists' point of view, one selec-

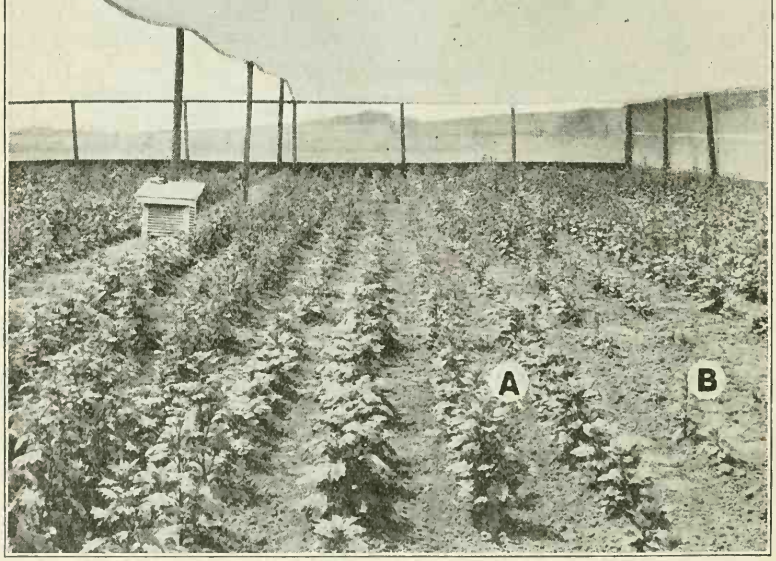

FIG. 10.—EXPERIMENTAL ASTER PLANTING ON ASTER-SICK SOIL AT RANDOLPH, WISCONSIN, 1929

The interior of the large cage or house (6+ by 60 by 98 feet) completely covered with cloth of 22 x 22 threads per inch is shown. Differences in susceptibility to the wilt disease is apparent between the two commercial strains in the right foreground (B) and the comparable selected strains to their left (A). (Similar contrasts appear in Figures 12 and 13.)

Table IV.—Wilt Resistance at the End of the Flowering Season in the American Branching Type in 1929 and 1930

Color and strain	1929		1930	
	Wisconsin per cent	California per cent[a]	Wisconsin per cent	California per cent[a]
White				
40	78	70	79	90
40-2	92[b]	....	64	70
40-2-2	....	....	77[b]	....
40-2-5	....	....	90[b]	....
45	....	....	86[b]	....
commercial	....	....	4	....
Pale Pink				
13-1-1-5	76[b]	....	71	90
13-1-1-5-2	....	....	58[b]	....
Pink				
39-4	76[b]	....	....	....
43-1	....	....	76[b]	....
K. (Kunkel)	89	80	77	70
commercial	25	....	30	....
Light Rose				
21-1-2-1	83[b]	....	37	60
21-1-2-1-1	....	....	66[b]	....
Deep Rose				
20-2-1	48	0+	....	60
20-2-1-1-1	....	....	35[b]	....
20-2-1-1-2	....	....	15[b]	....
20-2-2	39	10	....	60
20-2-2-4	....	....	57[b]	....
20-2-2-5	....	....	32[b]	....
commercial	14	....	14	....
Red				
26-5-4-a	83	70	....	90+
26-5-4-b	85	80	....	90+
26-5-4-1	75[b]	....	....	....
26-5-4-2	98[b]	....	84	90
26-5-4-2-1	....	....	87[b]	....
26-5-6	71	80	76	90+
Pale Lavender				
25-1-3	71	10	....	60
25-1-5	47	0+	....	70
25-1-5-3	58	....	....	....
Deep Lavender				
30-1-1	53	40	....	70
30-1-1-6	61[b]	....	....	....
30-1-1-7	61[b]	....	....	....
42	....	....	53	90
commercial	62	....	45	....
Purple				
37-1	84[b]	....	....	....
37-1-1	....	....	59[b]	....
37-1-2	....	....	68[b]	....
37-1-4	....	....	78[b]	....
commercial	....	30	....	....

a These figures are estimates provided by Miss Bodger and Dr. Jagger.
b Individual plant selection.

tion showed 83 per cent resistance. A commercial strain of crimson showed 14 per cent resistance. Of red in the same shade as the Heart of France but with the more desirable American Branching plant habit (less branching and less of yellow-centered flowers) the five strains tried showed over 50 per cent resistance. Of a pale lavender, two strains showed promise. Of a deeper lavender, three selections

gave 53, 61, and 61 per cent stands. A commercial strain of this shade proved equally promising. Of purple, one plant selection proved relatively resistant. Twelve of the above sixteen strains originated from five of the selections of 1925. Thus there were at hand in the American Branching type promising Wisconsin selected wilt-resistant aster strains in the range of colors (white, pink, rose, lavender, and purple) desired by the florists' trade. These strains, in general, were much more resistant than the comparable commercial stock.

Two other promising strains of American Branching appeared in the 1929 trials. One of these was courteously supplied by Kunkel (1929b) who, in 1928, had found a commercial strain of Semple pink of considerable promise. This strain proved excellent in resistance (Table IV). The other promising strain was the commercial American Branching lavender mentioned above which gave 62 per cent resistance.

In Heart of France in 1929 (Table V), seven out of eight selected strains gave over fifty per cent resistance both when flowering began and later in the season, as compared with a commercial strain of 6 per cent resistance. All of the selected strains owe their origin to one of the original selections of 1925. Thus, desirable wilt-resistant selections have been developed in the Heart of France type.

Figure 11 shows both the 1929 status and the development through several years of the wilt-resistant selections in the red American Branching and in the Heart of France types and serves to illustrate graphically the possible development of wilt-resistant strains by annually repeated selection.

In American Beauty, in which first selections were made in 1928, some progress appeared in 1929. In white, selections showed 40, 30, and 12 per cent disease resistance late in the season as compared with 2 per cent resistance in a commercial strain. The type of flower in these selections, however, was not so desirable from a commercial florists' viewpoint as that of the American Branching white men-

Table V.—Wilt Resistance at the End of the Flowering Season in the Red Heart of France Type in 1929 and 1930

Strain	1929		1930	
	Wisconsin per cent	California per cent[a]	Wisconsin per cent	California per cent[a]
2-1-1-a	79	70	....	90
2-1-1-b	58	70	....	90+
2-1-1-1	92[b]	....	80	80
2-2-1-a	62	80	....	90+
2-2-1-b	72	70	....	90
2-2-5-a	66	70	....	90
2-2-5-b	71	70	....	90+
5-1-1	16	20	....	90
commercial	6	....	....	....

a These figures are estimates provided by Miss Bodger and Dr. Jagger.
b Individual plant selection.

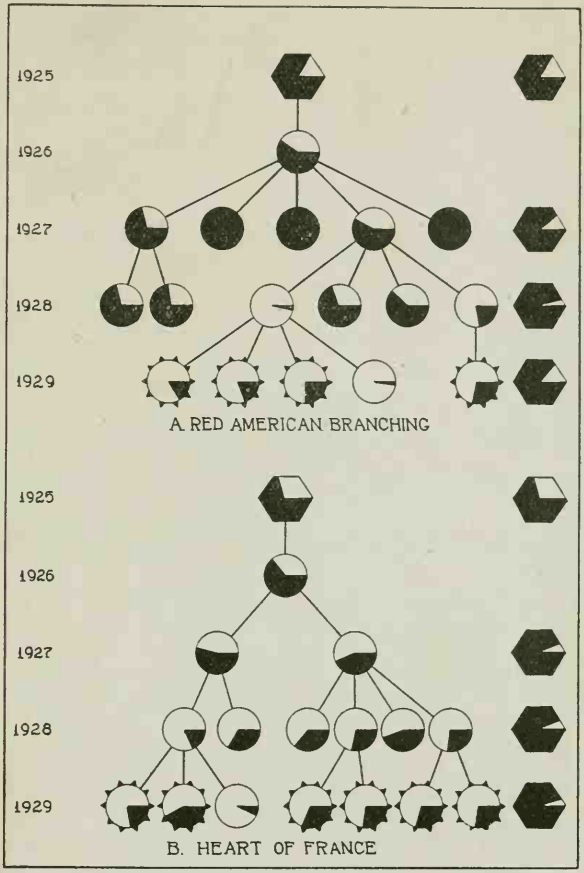

FIG. 11.—DISEASE RESISTANCE IN ANNUALLY REPEATED SELECTIONS IN RED AMERICAN BRANCHING (A) AND HEART OF FRANCE (B).

White Sector—Per cent healthy plants. *Black Sector*—Per cent plants with wilt. *Hexagon*—Results from use of commercial seed. The commercial stock used for the American Branching (A) in 1925 was of mixed colors. In 1927, 1928, and 1929 a commercial crimson was employed for comparison. Heart of France (B) commercial stock was bought under that name. *Smooth Circle*—Results from use of individual plant selected seed. *Toothed Circle*—Results from use of bulk selected seed. This shows that progress was made by repeated annual selections in both of these varieties.

tioned earlier. In shell pink one selection showed 57 per cent resistance. No 1928 selections of rose produced seed for the 1929 trials. In lavender no progress was made. In purple, however, a selection showed 22 per cent wilt resistance as compared with a commercial of only 2 per cent resistance. Thus in American Beauty some progress has been made in selections of certain desirable florists' colors but no selection showed a commercially profitable degree of resistance in 1929.

Of other aster types the Comet-like selection from Peerless pink mentioned before gave excellent results. The three bulk and two in-

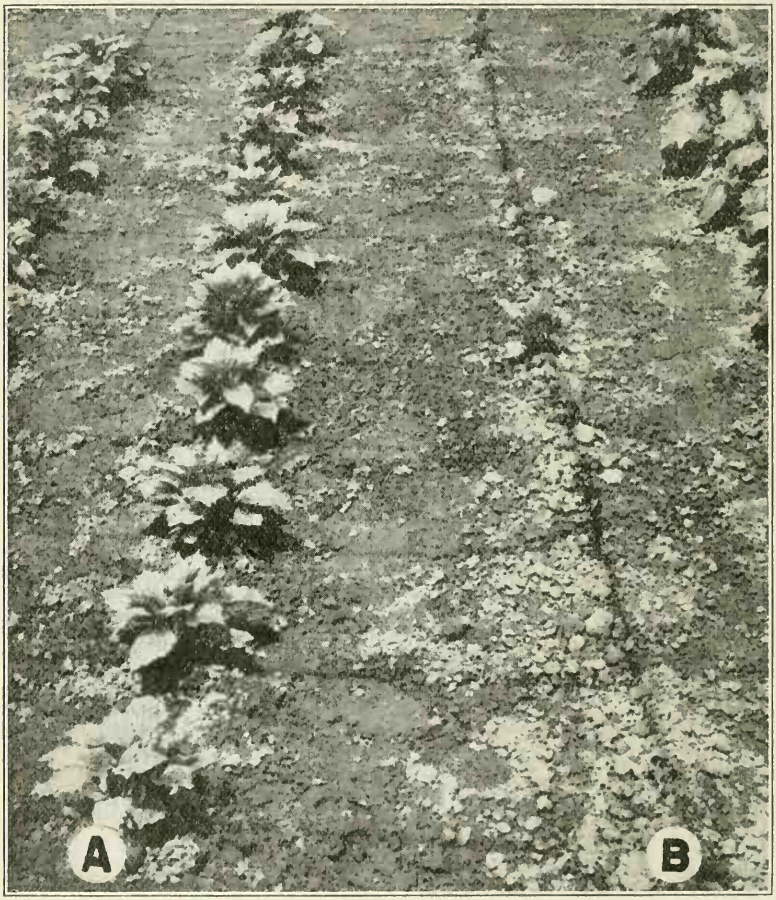

FIG. 12.—EARLY SEASON APPEARANCE OF WILT RESISTANT (A) AND COMMERCIAL (B) STRAINS OF THE COMET-LIKE SELECTION FROM PEERLESS PINK ON ASTER-SICK SOIL AT RANDOLPH, WISCONSIN, JULY 1929. (SEE FIGURE 13.)

dividual plant selections tried showed respectively 75, 62, 92, 82, and 53 per cent wilt resistance at the end of the flowering season. At the same time plants from the commercial stock of the same origin all died of the wilt. The behavior of one of these selected strains in contrast with the commercial strain in early July and in mid-August are shown in Figures 12 and 13 respectively. This is typical of the contrast between selected and comparable commercial strains in many instances (Figure 10). An individual plant selection from Royal purple also proved excellent. This showed 95 per cent resistance late in the season.

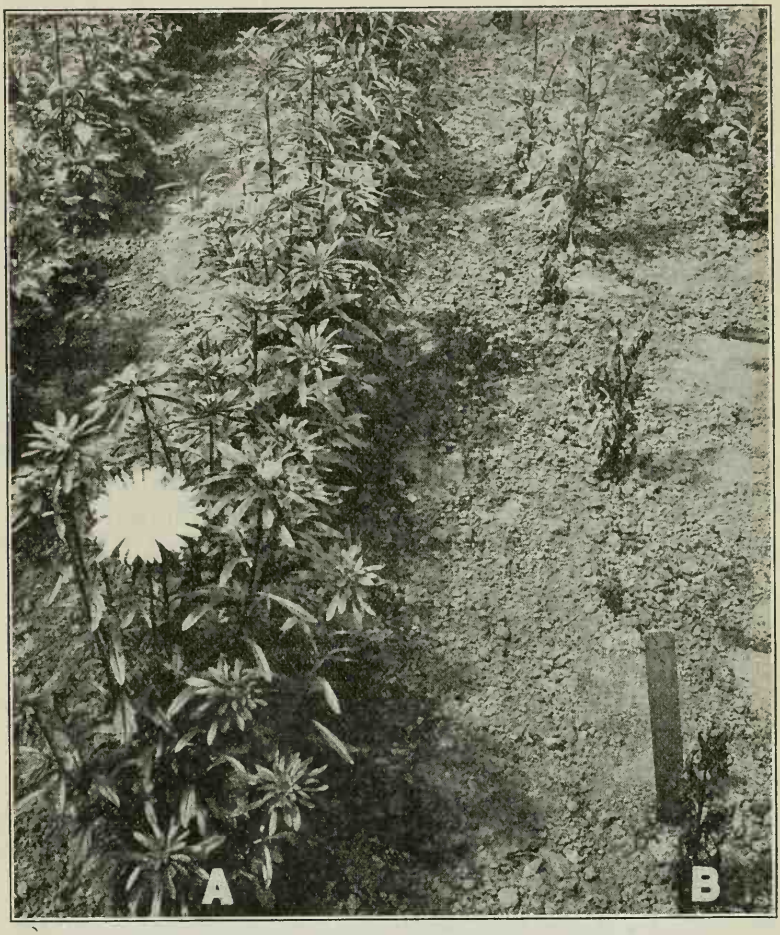

FIG. 13.—LATE SEASON APPEARANCE OF WILT RESISTANT (A) AND COMMERCIAL (B) STRAINS OF THE COMET-LIKE SELECTION FROM PEERLESS PINK ON ASTER-SICK SOIL AT RANDOLPH, WISCONSIN, AUGUST 1929. (SEE FIGURE 12.)

The contrast in behavior between the commercially satisfactory stand of the selected resistant strain and the complete loss of the commercial strain is marked both early and late in the season. A similar contrast appears in Figure 10.

As mentioned above, certain of the wilt-resistant strains were tested in 1929 not only in Wisconsin but also in other localities. The most extensive trials were made in an aster-sick field of Bodger Seeds, Ltd., El Monte, Cal.[10] The results obtained there are given

[10] In planning and securing data from these and tests of commercial varieties the authors are indebted to the continued courtesy of the Bodger Seeds, Ltd., and especially to the personal attention of Miss Elizabeth Bodger. Helpful advice was received at the outset from Professors R. E. Smith, W. T. Horne, and D. G. Milbrath of the California institutions. During the progress of the trials Dr. I. C. Jagger, as representative of the United States Department of Agriculture, cooperated with Miss Bodger both in 1929 and 1930 in the details of planting arrangements and in the taking of final notes upon the results. In addition profitable advice was received from Dr. W. J. Zaumeyer and Dr. Freeman Weiss of the United States Department of Agriculture following visits to these plots. All such aids were especially appreciated since neither of the writers visited El Monte between 1928 and 1931.

in Tables IV and V and agreed for the most part with those obtained in Wisconsin. Figure 14 shows the behavior of certain selections of the Heart of France type in California as compared with commercial strains. Kunkel's Semple pink proved resistant in Wisconsin and California as it had in New York. Some of the Wisconsin strains were also tested by Kunkel in New York and by Weiss in Washington, D. C. According to their reports strains which were wilt-resistant in Wisconsin and California were also resistant in the above localities. This agreement in the behavior of the strains serves as evidence for the statement made earlier that aster wilt may be due to essentially the same cause in many localities from coast to coast. If so, strains resistant in one locality would probably prove resistant in other regions.

Results in 1930

Trials in 1930 followed in general plan those of 1929 except in the following particulars. Certain of the promising wilt-resistant strains of 1929 were not continued in Wisconsin in 1930 either because they were essentially duplicate types, or because the shade or type of blossoms was not the most desirable from the commercial florists' point of view. Such reduction in the number of strains permitted an extension of the work to include certain colors of the Royal type, a variety much in demand among the eastern florists. Trials of certain of the promising Wisconsin selections were made by florists in a larger

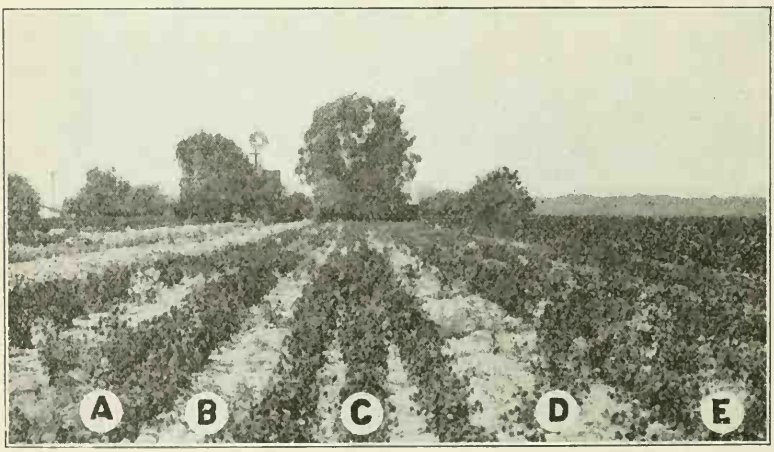

FIG. 14.—ASTER PLANTING ON ASTER-SICK SOIL OF BODGER SEEDS, LTD. AT EL MONTE, CALIFORNIA, 1929. HEART OF FRANCE TYPE.

(A.) Wisconsin selection 2-1-1-b (2 rows).
(B.) Commercial (1 row).
(C.) Wisconsin selection 2-2-1-a (3 rows).
(D.) Commercial (2 rows).
(E.) Wisconsin selection 2-2-1-b (2 rows).

The commercial strains (B and D) proved highly susceptible and the selected strains (A, C, and E) relatively resistant to wilt. The approximate stands are indicated in Table V.

number of widely different localities in order to determine further the wilt-resistant qualities of the strains and to increase the desirable strains for commercial distribution.

In the American Branching type nineteen out of twenty-two selections in Wisconsin gave at least a 50 per cent stand when flowering began and eighteen maintained this degree of resistance at the end of the blooming period (Table IV). These were of essentially the same colors as the commercially desirable promising selections of 1929, i.e., white, two shades of pink, rose, red, deep lavender, and purple. The five commercial strains tried showed 4, 30, 14, 45, and 30 per cent resistance late in the season.

In the Heart of France only one selection was tried in Wisconsin in 1930 and this had an 80 per cent resistance to wilt (Table V).

Since the American Beauty aster is rather late for the north-central state conditions, no extension of the work with that type was made. However, the shell-pink and purple selections of promise in 1929 were continued in 1930. Three selections of the shell pink gave 75, 77, and 82 per cent resistance at the end of the flowering season, and two purple selections 66 and 77 per cent resistance.

The other aster types of promise in 1929 again proved satisfactory. Two strains of the Comet-like selection from Peerless pink showed 63 and 83 per cent resistance late in the season. At the same time three strains of Royal purple were of 85, 80, and 76 per cent resistance. A commercial strain of Royal purple showed only 2 per cent wilt resistance.

As mentioned above, the trials of 1930 in Wisconsin were extended to include other colors of the Royal type. The results with commercial stock were as follows: rose 2 per cent, lavender 1 per cent, and pink 49 per cent wilt resistance. Preliminary selections were made from the resistant plants.

As in 1929 the behavior of the strains in other localities agreed with that in Wisconsin. Tables IV and V show the stands in California both for selections sent in 1929 and grown again in 1930, and for additional selections.

The results from annually repeated selection seem, therefore, conclusive in the major points of interest. They may be summarized as follows. In all the commercial varieties of China aster that have been tested, there is a considerable range of variation as to individual resistance to the wilt disease. With certain individuals at least this character is inheritable. By selecting such individuals, even from strains showing relatively low general resistance, and by annually repeating the trials upon aster-sick soil and then reselecting the most promising progeny, strains may be developed of a relatively high and stable degree of resistance.

More thorough trials have been made at Wisconsin in the American Branching type, this being the one of chief commercial interest in the Wisconsin-Chicago area. As a result, strains of American

Branching of a promising degree of resistance in the colors especially sought by commercial growers (white, pink, rose, lavender and purple) have been obtained. In other types early attention was given to the Heart of France. This is a red aster in which a high degree of resistance was easily secured from a susceptible commercial strain. This was likewise true with the corresponding red in the more desirable American Branching type. Less has been done with the somewhat earlier Royal type, the Comet, and the later American Beauty. In all of these, so far as tested, however, progress has been made.

The occurrence of wilt-resistant varieties of China asters among established commercial types

The occurrence of outstanding differences in varietal susceptibility to wilt has been noted from time to time, beginning with the very early reports upon the disease.[11] Search for evidence of this has been made as opportunity permitted from the beginning of the Wisconsin studies in 1925. The unfortunate necessity of limiting experimental plots in this state to areas which could be protected by cloth made advance by this means slow. As will be indicated later, developments in California of recent years have favored much more rapid and encouraging progress in this direction.

Tests of commercial varieties in the experimental plots at Madison and Randolph on a small scale have shown few to be wilt resistant. These tests have been in progress from 1925 to date. Table VI shows the results in detail. In all, thirty-two commercial strains have been tried. Of these only three have approached or exceeded 50 per cent wilt resistance. The remainder have produced stands varying from 0 to 30 per cent.

Observations were extended to other plantings in Wisconsin. Extensive surveys were made in 1927 among available aster plantations growing upon presumably aster-sick soils in the search for significant varietal resistance. These included notes upon the relative amount of wilt occurring in the various strains of aster in the horticultural garden at Madison (Table I). Although there were some differences as to wilt resistance between types there were even greater differences between colors of the same type. No very promising commercial strain was found here.

This same year an unusually good opportunity for comparison of commercial varieties was afforded by the plantings of the J. W. Jung

[11] As early as 1897 Stone and Smith quoted a letter to the effect that the Giant White Comet seemed free from the disease. In 1923 in Ohio an inclination toward resistance in the Comet variety was again noted (Martin 1925). Arnold (1917, 1919) observed that there were decided differences in varietal susceptibility. Weaver (1917) recommended that the Queen of the Market variety be planted on new soil each season because of the stem rot, whereas Vick's Royal might be planted several seasons on the same soil without having stem rot. Curtiss (1926) reported from Iowa that certain varieties showed a higher degree of resistance than others. While Jackson (1927) did not find special resistance in any variety at St. Catherines, he noted a report by Mr. F. L. Drayton that Heart of France showed considerable resistance at Ottawa. Kunkel (1929b) found one very resistant strain of a commercial variety. Ball (1930) recorded that Royal shell pink proved highly resistant, as well as Heart of France. White (1931) suggested Semple's shell pink, Heart of France, Express, and Royal varieties as having some resistance.

Table VI.—Wilt Resistance at the End of the Flowering Season in the Commercial Varieties Tested in the Experimental Plots at Madison and Randolph, Wisconsin, from 1925 to 1930

Strain	1925	1927	1928	1929	1930
American Branching					
mixed	16	....	....	....	....
white	....	....	....	....	4
pink	....	3	6	25	30
rose	....	....	....	....	14
crimson	....	11	8	14	....
lavender	....	22	8	62	45
purple	....	....	3	....	30
Heart of France	29	7	7	6	....
American Beauty					
white	....	....	3	2	....
pink	....	....	9	....	....
rose	....	....	1	....	....
lavender	....	....	15	....	....
purple	....	....	5	2	....
Comet					
pink	....	....	....	0	....
Royal					
pink	....	....	....	....	49
rose	....	....	....	....	2
lavender	....	....	....	....	1
purple	....	....	....	....	2

Seed Co. at Randolph. This concern had replanted to asters a considerable area that was infested with the aster wilt Fusarium. In this field some well marked differences in varietal susceptibility appeared (Figure 2). A Comet-like strain of Peerless shell pink proved especially promising (Figure 2A). Selections from the resistant plants were made and have been continued as discussed earlier. This was the first resistant commercial variety found in the Wisconsin studies.

A greater revelation of the possibilities offered by inspection of commercial seedsmen's plantings came to the senior author in the season of 1928. Visits were then made to a number of commercial seed growing fields in California, including the very extensive aster plantations of Bodger Seeds, Ltd. at El Monte. Through the courtesy of Mr. John Bodger and other members of the firm the plantings of that year were observed and the earlier experiences of the company were noted. In 1928 the asters were being grown on "new" soil. Although some wilt was scattered through most of the fields it was not serious. The asters had been planted on "new" soil because in the previous summer, 1927, some varieties had suffered very badly from wilt. These varieties had been placed in an "old" field which had unexpectedly proven to be infested with the aster wilt Fusarium. Examination of the 1927 records showed that wilt may be as highly destructive in California as in Wisconsin. It also revealed that there may be a wide range in loss from wilt among different varieties on soils similarly aster-sick. In general less than 25 per cent of the

normal yield of seed was obtained. The greatest extremes, as shown by the 1927 records, occurred in the American Branching type between the light flesh pink variety Mary Semple, apparently peculiarly susceptible as shown by a loss of over 99 per cent, and the apparently resistant lavendar variety showing a loss of less than 10 per cent. Two things seemed obvious. The first was that there already existed among these aster strains wide differences in relative susceptibility, including some presumably resistant types like the lavender. The second was that, where a susceptible strain like the Semple pink had been grown on sick soil, the seed from the surviving plants might carry a higher degree of resistance than previously. The experiences in Wisconsin substantiated these suppositions.

In 1929 Bodger Seeds, Ltd. made comparative trials on their aster-sick soil of a number of their commercial strains and certain selected strains sent from Wisconsin and elsewhere. These were repeated in 1930. The behavior of the selected strains from Wisconsin is shown in Tables IV and V and Figure 14. Of the fifteen commercial strains of Bodger seed planted in 1929 three showed a high degree of resistance. These results were so encouraging that further and more extensive trials were made on the same sick soil in 1930. The trials then included 148 commercial strains of Bodger seed of which 127 showed a rather uniform susceptibility whereas 21 showed promise of a commercial degree of resistance.

Thus, it appears that although certain commercial strains of aster may show a high resistance to wilt, most of them are at present very susceptible to this disease. On the other hand it is evident that where it is possible to test a large series of commercial varieties in generous numbers on Fusarium-infested soil, valuable wilt-resistant strains may be discovered. It has been practicable to do this only in a small way in Wisconsin because the necessity of screening the plants against yellows has restricted the size of the trial plantations. The results on the Bodger grounds in California in the three years as reported above have already led to the discovery of a number of desirable commercial strains and indicate the value of continued and even more extensive trials of existing commercial strains.

Discussion and conclusions on the control of aster wilt

The studies upon the possibilities of the control of aster wilt through the use of disease-resistant strains have led to certain conclusions.

It seems that with this vascular Fusarium disease as with other similar diseases studied at Wisconsin, e.g., that of cabbage yellows and pea wilt, there are differences in susceptibility between varieties or strains and more especially between individuals within the variety or strain of the host.

These differences, in many cases at least, are largely inheritable. Therefore through selection followed by repeated trials upon infested soil and annual reselection, encouraging progress has been made in the

development of wilt-resistant strains. In general, annual selections through three to five generations have been sufficient to secure a satisfactory degree of resistance for purposes of successful floriculture, even upon heavily infested soil.

There are numerous types of China aster in cultivation varying in season of blooming, floral type and color, and vegetative habit. So far the American Branching, Heart of France, American Beauty, Comet, and Royal types have received attention in the Wisconsin studies. These include the types most frequently used by the local commercial aster growers. All of these, however, have shown similar general susceptibility to wilt and all have yielded wilt-resistant strains by selection. Essentially like results may probably be expected with the other established commercial types.

There is no clear evidence of a correlation of color with resistance although it has been somewhat easier to establish resistant strains of red in the American Branching and Heart of France types. It may be noted that these red asters have high vegetative vigor.

As far as color is concerned but slight evidence has been secured of cross pollination of asters in the experimental plantings in Wisconsin. Flowers of different colors have developed immediately adjacent with infrequent admixture of color, although this may sometimes occur as Fleming (1929) has pointed out.

The progeny of individual plants frequently vary in flower type and habit of growth. Consequently in selecting for resistance other desirable characters must be kept constantly in mind.

Although resistance to wilt seems a fairly stable character, in no case have resistant strains shown complete immunity. That is, upon infested soil under conditions which favor disease development, such as high temperature, some wilt may be expected in even the most resistant strains. It appears that for the best results with resistant asters, as with cabbages, the mother seed at least should be produced continuously upon Fusarium-infested soil. In this way the degree of resistance may be not only maintained but presumably gradually increased. An indication of the stability of the resistant character and of the similarity of the wilt disease in widely separated places is afforded by the behavior of certain of the resistant strains developed in Wisconsin upon aster-sick soil in California, New York, Washington, and other places. In all cases their resistance has been essentially the same in the other localities as in Wisconsin.

SUMMARY

TWO DISEASES, aster yellows and aster wilt, seriously menace the culture of the China aster throughout the United States.

2. The symptoms of these two diseases are so similar at certain stages that aster growers often confuse them as one malady. They have, however, distinguishing characters which are herein described by which each may be recognized with certainty.

3. Both are infectious diseases, but they are introduced and spread in such different ways that they require entirely different methods of control.

4. Aster yellows is a virus disease, transmitted by the leaf hopper *Cicadula sexnotata.*

5. The virus overwinters in certain biennial and perennial host plants, including some common garden weeds. From these it is carried to the young asters in early summer by leaf hoppers. Thereafter, it is spread among the aster plants with increasing rapidity.

6. Control of aster yellows is dependent, therefore, upon shielding the aster plants from viruliferous leaf hoppers.

7. An effective shielding material was found in cloth not coarser than 22 x 22 threads per inch.

8. Two types of shield have been tried comparatively:

a. *Fences*: The use of cloth-covered side walls or "fences" six feet high (tops uncovered), combined with roguing, reduced the yellows somewhat but was not commercially satisfactory under Wisconsin conditions.

b. *Houses*: In Wisconsin trials for six years, aster yellows has been controlled by the use of cloth-covered cages or houses. The tops and sides of the enclosures were completely covered with cloth not coarser than 22 x 22 threads per inch. Trials using a somewhat coarser cloth for top cover showed some promise for commercial houses.

9. Aster wilt is caused by a parasitic fungus (*Fusarium conglutinans* v. *callistephi*). This may be carried on aster seed and, once introduced, persists indefinitely in the soil, making it aster-sick.

10. Use of clean seed and disease-free soil is, therefore, the simplest way to escape the wilt. This may be accomplished with reasonable success by painstaking home gardeners or even small com-

mercial growers, especially if they can use home grown or disinfected seed and plant on new soil each year. But with most commercial growers the hazards are unavoidably great.

11. The use of wilt-resistant strains of aster seems to be the most promising way to avoid incrasing difficulty from this wilt for commercial growers and for many home gardeners.

12. Wilt-resistant strains of aster have been developed at Wisconsin by annually repeated selections during the last six years. Those that are now stabilized include the several basic colors and a fairly wide range in flower type and growth habit, as represented by American Branching, Heart of France, Comet, Royal, and American Beauty. This seems to justify the opinion that resistance can thus be developed in the standard types and colors of asters.

13. Special attention has been given to the development of wilt resistance in a series of types suitable for the commercial cut flower trade of the middle west. This has been most satisfactorily accomplished through securing American Branching types in the important florists' colors, viz., white, pink, rose, lavender, and purple.

14. In additon to limited trials of miscellaneous commercial strains at Wisconsin, data have been secured from larger field trials by a commercial seed firm in California which justify confidence that further large scale trials on suitable aster-sick soil will discover numerous highly wilt-resistant strains already in existence among standard commercial asters.

15. The probability of this is increased by the known facts that at times aster wilt has occurred during recent years in highly destructive degree in some seed growing areas, both eastern and western. The "survival of the fittest" has doubtless operated to bring about increased resistance in commercial strains even though the nature of the trouble was not clearly recognized at the time.

16. Trials of resistant Wisconsin strains on aster-sick soils in other sections extending from New York and Washington in the east to California in the west indicate the stability of the resistant character. It appears that there is essentially one type of pathogen and that disease expression is not so affected by environment as to overcome the resistance of well-established strains.

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Stripe Resistance and Yield of Smooth-Awned Barley Hybrids

Agricultural Experiment Station of the University of Wisconsin,
Madison

Contents

Stripe Resistance and Yield of Smooth-Awned Barley Hybrids

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THE EARLY DEVELOPMENT of barley in Wisconsin was centered around the introduction and improvement of the varieties Oderbrucker and "Manshury" (Manchurian types) by the Agronomy Department. Of several pure lines from these varieties, Pedigrees 5 and 6 from the Oderbrucker were selected for high yield and excellent malting quality. These barleys had two objectionable characteristics however, barbed awns and susceptibility to the stripe disease caused by *Helminthosporium gramineum* Rabh. In 1917, the Wisconsin Experiment Station started on a new line of attack in barley breeding, the production of a white, six-rowed, smooth-awned barley by hybridization and selection. The rough-awned Oderbrucker, *Hordeum vulgare* var. *pallidum typica*[2] Ser. which was the standard variety in the state was crossed with a small, black, smooth-awned barley, *H. vulgare* var. *nigrum leiorrhynchum* Kcke., primarily to combine the smooth-awned condition with the desirable characters of the Oderbrucker barley. Selection within the segregating hybrid lines had proceeded far enough by 1925 to place several of the more desirable hybrid strains in preliminary yield trials. The selection, X39-5, which outyielded Oderbrucker for three consecutive years (see Table III) was increased and distributed as Wisconsin Pedigree No. 37. Later, the selection, X39-9-3, which in two years' plot trials had been superior to Pedigree 37 in yield and stripe resistance, was increased and distributed as Wisconsin Pedigree No. 38 and was named "Wisconsin Barbless".

Preliminary field counts in 1926 showed certain of the hybrid selections, notably the X39 group, relatively free from the stripe disease. Other selections, such as certain of the X105 group, had more diseased plants than the Oderbrucker parent variety. The results indicated, therefore, a range in reaction to the stripe disease from susceptible to practically immune selections within the progenies of the crosses. The question, therefore, naturally arose whether these hybrid selections were more resistant to stripe or were escap-

[1] Cooperative investigations between the Departments of Agronomy and Plant Pathology, University of Wisconsin and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Citation by number to literature cited, Harlan. (2)

FIG. 1.*—STRIPED PLANTS OF ODERBRUCKER PEDIGREE 6.

ing the disease. This was a question of practical significance since the stripe disease was becoming of increasing importance and heretofore there had been no report of commercial varieties or selections showing resistance to this disease.

The stripe disease had increased in the Upper Mississippi Valley spring barley section to where it had become one of the most important diseases in barley production. Field surveys showed an average estimated loss of 2.1, 2.5, and 1.9 per cent[3] of the crop in this area for the years 1924 to 1926 inclusive. Seed treatments for the control of the seed borne infection were unsatisfactory as well as difficult and expensive to use with a crop like barley, where most growers produced their own seed. The development of stripe resistant barley varieties was becoming increasingly important therefore, if this loss to Wisconsin barley growers was to be prevented. The logical way to proceed was to combine this stripe study with the intensive agronomic study being made upon the new smooth-awned hybrid selections.

A parallel study was made with the scab disease caused by *Gibberella saubinetii* (Mont.) Sacc. A number of the smooth-awned hybrid selections and Oderbrucker have been tested for resistance to the scab disease by inoculating under muslin cages kept at a high humidity by sprinkling with water. The percentages of scabbed kernels developing under these very favorable conditions for disease production follow: Oderbrucker, Pedgree 5-1, 74.9 per cent; Oderbrucker, Pedigree 6, 75.8 per cent; Pedigree 37, 73.5 per cent; and Wisconsin Barbless, Pedigree 38, 73.4 per cent. From the averages of a three years' cage test and from field observations where the percentage of scab is very much less, there seems to be no significant

[3] Data taken from the Plant Disease Bulletin Supplement, U.S.D.A., 1925-1927.

* Photographs and graphs were made by Eugene H. Herrling, Department of Plant Pathology.

difference in the resistance of these varieties. In regard to loose smut reaction, field observations indicate that Pedigree 37 and Pedigree 38 show the same susceptible tendency found in most of the other smooth-awned barleys.

Materials and Methods

THE HYBRIDS used in these studies were obtained by crossing pedigreed strains of Oderbrucker, *Hordeum vulgare* var. *pallidum typica* Ser., with Leiorrhynchum, *Hordeum vulgare* var. *nigrum leiorrhynchum* Kcke, a six-rowed, black, smooth-awned variety. In field plots, Oderbrucker, Wisconsin Pedigree 6, was rather susceptible to stripe, whereas Oderbrucker, Wisconsin Pedigree 5, selected from the same original lot, was more resistant. Both of these lines were used in makng crosses with Leiorrhynchum before their reaction to stripe had been ascertained.

TABLE I.—PARENTAGE OF VARIOUS BARLEY CROSSES AND DATES WHEN THE HYBRIDS WERE MADE

Cross	Pistillate parent	Staminate parent	Year cross was made
X39[a]	Ped. 5	Leiorrhynchum	1917
X57	Leiorrhynchum	Ped. 6	1920
X60	Leiorrhynchum	Ped. 6[b]	1922
X66	X39-8[c]	Ped. 6	1923
X67	X39-11	Ped. 6	1923
X69	X57-10-4	Ped. 5-1[c]	1923
X102	X60-1	Ped. 6	1924
X104	X39-9-10	Ped. 6	1924
X105	X39-3-9	Ped. 6	1924
X106	X57-5-2	Ped. 6	1924

[a] The parentage of X39 was incorrectly published in the Journal of the American Society of Agronomy 1931, 23:396-401, p. 400, as a cross between Pedigree 5 Oderbrucker male and Leiorrhynchum female made in 1916.

[b] Pedigree number not recorded, but probably Pedigree 6.

[c] The number or numbers following the dash after X numbers or Pedigree numbers represent individual plant selections.

Head selections were made from the superior plants in the segregating populations from the crosses. The grain from the heads selected was sown in head rows in the nursery the following year. If the plants in the head row showed heterozygosity, reselections were made, and the most desirable heads propagated. When relatively homozygous lines were established, they were grown in triplicate rod rows the next season. When undesirable plant characters appeared in the rod row trials, reselections were made if superior individuals appeared; if not, the strain was discarded.

The test for yield and quality was made in 1/20th acre plots. Many of the surviving selections were eliminated in the yield plot where competition was more nearly comparable to that in the field. Strains which were conspicuously inferior were eliminated early in the test. See Table V. The final test to determine adaptation to

FIG. 2.—COMPARATIVE HEADS OF ODERBRUCKER, PEDIGREE 5-1, AND WISCONSIN BARBLESS, PEDIGREE 38.
Awns of central spikelets have been removed to show relative size and arrangement of kernels.

different soil and climatic areas of the state was made by the Branch Experiment Stations and also by farmers.

Head selections were made to secure the Oderbrucker quality combined with the smooth awns. Selection studies were made for size and color of head; size, shape and color of kernel; smoothness of awn; length of internodes of the culm and rachis; strength of straw; date of heading and maturity; disease resistance and, finally, yield in both the rod rows and plots. The results of the agronomic studies were reported in a previous paper (4). Some of the hybrid selections showing desirable qualities were again crossed with Oderbrucker, Pedigree 5 or 6, and reselected.

Detailed studies on resistance to the stripe disease were made at several locations in the state. Rod row plantings of the various hybrid selections were made with stripe-infected Oderbrucker sown on each side of the hybrid selection to secure maximum uniform distribution of conidial inoculum during the period of floral infection. The seed thus secured was sown at the different locations under conditions favorable for stripe development. Stripe counts were made in the hybrid selections and in Oderbrucker controls soon after the barley was headed when the disease was most conspicuous.

Agronomic Characters and Yield

THE PLANT CHARACTERS of the hybrid selections differed somewhat from the Oderbrucker type in that the best yielding hybrid selections matured four to five days later than the Pedigreed Oderbrucker. As late maturing barleys are often injured by midsummer heat and drouth, selection for earliness was continually practiced in the barbless hybrids, but the long head and large kernel appeared to be associated with later maturity. However, reports over the state from the crop of 1931, a record season of heat and drouth,

indicated that the Pedigrees 37 and 38 withstood the drouth and heat fully as well as the Oderbrucker.

The number and size of the barbs on the awns varied within the hybrid selections. The smoother variants, having fine barbs only near the tip of the awn, were selected for continued line propagation. These selections have been found somewhat more difficult to thresh than the Oderbrucker due to the smooth and somewhat flexible awn and the slightly looser attachment of the hull. Setting the concaves in the separator close enough to remove all the awns resulted in some damage to the kernels by peeling the hull. Due to genetic linkages it has been impossible to eliminate the slightly grayish color of the hull of the kernel as well as a slightly brownish discoloration from weathering. Crossing the hybrid selections back to the Oderbrucker strains and reselecting have improved the color somewhat. Strains selected so far have not given the pure white kernel characteristic of the Oderbrucker.

Kernel Weight

The consistent increased yield of the Wisconsin Barbless, Pedigree 38, over the Oderbrucker suggested a comparative study of the weight of kernel and hull in relation to this increased grain production. Grain from the yield plots at Madison, Wisconsin, was used for comparative study over a three-year period which included the hot, dry season of 1931 relatively unfavorable for barley production.

The kernel weight of the two barley varieties was not significantly different under favorable conditions for production of the crop. In general, Oderbrucker, Pedigree 5-1, developed a slightly larger kernel than the Wisconsin Barbless, the latter being 1.45 per cent lighter in the two favorable seasons than the standard Oderbrucker. See Table II.

TABLE II.—KERNEL WEIGHTS OF WISCONSIN BARBLESS, PEDIGREE 38, AND ODERBRUCKER, PEDIGREE 5-1, GROWN AT MADISON, WISCONSIN, SEASONS OF 1930 TO 1932.

Season and place grown	Average weight of 100 kernels in grams		Difference in kernel weight—Ped. 38 compared with Ped. 5-1	
	Oderbrucker Ped. 5-1	Wisconsin Barbless, Ped. 38	Grams	%
1930 Yield plots.....	3.3651	3.2819	—.0832	— 2.4
1931 Yield Plots.....	2.6539	3.2520	+.5981	+22.5
1932 Yield plots.....	3.5728	3.5531	—.0197	— 0.5

This difference in kernel weight was probably due in part to the increased number of kernels per head in Pedigree 38. In the unfav-

orable season of 1931, however, a very significant difference in kernel weight was found in favor of the Wisconsin Barbless, Pedigree 38, which was 22.5 per cent heavier than the Oderbrucker, Pedigree 5-1. While yield was lowered in both varieties, the kernel weight of Pedigree 38 was reduced 4.8 per cent based on the average of the two favorable seasons in contrast with 23.5 per cent in Pedigree 5-1. In other words, the size and weight of the kernel in Pedigree 38 were maintained even under unfavorable conditions. Since grade and price of malting barley are closely correlated with kernel uniformity and size, this is an important factor in marketing barley for commercial uses. During the past four years, Pedigree 38 has shown this advantage in uniformity of kernel, size and weight, in both plots and fields.

Hull Percentage

The percentage of hull[4] based upon kernel weight was slightly higher in Pedigree 38 than in the Oderbrucker. In the two favorable years, the hull of Pedigree 38 averaged 1.52 per cent higher than that of Pedigree 5-1. See Table III. In 1931, because of the larger sized berry of Pedigree 38, the two varieties contained approximately the same percentage of hull. The hull of the Pedigree 38 was not as firmly attached (especially toward the tip of the kernel) as in Oderbrucker. This condition resulted in some peeling in the rough treatment of dry grain and in excessive mixing and handling of the steeped moist grain. Considerable variation in the adhesion of the hull to the kernel occurred in the hybrid selections; therefore, it should be possible to select a strain with the hull as firmly attached as in the standard Oderbrucker.

TABLE III.—AVERAGE PERCENTAGE OF HULL BY WEIGHT ON ONE HUNDRED KERNELS SAMPLES OF ODERBRUCKER (PED. 5-1) AND THE WISCONSIN BARBLESS (PED. 38) BARLEY. GROWN AT MADISON, WISCONSIN, SEASONS OF 1930 TO 1932.

Season and place grown	Average percentage of hulls on kernels of		Difference. Ped. 38 compared with Ped. 5-1 in per cent
	Ped. 5-1	Ped. 38	
1930 Yield plot	9.66	10.88	+1.22
1931 Yield plot	11.87	11.82	—0.05
1932 Yield plot	8.99	10.81	+1.82
Average difference in 3 seasons			+1.00

Chemical Composition of Kernel

Kernel composition and malting quality of Pedigrees 37 and 38 have been about the same as Oderbrucker. The starch and crude

[4] Duplicate 100 kernel samples were steeped for 50 minutes in water at 100° F. After the steeping, the hulls (lemma and palea) were stripped off, dried, and weighed.

fiber have been likewise similar to Oderbrucker, the crude fiber being slightly less. Malting trials on small samples and bulk lots of the hybrid selections, Pedigrees 37 and 38, have given results equal to the standard Oderbrucker.

Comparative studies on composition showed Pedigree 38 to be slightly lower in protein than the Oderbrucker. The protein content of the Oderbrucker, Pedigree 5-1, ranged from 10.56 per cent in 1930 to 15 per cent in 1928, averaging 13.03 per cent for the four seasons; the Pedigree 38 ranged from 9.19 in 1930 to 13.69 in 1928 averaging 11.62 per cent or 1.41 per cent less protein than Pedigree 5-1, Oderbrucker. See Table IV. The high protein content in 1928 probably was due to soil conditions as barley followed sweet clover in the rotation that year; whereas, the high protein in 1931 was due primarily to the hot, dry season. The protein content of both varieties in the years studied was well within the range desired for commercial use and feed.

TABLE IV.--TOTAL NITROGEN AND PROTEIN CONTENT OF ODERBRUCKER (PED. 5-1) AND WISCONSIN BARBLESS (PED. 38) BARLEY GROWN AT MADISON, WISCONSIN.

Season and place grown	Average total nitrogen %		Average protein (Factor 6.25) %		Difference, Ped. 38 compared with Ped. 5-1 in per cent
	Ped. 5-1	Ped. 38	Ped. 5-1	Ped. 38	
1928 Yield plots	2.40	2.19	15.00	13.69	—1.31
1930 Yield plots	1.69	1.47	10.56	9.19	—1.37
1931 Yield plots	2.20	2.14	13.75	13.37	—0.38
1932 Yield plots	2.05	1.64	12.81	10.24	—2.57
Average for the 4 seasons			13.03	11.62	—1.41

Yield

The yields of the superior hybrid selections have been consistently higher than the Oderbrucker, Pedigree 5-1 and Pedigree 6, both in plot trials and field tests. After three years' yield trials at Madison, Pedigree 37 was sent to selected farmers to test its suitability to different regions of the state. Pedigree 38 entered the yield trial at Madison in 1928 and has consistently outyielded Pedigree 37 over a five-year period. Trials with farmers throughout the state have corroborated the Station tests. Two other selections, X39-9-3-4 and X57-12-4-13, have given yields about equal to Pedigree 38 and have been equally resistant to stripe. Other selections gave good yields in 1932 and will be tested further at Madison. Table V.

The three years' data on yields from the branch stations, while showing a great variation from season to season, due to unfavorable conditions especially in 1931, have shown Pedigree 38 consistently higher than the Oderbrucker, Pedigree 5-1. Table VI.

TABLE V.—COMPARISON OF YIELDS OF SMOOTH-AWNED HYBRID SELECTIONS AND ODERBRUCKER AT MADISON, WISCONSIN

Selection	Yield in bushels per acre									
	1925	1926	1927	1928	1929	1930	1931	1932	8 yr. Av.	5 yr. Av.
Oderbrucker, Wis. Ped. 6 (check)..	58.5	41.5	37.7*	49.0	29.5	41.6	39.5	34.9	41.5	38.9
White barbless selections:										
X39-5 (Ped. 37)	69.0	60.3	47.8	41.7	43.7	40.7	34.6	49.5	48.8	42.0
X39-9	65.4	52.0	53.1	.....	.....	.....	.....	.....	.....	.....
X39-2	60.4	55.7	53.8	40.3	.....	.....	.....	.....	.....	.....
X39-8	58.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
X39-11	52.5	42.9	.....	.....	.....	.....	.....	.....	.....	.....
X39-13	49.9	.....	.....	.....	.....	.....	.....	.....	.....	.....
X39-6	48.1	.....	.....	.....	.....	.....	.....	.....	.....	.....
X39-9-3 (Ped. 38)	.....	.....	.....	46.4	47.7	45.5	37.8	55.5	.....	46.6
X39-9-3-4	.....	.....	.....	67.2	44.1	46.7	36.2	51.8	.....	49.2
X60-2-1	.....	.....	.....	53.1	37.3	44.3	35.5	.....	.....	.....
X60-2-3	.....	.....	.....	52.4	37.0	45.2	36.1	.....	.....	.....
X57-27-5-5	.....	.....	.....	49.2	33.5	38.7	.....	.....	.....	.....
X57-12-4-13	.....	.....	.....	42.9	47.4	59.1	34.4	49.7	.....	46.7
X105-2-4-1	.....	.....	.....	.....	30.1	36.3	.....	.....	.....	.....
X105-2-4-2	.....	.....	.....	.....	29.4	39.3	32.0	.....	.....	.....
X39-9-3-6-8	.....	.....	.....	.....	.....	.....	.....	58.2	.....	.....
X39-9-3-6-7	.....	.....	.....	.....	.....	.....	.....	55.5	.....	.....
Ped. 37-4	.....	.....	.....	.....	.....	.....	.....	52.6	.....	.....
X69-2-6	.....	.....	.....	.....	.....	.....	.....	47.4	.....	.....
X69-2-9	.....	.....	.....	.....	.....	.....	.....	41.1	.....	.....

* Beginning with 1927, the Pedigree 5-1 Oderbrucker was used as a check.

TABLE VI.—YIELDS OF PEDIGREE 37, PEDIGREE 38, AND ODERBRUCKER, PEDIGREE 5-1, AT THE WISCONSIN BRANCH EXPERIMENT STATIONS

Station	Bushels per acre		
	Oderbrucker Ped. 5-1	Pedigree 37	Pedigree 38
1930			
Ashland	21.3	34.8	38.0
Marshfield	51.0	.....	80.8
Sturgeon Bay	.....	.....	46.0
1931			
Ashland	14.2	.....	18.7
Marshfield	29.2	28.3	34.2
1932			
Ashland	38.5	.....	40.0
Marshfield	25.8	30.8	33.3

The yields of the two selections, Pedigree 37 and 38, in farm trials have been considerably higher than the Oderbrucker. In 1930 several farmers reported yields above 60 bushels per acre from the Pedigree 38. The heat and drouth in 1931 reduced barley yields throughout the state; the yields of Pedigree 38 reported, however, were without exception higher than those obtained with Oderbrucker. In three widely separated counties where yields of Pedigree 38 and Oderbrucker were compared on farms in the same locality, 28 farmers reported an average yield of 31.7 bushels per acre for Pedigree 38; 22 growers, an average yield of 26.7 bushels per acre for the Oderbrucker. Again in 1932, Pedigree 38 out-yielded the standard varieties. County agents and farmers in the better barley sections of the state reported around 60 bushels per acre with five reporting 70 bushels or higher.

Since the smooth-awned, hybrid selections have given high yields of good quality grain in both test plots and field trials, Wisconsin Barbless, Pedigree 38, has been accepted as the standard variety for the state.

Stripe Resistance Studies

DATA ON STRIPE infection, taken in 1927, Table VII, suggested differences in resistance to stripe. In order to study the disease reaction of these crosses under different environmental conditions, plots were sown in 1928 in South Central Wisconsin at Janesville and Madison and in the lake shore region near Milwaukee, Cleveland, and Sturgeon Bay. These locations were selected because stripe is a very important factor in barley production along the shore of Lake Michigan as well as in the vicinity of Janesville, and it was thought that the maximum amount of infection could be obtained at these places. (Fig. 3). Seed grain for the 1928 sow-

FIG. 3.—MAP OF WISCONSIN SHOWING LOCATION OF STRIPE PLOTS IN 1928 AND 1929.

ings came from the plot supplying the data in Table VII. The opportunity for infection, therefore, should have been relatively uniform among the various selections. In order further to expose the hybrids to a uniform and maximum amount of infection, a row of stripe-infected Pedigree 6 barley was sown alternately with each row of hybrids, so that there was a row of stripe-infected Pedigree 6 on either side of the hybrid selection. In all locations the same quantity of seed was used in sowing each rod row, which made counts of striped plants comparable. By using the same uniformly infected seed at the different locations, it seemed possible that any large differences from place to place in any given hybrid selection or in the check, Pedigree 6, could be attributed to environmental conditions.

TABLE VII.—NUMBER OF STRIPED BARLEY PLANTS IN THREE ROD ROWS OF BARLEY SELECTIONS GROWN AT MADISON, WIS., 1927

Selection	Number of striped plants in—			
	Series 1	Series 2	Series 3	Total
X39-9-3-4	0	0	0	0
X57-12-4-13	0	0	0	0
X57-24-6-9-3	3	0	0	3
X57-24-6-10-1	0	1	0	1
X57-24-6-10-5	0	0	0	0
X57-24-10-2-3	0	2	1	3
X57-24-6-6-4	0	1	0	1
X57-24-6-6-2	3	1	2	6
X60-2-1	1	0	3	4
X60-2-3	0	1	2	3
Pedigree 6-2	2	3	2	7
Pedigree 6-3	....	0	....	0
Pedigree 6-6	0	0	....	0
Pedigree 6-7	3	2	....	5
Pedigree 5, (controls)	4.2[a]	....	....	....

[a] Average per row for 41 rod rows distributed every fifth row throughout the plot.

TABLE VIII.—NUMBER OF STRIPE-INFECTED BARLEY PLANTS PER ROD ROW IN HYBRID SELECTIONS AND PEDIGREE 6 GROWN AT VARIOUS LOCATIONS IN 1928 FROM SEED GROWN AT MADISON, WIS. IN 1927

Hybrid selection and pedigree nos.	Janesville	Milwaukee	Madison Series 1	Madison Series 2	Madison Series 3	Cleveland	Sturgeon Bay	Total
Hybrid selections								
X39-5-8-4-1	0	0	0	0	0	0	0	0
X39-5-8-4-2	0	0	0	0	0	0	0	0
X39-5-8-4-3	0	0	0	0	0	0	0	0
X39-9-3	0	0	—	—	—	0	0	0
X39-9-3-4	0	0	—	—	—	0	0	0
X39-9-3-6-1	0	0	0	0	0	0	0	0
X39-9-3-6-2	0	0	0	0	0	1	0	1
X39-9-3-6-4	0	0	0	0	0	0	0	0
X39-9-3-6-5	0	0	0	0	0	0	0	0
X39-9-3-6-7	0	0	0	0	0	0	0	0
X39-9-3-6-8	0	0	0	0	0	0	0	0
X39-9-10V-1	0	0	0	0	0	0	0	0
X39-9-10V-5-3	0	0	0	0	0	0	0	0
X39-9-10V-5-6	0	0	0	0	0	0	0	0
X57-5-3	0	0	—	—	—	0	2	2
X57-12-4-13	0	0	—	—	—	0	1	1
X57-12-5-2-1	0	0	0	0	0	0	0	0
X57-24-6-6-4	0	0	0	2	0	0	2	4
X57-24-6-9-3	1	0	2	0	0	2	0	5
X57-24-6-10-5	0	0	1	0	0	1	2	4
X57-27-5-5	0	0	—	—	—	0	3	3
X60-2-1	0	0	—	—	—	0	1	1
X60-2-3	0	0	—	—	—	0	0	0
X66-1-3	0	0	0	0	0	0	0	0
X66-1-4	0	0	0	0	0	0	0	0
X66-1-7	0	0	0	0	0	0	0	0
X67-1-1	0	0	0	0	0	0	0	0
X69-1-2	2	0	0	0	0	0	0	2
X69-1-4	1	0	0	0	0	0	1	2
X69-2-2	0	0	0	0	1	0	0	1
X69-2-3	0	2	0	0	0	0	0	2
X69-2-6	2	0	0	0	0	0	0	2
X69-2-7	0	0	0	0	0	0	0	0
X69-2-9	1	0	0	0	1	0	0	2
X69-2-10	0	0	0	0	0	0	0	0
X102-1-5-4	1	0	0	0	0	0	0	1
X102-1-6-1	3	0	0	0	0	0	1	4
X102-1-6-2	5	1	1	0	2	2	2	13
X102-1-6-3	0	0	0	0	0	0	0	0
X102-1-8-1	1	1	0	0	0	2	1	5
X102-2-1	0	0	0	0	1	0	0	1
X102-2-2	0	0	0	0	0	0	0	0
X102-2-3	1	0	0	0	1	1	0	3
X102-2-3-4	0	0	0	0	0	0	0	0
X102-2-4-1	0	0	0	0	0	0	0	0
X102-2-4-2	1	0	0	0	0	0	1	2
X102-2-4-3	0	0	0	0	0	0	0	0
X104-1-1-1	0	1	0	0	1	0	0	2
X104-1-1-2	0	4	0	0	2	0	1	7
X104-1-1-3	1	0	0	0	1	0	0	2
X104-1-1-4	0	0	1	0	0	1	1	3
X104-1-2-1	0	0	0	0	0	0	0	0
X104-1-2-2	1	0	0	0	0	0	1	2
X104-1-2-3	0	0	0	0	0	1	1	2
X104-1-2-4	1	0	0	0	0	0	0	1
X104-1-2-5	0	0	0	1	0	0	0	1
X104-1-2-6	0	0	0	0	0	0	1	1
X104-1-3	2	0	0	0	0	1	0	3
X105-1-2-3	0	1	0	1	0	0	1	3
X105-1-4-1	0	0	0	0	0	0	0	0
X105-1-4-2	1	0	0	0	0	0	0	1

TABLE VIII.—CONTINUED

Hybrid selection and pedigree nos.	Janesville	Milwaukee	Madison Series 1	Madison Series 2	Madison Series 3	Cleveland	Sturgeon Bay	Total
Hybrid selections								
X105-1-5-1	0	1	0	0	0	0	0	1
X105-1-5-2	0	0	0	0	0	0	0	0
X105-1-5-3	1	0	0	0	0	0	0	1
X105-2-1-1	1	0	0	0	1	0	1	3
X105-2-1-2	0	1	1	0	1	2	1	6
X105-2-1-3	1	1	4	1	1	1	0	9
X105-2-2	2	0	2	0	4	4	2	14
X105-2-2-1	1	0	1	3	0	0	0	5
X105-2-2-2	3	1	0	0	0	2	1	7
X105-2-4-1	5	2	—	—	—	2	1	10
X105-2-4-2	3	2	—	—	—	6	4	15
X105-2-6-1	2	2	1	0	0	2	1	8
X105-2-6-3	4	3	0	1	1	0	1	10
X105-2-6-4	0	3	0	0	0	1	1	5
X105-2-7	2	1	3	2	0	2	1	11
X105-2-7-2	1	0	0	0	0	0	0	1
X105-2-7-3	0	0	0	0	0	0	0	0
X106-1	0	0	0	0	0	0	1	1
X106-2-1	1	1	0	1	0	0	0	3
X106-2-2	2	0	2	1	0	0	0	5
X106-2-3	2	0	1	0	0	1	1	5
X106-2-4	1	0	0	0	1	0	1	3
X106-2-5	2	0	1	0	0	1	0	4
X106-2-6	4	0	0	0	0	0	0	4
X106-2-7	1	0	0	0	0	0	0	1
Pedigree 6 Av. per rod row	16.8	8.6	15.4	16.8	16.9	26.9	28.9	—

Seed from the selections, grown at the five stations in 1928, was sown in a uniform plot at Madison in 1929 in order to test the possible effect of the several environments on infection. In this experiment, also, stripe-infected Pedigree 6 was included for comparison and for supplying inoculum.

Experimental Data and Results

At each location in the 1928 tests, there were approximately 250 plants per rod row. The stripe-infected plants were counted and the data are given in Table VIII and summarized in Table IX.

Because of insufficient seed, two lots of stripe-infected Pedigree 6 were used, one grown at Janesville in 1925, and the other grown at Janesville in 1926. The first lot was used at Janesville, Milwaukee, and Madison, and the second at Cleveland and Sturgeon Bay. Apparently, the 1926 Pedigree 6 seed was the more heavily infected, judging by the relatively greater number of striped plants developing in the controls at Cleveland and Sturgeon Bay where this seed was used.

The hybrid selections, from the presumably uniformly inoculated 1927 Madison crop, showed some differences in stripe infection at the different locations. This suggested the importance of environment on the development of the disease.

TABLE IX.—SUMMARY OF THE NUMBER OF STRIPED PLANTS OF PEDIGREE 6 AND HYBRID SELECTIONS GROWN IN ROD ROWS IN 1928 AT DIFFERENT LOCATIONS IN WISCONSIN.

Location of plots	Rod Rows of—		Striped plants in—		Average number striped plants per row	
	Ped. 6	Hybrid selections	Ped. 6	Hybrid selections	Ped. 6	Hybrid selections
	Number	Number	Number	Number		
Janesville	88[a]	86	1481	64	16.83	.74
Milwaukee	88[a]	86	760	28	8.64	.33
Madison (Av of 3 series)	78[a]	77	1276	17.61	16.35	.23
Cleveland	88[b]	86	2369	36	26.92	.42
Sturgeon Bay	88[b]	86	2545	40	28.92	.47

[a] Seed grown at Janesville in 1925.
[b] Seed grown at Janesville in 1926.

Only one striped plant occurred in the 14 selections from the X39 cross. Pedigree 5 was the pistillate parent and Leiorrhynchum the staminate in this cross. Leiorrhynchum was the pistillate parent and Pedigree 6 the staminate of hybrid X57. Of the seven X57 selections all except one showed some stripe infection. Selections of X66 with Pedigree 6 as the staminate parent failed to develop stripe in 1928, but showed susceptibility in 1929. This indicates the greater genetic resistance of Pedigree 5 over Pedigree 6. One selection from X60 showed stripe infection in 1928 while the other did not. Cross X69 was a hybrid between a selection of X57 (Leiorrhynchum X Pedigree 6) and Pedigree 5. Only two of the eight selections from X69 were free from stripe in 1928. Hybrid X102, a cross between X60-1 and Pedigree 6, showed eight selections with stripe and four with no stripe in 1928. Hybrids X104 and X105 both had Pedigree 5 and Pedigree 6 in their parentage and were similar to hybrids X66 and X67. Only one selection of X104 was free from stripe, while three in cross X105 were free. Most of the selections from X105 were very susceptible. In X106, a cross between X57-5-2 and Pedigree 6, Pedigree 6 entered into the recombination twice and all eight selections tested were infected with stripe.

These 1928 data indicate rather clearly that stripe resistance and susceptibility are inherited, although the nature of the inheritance is not shown. Genau[1] observed that, after head inoculation, varietal differences in stripe infection ranged from 0 to 60 per cent.

Environment influenced stripe development in the 1928 experiments and perhaps modified the expression of resistance to stripe. The effect of environment during the seedling stage was evident by the difference in the number of striped plants occurring at different locations where the same seed lot had been used (Table IX). About twice as much stripe occurred in both hybrids and controls at Janesville as at Milwaukee; whereas, in comparing Janesville and

TABLE X.—STRIPE-INFECTED BARLEY PLANTS PER ROD ROW IN HYBRID SELECTIONS AND ODERBRUCKER PEDIGREE 6 GROWN AT MADISON, CLEVELAND, AND MARSHFIELD, WIS. IN 1929, FROM SEED GROWN AT VARIOUS LOCATIONS IN 1928.

Hybrid selection or pedigree nos.	Number of stripe-infected plants per rod row at Madison from seed grown at—												Summary of striped plants from all locations	
	Madison		Janesville		Milwaukee		Cleveland		Sturgeon Bay		Cleveland[b]	Marshfield[b]	Total number	Per cent[c]
	S 1[a]	S 2[a]	S 1	S 2	S 1	S 2	S 1	S 2	S 1	S 2				
Ped. 6[d]	20	10	14	19	18	13	26	21	16	11	3	11	182	6.07
X39-5-8-4-1	0	0	0	0	0	0	0	0	0	0	0	0	0	.00
X39-9-3-6-1	1	1	0	0	1	1	1	1	0	0	0	0	6	.20
Ped. 6[d]	16	19	31	22	15	22	25	13	16	15	3	7	204	6.80
X39-9-3-6-4	0	1	2	0	1	0	0	2	1	0	2	0	9	.30
X39-9-3-6-5	0	1	1	0	0	2	0	2	0	2	0	2	10	.33
Ped. 6[d]	16	15	16	13	20	17	16	16	13	17	4	7	170	5.67
X39-9-3-6-7	1	0	1	0	2	0	1	1	3	0	0	1	10	.33
X39-9-3-6-8	1	1	0	0	1	6	1	2	0	0	1	0	13	.43
Ped. 6[d]	17	21	19	18	16	15	16	11	22	22	5	8	190	6.33
X39-9-10V-5-3	1	0	1	0	0	0	3	0	1	0	0	0	6	.20
X39-9-10V-5-6	2	2	0	0	0	1	0	0	1	0	0	2	8	.27
Ped. 6[d]	11	21	18	16	19	17	13	9	9	15	1	7	156	5.20
X57-24-6-6-4	4	1	8	4	3	3	10	13	2	2	1	4	55	1.88
X66-1-4	21	14	28	12	4	8	4	7	5	4	1	5	113	3.77
Ped. 6[d]	19	14	22	22	13	23	24	21	19	19	5	7	208	6.93
X69-1-2	5	5	6	11	2	2	4	2	0	1	2	2	42	1.40
X69-2-2	1	1	1	1	1	3	4	2	2	2	4	0	22	.73
Ped. 6[d]	15	20	27	21	19	16	19	14	12	18	5	12	198	6.60
X69-2-3	0	0	1	1	0	0	3	2	0	1	2	0	10	.33
X69-2-6	0	2	2	3	0	0	1	0	0	1	4	0	13	.43
Ped. 6[d]	19	18	19	20	27	13	8	17	19	17	6	2	185	6.17
X69-2-7	1	1	0	0	0	2	2	1	0	0	2	1	10	.33
X69-2-9	7	2	1	3	2	2	1	6	2	7	4	1	38	1.27
Ped. 6[d]	18	17	12	17	14	29	14	14	14	13	7	6	175	5.83
X69-2-10	1	2	0	2	1	0	0	6	1	0	1	0	14	.47
X102-1-5-4	5	3	6	4	6	8	13	4	7	8	11	4	79	2.63
Ped. 6[d]	22	18	15	14	13	14	20	19	19	7	6	10	177	5.90
X102-3-4	2	2	1	1	1	1	1	4	2	1	0	0	16	.53
X104-1-2-3	5	11	9	5	8	11	17	19	5	2	11	6	109	3.63
Ped. 6[d]	15	22	23	11	19	14	15	19	19	9	3	4	173	5.77
X104-1-2-4	7	2	4	5	2	7	5	4	1	2	8	2	49	1.63

TABLE X—CONTINUED

Hybrid selection or pedigree nos.	Number of stripe-infected plants per rod row at Madison from seed grown at—												Summary of striped plants from all locations	
	Madison		Janesville		Milwaukee		Cleveland		Sturgeon Bay		Cleveland[b]	Marshfield[b]	Total number	Per cent[c]
	S 1[a]	S 2[a]	S 1	S 2	S 1	S 2	S 1	S 2	S 1	S 2				
X104-1-2-5	1	2	2	0	0	2	1	1	2	0	2	0	13	.43
Ped. 6[d]	25	15	18	15	21	18	19	19	16	13	12	7	198	6.60
X105-2-1-3	10	10	18	1	5	11	8	5	13	15	6	5	107	3.57
X105-2-2-1	9	6	18	20	15	19	16	17	12	14	21	6	183	6.10
Ped. 6[d]	21	16	29	12	17	22	18	14	17	16	11	6	199	6.63
X105-2-2-2	6	11	25	22	14	19	22	32	11	19	19	7	207	6.90
X105-2-6-3	15	17	9	17	12	7	20	8	10	5	8	2	130	4.33
Ped. 6[d]	16	19	17	23	18	19	7	22	18	23	6	3	191	6.37
X105-2-6-4	6	8	9	15	7	13	18	15	5	7	12	1	116	3.87
X106-2-1	1	7	5	15	4	1	6	4	1	5	2	3	54	1.80
Ped. 6[d]	21	16	22	18	16	13	21	16	11	17	10	4	185	6.17
Ped. 6 Composite[e]	15	19	40	30	18	23	38	42	40	43	—	—)		
Ped. 6 Composite[e]	21	19	24	31	26	17	36	30	42	48	—	—)	1033	11.81
Ped. 6 Composite[e]	15	21	45	31	14	22	43	40	37	28	—	—)		
Ped. 6 Composite[e]	13	—	23	—	20	—	43	—	36	—	—	—)		
Striped plants in hybrid selections. Total number.	226		300		221		322		185					
Av. percentage per rod row.	3.23		4.29		3.16		4.60		2.64					
Striped plants in Pedigree 6. Total number.	123		224		140		272		274					
Av. percentage per rod row.	7.03		12.80		8.00		15.54		15.66					

[a] S-1 and S-2 = Series 1 and Series 2, respectively; [b] One series only from seed grown at Madison in 1928; [c] Percentages are based on an average of 250 plants per rod row; [d] Pedigree 6 control grown at Madison in 1928; [e] Pedigree 6 composite from seed grown in 1928 at each of the locations.

Madison the stripe infection in Pedigree 6 was practically equal. In the hybrid selections, however, there was approximately three times as much stripe at Janesville as at Madison. There was no apparent reason for the difference in the ratio of striped plants in the hybrid selections and in Pedigree 6 at the two locations. Less stripe also occurred in the hybrid selections at Cleveland and Sturgeon Bay than at Janesville. This might suggest differential environmental reaction in the various strains of barley.

The effect of high soil moisture on stripe development was observed at Janesville. Twenty rows of Pedigree 6 at one end of the plot were on lower ground, considerably higher in moisture content than the rest of the plot. In these twenty rows there was an average of 11.6 striped plants per row; whereas, the average for all rows of Pedigree 6 in the plot was 16.83. The other plots did not show differences in the percentages of stripe that could be attributed to differences in soil conditions. These results agreed with those reported by Leukel et al[3], summarized as follows: "Relatively dry soil (less

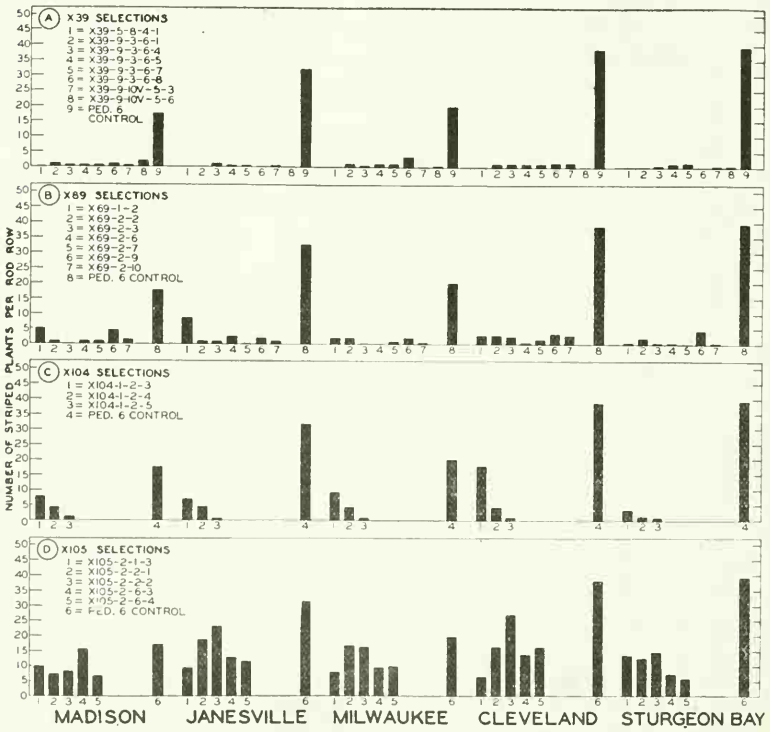

FIG. 4.—GRAPHS SHOWING THE RANGE OF STRIPE INFECTION IN THE VARIOUS SELECTIONS WITHIN THE HYBRIDS X39, X69, X104, AND X105, GROWN AT MADISON, WISCONSIN, IN 1929. THE SEED WAS PRODUCED UNDER UNIFORM CONDITIONS AT EACH OF THE FIVE STATIONS INDICATED IN 1928.

than 20 per cent saturation) during the period of emergence seemed to favor stripe development as compared with very wet soil."

Seed of the Pedigree 6 grown at each of the five locations in 1928 was bulked and sown in 1929 for comparison with 28 hybrid selections. Seed of stripe-infected Pedigree 6 grown at Madison in 1928 was sown every third row as a control. The seed grown at each of these different locations was sown in two series (S-1 and S-2) at Madison to study the effects of the different environments in 1928 upon the floral infection with the stripe fungus. In addition to this, a similar series of Pedigree 6 and hybrid selections was sown at Cleveland and Marshfield in 1929 with seed grown at Madison in 1928. The arrangements of rows and the results are given in Table X.

The X39 selections again showed a low number of striped plants per row, X39-5-8-4-1 showing no striped plants in any of the 1929 plantings. (Fig. 4A). The other selections of X39 had a total of 6 to 13 plants for the 12 rod rows or approximately 0.0 to 0.43 per cent. The X57 selection was more susceptible having 1.88 per cent stripe. The selection of hybrid X66 from Maidson and Janesville seed was heavily infected but was somewhat less so from other locations. And again, the selections of hybrid X69 were uniformly low in stripe infection from all locations. (Fig. 4B). One of the selections from hybrid X102 was higher than the other selection in stripe infection at all of the locations. One of the selections from the X104 cross was uniformly low in infection at all stations and one was intermediate. On the other hand, selection X104-1-2-3 was higher at all stations with a marked increase in infection in the seed produced at Cleveland. (Fig. 4C). Infection in hybrid X105 was higher than in any other hybrid and the selections showed some variation in infection from the different seed sources. The reactions of the X105 selections grown at the five locations are shown graphically in figure 4D. Infection in several of the selections parallels that in Pedigree 6 while others fail to show any such correlation. This is illustrated by comparing X105-2-2-2, practically as susceptible as the control, with X105-2-1-3, fairly resistant. The two selections were infected about equally when grown at Madison under the conditions where they had been selected; but, when grown at Janesville and Cleveland, differences occurred that were large enough to appear significant. The infection in the X105 selections at Sturgeon Bay was practically the same as that at Madison. A comparison of infection in the various strains grown in the different sections of the state suggests, insofar as resistance to stripe is concerned, a less stable condition of some strains under different environmental complexes.

The effect of environment during germination and seedling development was emphasized again in comparing the amount of stripe obtained from uniformly infected seed grown at Madison and sown at Madison, Cleveland, and Marshfield in 1929. The Pedigree 6 grown at Cleveland developed less than one-third the stripe that oc-

curred at Madison. The infection developing at Marshfield like that at Cleveland was also very low. In contrast with this, a number of the hybrids increased in the amount of stripe under Cleveland conditions and two of them actually had more than the checks. (Fig. 5). Under Marshfield conditions, an unusually low infection developed during the spring of 1929 which may be partly explained by the warm weather prevailing at the late date of planting (May 14).

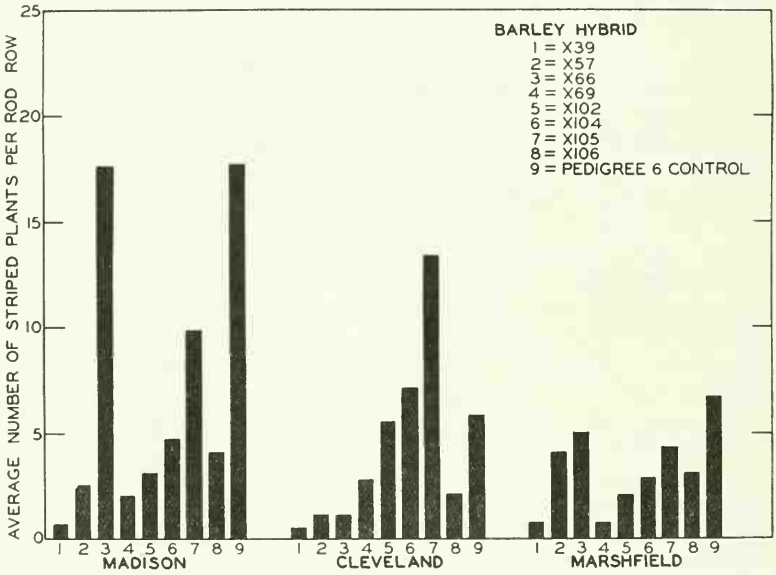

FIG. 5.—GRAPH SHOWING THE AVERAGE NUMBER OF STRIPE-INFECTED PLANTS PER ROD ROW FOR ALL SELECTIONS WITHIN EACH HYBRID AND PEDIGREE 6 GROWN IN 1929, AT LOCATIONS INDICATED, FROM SEED GROWN IN 1928 UNDER UNIFORM CONDITIONS AT MADISON.

TABLE XI.—THE EFFECT OF ENVIRONMENT ON STRIPE DEVELOPMENT. UNIFORMLY INFECTED 1928, MADISON SEED WAS GROWN IN 1929 AT MADISON, CLEVELAND AND MARSHFIELD.

	Average % per rod row of stripe-infected plants at		
	Madison	Cleveland	Marshfield
Hybrid selections	5.23	1.77	0.77
Pedigree 6	7.03	2.32	2.69

The effect of environment upon floral infection is well illustrated in comparing the range of stripe infection in Pedigree 6 grown at the different locations. (Fig. 6). For example, the amount of infection at Madison and Milwaukee was rather low, that at Janesville intermediate, and that at Cleveland and Sturgeon Bay about equally high. Since there was an abundance of striped plants at all places

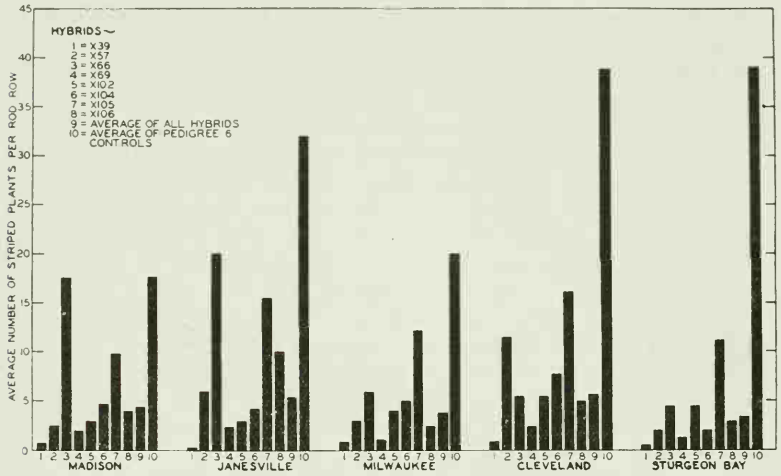

FIG. 6.—GRAPH SHOWING THE AVERAGE NUMBER OF STRIPED PLANTS PER ROD ROW FOR ALL SELECTIONS WITHIN EACH HYBRID AND PEDIGREE 6 GROWN IN 1929 AT MADISON FROM SEED GROWN IN 1928 AT THE STATIONS INDICATED.

in 1928, the difference in infection must have been due largely to environmental conditions at the different locations, although sufficient weather data were not obtained to explain the differences. Figure 6 shows that the average number of striped plants per row for all hybrid selections follows the general trend of that for the average number of striped plants in Pedigree 6, except at Sturgeon Bay. The average of striped plants per row in Pedigree 6 from Cleveland and Sturgeon Bay was 38.9 and 39.1 respectively; whereas, the averages of striped plants from all hybrid selections were 5.75 from Cleveland and 3.30 from Sturgeon Bay. The reason for the lower amount of disease in the hybrid selections at Sturgeon Bay is not apparent, unless there is a difference in the physiology of the infection in the hybrids and Pedigree 6 or a marked difference in the period of development when the two become most susceptible to stripe infection under that particular environment.

Preliminary Artificial Inoculations

Winkelmann[5] infected barley artificially by dusting spores on the heads which were covered with parchment bags to maintain a high humidity. He was able to get infection over a period of four weeks after flowering, the most effective period being shortly after flowering.

A few of the most resistant hybrid selections and Pedigree 6 were inoculated artificially in 1928. Conidial suspensions were prepared from stripe-infected plants and applied to the heads by different methods and at different stages of development of the heads. Most of the inoculations were made over the period from blossoming to the

soft dough stage. To determine if a high humidity previous to inoculation would increase the percentage of infection, one-half of the heads were moistened with tap water and covered with glassine bags for 24 hours before they were inoculated; the other half were given no previous treatment. Two methods of inoculation were used: (1) spraying the conidial suspension into the flowers with an atomizer; (2) dipping the heads into a conidial suspension. In all instances the heads were covered with glassine bags following inoculation and left covered until mature. The seed was harvested and sown in the greenhouse and in the field at Madison in 1929 to determine the amount of stripe infection in each seed lot.

Spraying the heads with water and placing them in glassine bags one day prior to inoculation had no appreciable effect upon infection: 119 kernels from heads bagged yielded 29 per cent striped plants in contrast with 32 per cent striped plants from heads not previously bagged before inoculation. Both the spraying and the dipping methods of inoculation were effective in producing infection.

By artificial inoculation, the resistance of the smooth-awned selections as shown under field conditions was confirmed. All of the inoculated heads of Pedigree 6 gave much higher percentages of stripe infection than the naturally infected adjacent heads, averaging 30 per cent in the former in contrast with 7 per cent in the latter. There were 250 kernels from similar inoculations of X39 selections and 8.9 per cent of them produced striped plants. Likewise, 108 inoculated kernels of X57 selections gave 4.6 per cent diseased plants. The natural infection of adjacent plants of these hybrid selections averaged less than 1 per cent which was, as in Oderbrucker, Pedigree 6, considerably less than the artificially inoculated plants. These results indicated that while the smooth-awned selections were not immune to the stripe disease, they were highly resistant.

Discussion

IN THE DEVELOPMENT of a smooth-awned, white, hybrid from crosses of the Pedigreed Oderbrucker with the black smooth-awned Leiorrhynchum barley, several interesting characters appeared among the economically important selections.

A detailed study has shown a wide variation in reaction to stripe disease, some of the hybrids being nearly immune and some as susceptible as the Oderbrucker parent. The highest yielding selections were invariably four to five days later in maturity than the Oderbrucker. Although this character is usually unfavorable to a crop in years of summer drouth, the Pedigrees 37 and 38 yielded equally well or better than the Oderbrucker in the dry season of 1931. While the hull is slightly darker in color, this characteristic is not objectionable unless intensified by weathering. The smoothness and flexibility of the awn, with a somewhat looser hull than the Oderbrucker, have made threshing without peeling difficult. Preliminary tests

of malting quality indicate that they are equal to Oderbrucker, which is the standard for malting quality. The Pedigrees 37 and 38 have consistently outyielded the Oderbrucker, frequently the increased yield being as much as 10 to 20 per cent. Reports from County Agents and farmers indicate that Wisconsin Barbless, Pedigree 38, has almost displaced other barley varieties in the state during the past four years due to increased yields and stripe resistance.

The data obtained in the stripe resistance studies indicate a range from susceptible to highly resistant selections with environment influencing the percentage of infection. The X39 selections, which include Pedigree 38, are highly resistant to stripe, and one selection of this group has been free from stripe for three successive years under all conditions to which it has been subjected. When any of the X39 and X57 selections were crossed back to either of the Oderbrucker parents, the susceptibility to stripe was increased. Selections from such back crosses closely approached the Oderbrucker type in appearance and ranged in stripe reaction from those fairly resistant to those almost as susceptible as the Oderbrucker parents. (Figs. 4A and 4D). The stripe infection in a given selection seems to be correlated with environment during the flowering and seedling stages of plant development. The environment apparently affects the hybrid selections differently than Oderbrucker, Pedigree 6, as shown by differences in stripe infection at Cleveland and Sturgeon Bay. The results emphasize the necessity for a more complete study under controlled conditions of the influence of environment upon (1) floral infection and (2) progress of infection during germination and seedling growth.

Certain stable lines selected from crosses of Oderbrucker X Leiorrhynchum are resistant to barley stripe, the selections varying from highly resistant to susceptible. Environment plays an important and complex rôle in stripe development both at the period of floral infection and in the seedling stage. A given line fluctuates widely in stripe infection and stripe development under different environmental conditions. The same environment seems to affect stripe development differently in the various selections.

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