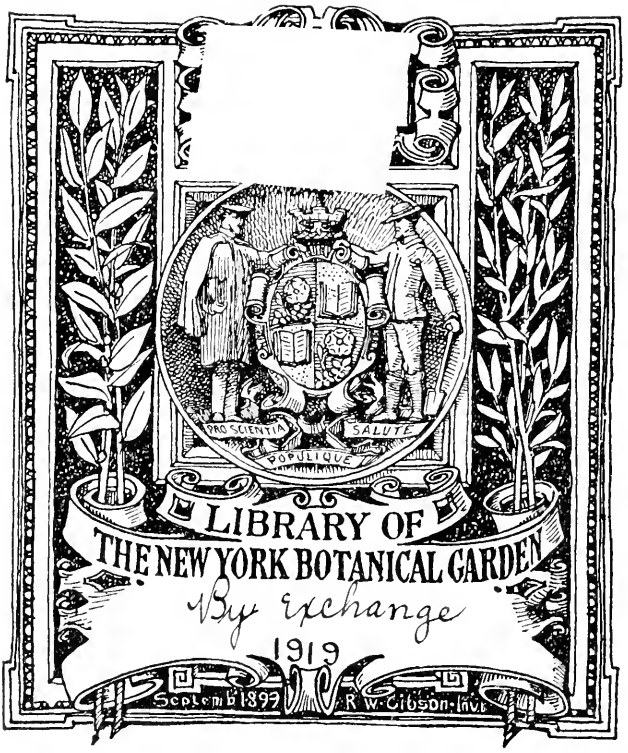
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JOURNAL OF AGRICULTURAL RESEARCH

VOLUME XVI

JANUARY 6—MARCH 31, 1919

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN
AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

EDITORIAL COMMITTEE OF THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from State Experiment Stations should be addressed to H. P. Armsby, Institute of Animal Nutrition, State College, Pa.

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ERRATA AND AUTHORS' EMENDATIONS

Page 103, line 15, "C I 2879" should read "C I 5879."

Page 104, line 19, "C I 4103" should read "C I 4013."

Page 161, line 31, "*A. labena* n. sp." should read "*Labena* n. sp."

Page 168, the cuts of figures 1 and 2 should be transposed.

Page 229–244, throughout the paper "*Pegomyia affinis* Stein." should read "*Pegomyia vanduze* Mallock 1919."

Page 248, Table III, column 2, line 11, "4¾" should read "3¾."

Page 252, Plate 31, A, should be inverted.

Page 260, Table I, the figures in column 2 should read, from top to bottom, "1.39, 1.94, 1.89, .24, .23, .21, .17, .19, .21, .55"; the figures in column 3 should read ".50, .10, .17, .05, .10, .11, .10, Trace, .03, .10."

Page 261, line 10, "0.27" should read "0.26" and "0.17" should read "0.08."

Page 280, paragraph 2, line 15, "41, B" should read "40, A."

Page 285, paragraph 2, line 5, "38, D" should read "38, C."

Page 286, paragraph 3, line 11, "tube" should read "tuber."

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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from State Experiment Stations should be addressed to H. P. Armsby, Institute of Animal Nutrition, State College, Pa.

JOURNAL OF AGRICULTURAL RESEARCH

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DETERMINATION OF ACIDITY AND TITRABLE NITROGEN IN WHEAT WITH THE HYDROGEN ELECTRODE

By C. O. SWANSON, *Associate Chemist*, and E. L. TAGUE, *Assistant Chemist, Department of Chemistry, Kansas Agricultural Experiment Station*

Acidity in wheat flour is usually determined by making a water extract of the flour at a definite temperature for a definite time and titrating this extract with a standard alkali, using phenolphthalein as an indicator. Since the quantity of alkali neutralized is governed to a considerable extent by the temperature and duration of extraction, a large number of workers extract at 40° C. for two hours. This method can also be used for wheat (*Triticum aestivum*), the grain being first finely ground.

Because wheat contains the enzym phytase, as has been shown by several investigators,[1] it is to be expected that the duration of extraction and the temperature used will influence the amount of standard alkali neutralized.

Acidity in wheat or wheat flour is not due to the presence of free acids as that term is ordinarily understood. The varying amounts of alkali neutralized in different samples are supposed to be due to the presence of phosphates in less or greater amounts. This statement is supported by the fact that the greater the acidity the greater the amount of phosphorus in the extract.[2]

Water extracts of wheat and wheat flour as ordinarily made are slightly turbid. This turbidity depends to some extent on the nature of the flour. Extracts from wheat and low-grade flour give clearer extracts than those from high-grade flour. This is probably due to the presence of greater amounts of electrolytes in the ground wheat and low-grade flour, as these would help to coagulate the turbid or colloidal matter.

Because of the turbidity and the colloidal nature of wheat and flour extracts, absorption plays a part in the determinations of acidity when the colorometric method is used.

[1] ANDERSON, R. J. CONCERNING THE ORGANIC PHOSPHORUS COMPOUND OF WHEAT BRAN AND THE HYDROLYSIS OF PHYTIN. N. Y. State Agr. Exp. Sta. Tech. Bul. 40, 31 p. 1915.

[2] SWANSON, C. O. ACIDITY IN WHEAT FLOUR; ITS RELATION TO PHOSPHORUS AND TO OTHER CONSTITUENTS. *In* Jour. Indus. and Engin. Chem., v. 4, no. 4, p. 274–278. 1912.

METHOD OF MAKING THE EXTRACT

A good grade of Kansas hard wheat was used for this work. The wheat was finely ground, untempered in a burr mill. Fifty gms. of this ground material were weighed into a quart Mason jar and heated to the temperature used in making the extraction. Five hundred cc. of carbon-dioxide-free water, previously heated to the temperature employed, were then added, together with 5 cc. of toulene as a preventive of bacterial action. The whole was thoroughly shaken and placed in a thermostat. The shaking was repeated every minute for the first 5 minutes, then every 15 minutes during the time of extraction. At the end of the extraction period the contents of the jar were poured into centrifuge cups and centrifuged for 5 minutes at 2,000 revolutions per minute. The supernatant liquid was then poured through a filter for the purpose of removing light floating particles. The filtrate was used for the following determinations:

1. The hydrogen-ion concentration or the P_H value of the extract.
2. The cubic centimeters of *N/20* barium hydroxid used to titrate to the absolute neutral point of P_H 7.
3. The cubic centimeters of alkali used to titrate to the point of color change for phenolthalein or P_H8.3.
4. The cubic centimeters of alkali used to titrate to the point of color change for thymolphthalein or P_H9.3.
5. The amount of alkali necessary to reneutralize after the addition of neutral formaldehyde or the Sörensen method of determining amino nitrogen.
6. The total phosphorus in the extract.
7. The phosphorus in the extract precipitated by magnesia mixture. This may be considered phosphorus in the inorganic form. This method of determination is based on that used for the determination of inorganic phosphorus in animal tissues.

APPARATUS USED IN DETERMINING HYDROGEN-ION CONCENTRATION

Our apparatus contains the following pieces: One Kohlrausch slide wire bridge; one type B, No. 2500 Leeds and Northrup galvanometer; one Weston millivoltmeter and multiplier; Edison storage cells; and the hydrogen and the normal calomel electrodes made according to the directions of Hildebrand.[1] Hydrogen made by the electrolytic process was used. As a precaution against impurities, the gas was washed in a train of alkaline permanganate and pyrogallic acid.

[1] HILDEBRAND, JOEL H. SOME APPLICATIONS OF THE HYDROGEN ELECTRODE IN ANALYSIS, RESEARCH, AND TEACHING. *In* Jour. Amer. Chem. Soc., v. 35, no. 7, p. 847-871, 15 fig. 1913.

PERIODS OF TIME AND TEMPERATURES USED

After some preliminary work the following periods were chosen: 5, 30, and 60 minutes, 2, 4, 8, 16, and 24 hours. Extractions were made at the following temperatures: 5°, 20°, 40°, and 50° C. Other temperatures and periods were tried in the preliminary work, but the results obtained were found of no added value.

METHOD OF DETERMINING THE HYDROGEN-ION CONCENTRATION

One hundred cc. of the solution prepared as above—namely, the extract of 10 gm. of ground wheat—were pipetted into the hydrogen-ion cell. This is closed with a rubber stopper through which the electrodes, as well as the tip of the burette, are inserted. In this way the carbon dioxid from the air is excluded. The hydrogen gas is bubbled through until equilibrium is reached. The reading of the voltmeter gives the figure for calculating the actual hydrogen-ion concentration of the solution. To facilitate this calculation, we have made a table giving the hydrogen-ion concentration corresponding to the volt readings from 0.281 to 1.090. It takes about an hour to obtain equilibrium, but this can be shortened by previously saturating the electrode with hydrogen.

Barium hydroxid (*N/20*) was then added till the solution showed a volt reading of 0.686, indicating a hydrogen-ion concentration of P_H 7. This represents the absolute neutral point. The alkali was again added till the volt reading was 0.760, indicating a P_H value of 8.3. This corresponds to the acidity as commonly determined by the use of phenolphthalein as indicator. In several trials this indicator was added at this point, and gave the usual color change. The alkali was again added until the volt reading was 0.820, indicating a P_H value of 9.3. This is the point of color change of thymolphthalein, and this was actually determined by the use of the indicator. At this point 25 cc. of formaldehyde solution were added. This was made by mixing one part of 40 per cent formaldehyde with two parts of carbon-dioxid-free water and neutralizing to the hydrogen-ion value P_H 9.3 before using. The hydrogen gas was bubbled through until equilibrium was reached, and the P_H value noted. Barium hydroxid (*N/20*) was then added until a P_H value of 9.3 was again reached.

HYDROGEN-ION CONCENTRATION OF WHEAT EXTRACT

The results obtained by these methods at temperatures varying from 5° to 50° C., and for periods varying from 5 minutes to 24 hours are given in Table I.

TABLE I.—*Hydrogen-ion concentration of wheat extracts made at different temperatures and periods, together with the quantity of N/20 barium hydroxid used in the titrating to change the hydrogen-ion concentration*

Temperature.	Time.	Volt reading.	P_H values.	Quantity of N/20 barium hydroxid used to titrate to—				
				0.686 or P_H 7.	0.760 or P_H 8.3.	0.820 or P_H 9.3.	Volt reading after adding formaldehyde.	0.820 or P_H 9.3 after adding formaldehyde.
°C.				Cc.	Cc.	Cc.	Cc.	Cc.
5	5 minutes	0.663	6.60	0.5	1.3	2.8	0.778	1.5
5	30 minutes	.661	6.57	.5	1.6	3.1	.785	1.5
5	60 minutes	.666	6.65	.4	1.5	3.0	.790	1.4
5	24 hours	.661	6.57	1.5	5.5	9.0	.778	2.2
20	5 minutes	.662	6.58	.8	2.1	3.3	.763	2.2
20	30 minutes	.662	6.58	1.1	3.1	4.9	.761	2.9
20	1 hour	.667	6.67	1.3	4.5	7.3	.765	3.1
20	2 hours	.663	6.60	1.5	5.3	8.8	.768	3.5
20	4 hours	.661	6.57	2.2	7.9	11.8	.770	3.7
20	8 hours	.661	6.57	3.4	9.3	13.5	.762	4.4
20	16 hours	.661	6.57	3.4	14.3	19.0	.770	4.7
20	24 hours	.656	6.48	4.5	16.8	22.4	.770	4.9
40	5 minutes	.650	6.36	1.9	3.9	5.4	.780	1.7
40	30 minutes	.655	6.46	3.4	7.4	10.7	.771	3.3
40	1 hour	.654	6.44	4.4	11.1	15.7	.772	3.7
40	2 hours	.643	6.26	4.9	14.3	19.1	.762	4.3
40	4 hours	.654	6.44	5.2	17.3	22.4	.772	4.1
40	8 hours	.653	6.43	5.7	17.9	23.8	.776	4.0
40	16 hours	.651	6.39	6.3	18.0	23 9	.776	4.1
40	24 hours	.654	6.44	6.9	18.2	24.1	.780	4.2
50	5 minutes	.651	6.39	3.2	6.5	9.0	.762	3.5
50	30 minutes	.652	6.41	3.5	10.4	14.9	.770	3.6
50	1 hour	.653	6.43	4.0	13.7	18.2	.774	3.1
50	2 hours	.648	6.34	5.8	16.3	21.4	.774	3.2
50	4 hours	.655	6.46	5.3	16.4	21.5	.780	3.0

The data in Table I show the following results:

1. The average hydrogen-ion concentration of the extracts obtained at 5° C. is P_H 6.60; at 20° C. it is P_H 6.59; at 40° C. it is P_H 6.43; and at 50° C. it is P_H 6.52. Thus, the higher temperature gives an extract of slightly higher hydrogen-ion concentration.

2. The hydrogen-ion concentration does not increase with the duration of digestion. The average volt readings at 40° C. for 5 minutes, 30 minutes, and 1 hour is 0.653, corresponding to P_H 6.43. The average volt readings for 8, 16, and 24 hours are also 0.653, corresponding to P_H 6.43. The longer or shorter period of digestion does not increase nor decrease the hydrogen-ion concentration. The slight variations obtained at 20° and 50° for the different periods are so small that they do not modify the above statement.

3. A volt reading of 0.760 (P_H 8.3) corresponds to the point of color change of phenolphthalein. The reading 0.820 (P_H 9.3) corresponds to the point of color change for thymolphthalein. This was determined by adding these indicators at the points mentioned.

4. The amount of *N/20* of barium hydroxid used to titrate to P_H 8.3 is greater than the amount used to titrate to P_H 7, and the amount used to titrate to P_H 9.3 is greater than the amount used to titrate to P_H 8.3, and these differences show a progressive increase as the time of digestion is increased from 5 minutes to 24 hours.

5. The values obtained at 20° C. are lower than those at 40° and at 50°. At 5° they are the lowest. At this temperature very little or no hydrolysis takes place. Hydrolysis, as well as proteolysis, is most active at 40°.

6. The amount of *N/20* barium hydroxid required to titrate to P_H 7 at 40° C. increased but little after 16 hours. In titrating to P_H 8.3 or P_H 9.3 there is very little increase after 4 hours. For example, hydrolysis is slightly slower at 20° than at 40°, and also slower at 40° than at 50°.

7. At the end of 4 to 8 hours at 40° C., when hydrolysis is practically complete, three times the number of cubic centimeters are required to titrate to P_H 8.3 as are necessary to titrate to P_H 7, and four times as many are required to titrate to P_H 9.3.

8. The number of cubic centimeters used to determine the titrable nitrogen after the addition of formaldehyde shows a progressive increase with the duration of digestion, but the maximum is reached in about two hours, at 40° C. At 20° the increase is much slower and continues to the end of 24 hours. At 50° the final results obtained are lower than those at either 20° or 40°, but show no increase or decrease corresponding with the time. At 5° there is very little increase from 5 minutes to 24 hours. The number of cubic centimeters required to titrate to a definite point were only about one-third as many as those required to titrate to the same point at 40°.

9. The most outstanding result shown in Table I is the fact that, while the hydrogen-ion concentration shows no increase with the duration of digestion, the amount of *N/20* barium hydroxid used to neutralize to a given hydrogen-ion concentration increases in proportion to the duration of the time of digestion. A definite limit, however, is soon reached. This limit is reached soonest at the highest temperature.

10. The digestion of ground wheat in water produces a substance which is not ionized; yet it will neutralize definite amounts of standard hydroxid, and these quantities correspond to a certain extent with the duration and temperature of digestion. When the standard alkali is added, it is ionized and the quantity present can be determined by the method of titration.

The results obtained with various concentrations at 5°, 20°, and 40° C. are graphically presented in figures 1, 2, and 3.

Figure 1 shows the results of titrating to P_H 7. At 20° the increase is slow and gradual. At 40° it is very rapid up to two hours, and then it is very gradual.

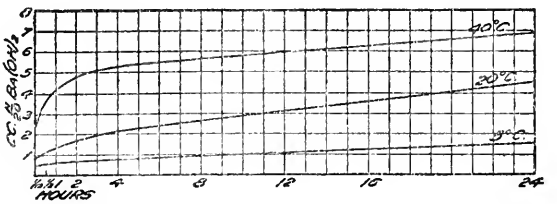

FIG. 1.—Graphs showing the hydrogen-ion concentration of wheat extract on titration to P_H 7.

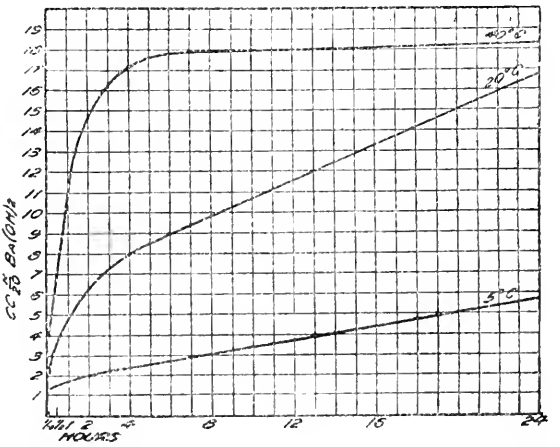

FIG. 2.—Graphs showing the hydrogen-ion concentration of wheat extract on titration to P_H 8.3.

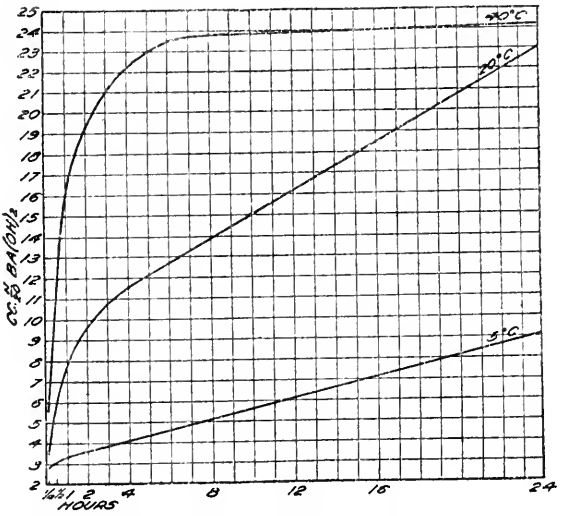

FIG. 3.—Graphs showing the hydrogen-ion concentration of wheat extract on titration to P_H 9.3.

Figure 2 shows the results of titrating to P_H 8.3. At 5° the amount of standard alkali required increases very slowly. At 20° the increase is very rapid up to four hours, followed by a gradual increase up to 24 hours. At 40° the increase is most rapid up to four hours. After that the increase is slight or practically none. The final results at 20° and 40° are not much different.

Figure 3 shows the results of titrating to P_H 9.3. The general direction of the curves are similar to those in figure 2, except that the values are higher.

DETERMINATION OF NITROGEN IN AMINO FORM BY FORMALDEHYDE METHOD

Some investigators neutralize to litmus [1] and others to phenolphthalein [2] before adding the formaldehyde. In Table I the results obtained by neutralizing to three points: P_H 7, P_H 8.3, and P_H 9.3, are given. That the latter two are the points of color change for phenolphthalein and thymolphthalein, respectively, in these extracts, was determined experimentally. At P_H 9.3 the formaldehyde was added and then the titration repeated until the concentration was again P_H 9.3. The results of this last titration are given in the last column of Table I. The amounts given times 0.7 gives the milligrams of nitrogen in the amino form. If the formaldehyde had been added at P_H 8.3 and then the titration continued to the P_H 9.3, a larger amount of *N/20* barium hydroxid would have been neutralized. Still larger amounts would have been neutralized if the formaldehyde had been added at the strictly neutral point, P_H 7, and then the titration continued to the P_H 9.3 point. These amounts are given in Table II, which has been calculated from Table I.

TABLE II.—*Quantity (in cubic centimeters) of N/20 barium hydroxid required had the neutralization been made to the points indicated before the formaldehyde was added*

Duration of extraction.	First neutralization to PH 8.3.			First neutralization to PH 7.		
	5° C.	20° C.	40° C.	5° C.	20° C.	40° C.
	Cc.	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
5 minutes	3.0	3.4	3.2	3.8	4.7	5.2
30 minutes	3.0	4.7	6.6	4.1	6.7	10.6
1 hour	2.9	5.9	8.3	4.0	9.1	15.0
2 hours	.....	7.0	9.1	.....	10.8	18.5
4 hours	.....	7.6	9.2	.....	13.3	21.3
8 hours	.....	8.6	9.9	.....	15.3	22.1
16 hours	.....	9.4	10.0	.....	20.3	23.9
24 hours	5.7	10.5	10.1	9.7	22.8	21.4

[1] ALLEN'S COMMERCIAL ORGANIC ANALYSIS, v. 8, p. 479. Philadelphia, 1918.

[2] ABDERHALDEN, EMIL, ED. HANDBUCH DER BIOCHEMISCHEN ARBEITSMETHODEN. Bd. 3, Hälfte 1, p. 228. Berlin, Wien, 1910.

The figures given under first neutralization to P_H 8.3 are obtained as follows: The number of cubic centimeters of *N/20* barium hydroxid obtained by titrating to P_H 8.3 are subtracted from the number of cubic centimeters obtained by titrating to P_H 9.3. This difference is added to the figures in the last column of Table I. The figures so obtained are assumed to be the same as if the formaldehyde had been added at the concentration P_H 8.3 and then the titration resumed till the concentration P_H 9.3 was obtained. The figures under first neutralization to P_H 7 were calculated in the same way except that the differences between the number of cubic centimeters in the columns under P_H 7 and P_H 9.3 in Table I were used.

The calculations made on these assumptions show that the "formol" or titratable nitrogen obtained by first titrating to P_H 8.3 and then adding the formaldehyde is over twice that obtained by titrating first to P_H 9.3 and then adding the formaldehyde. And further, if the formaldehyde is added at P_H 7 and the titration is then continued to the concentration P_H 9.3 the amount is over four times as great.

The results obtained by this method of calculation raises the question, To what point of concentration should the solution be titrated before the formaldehyde is added? To throw light on this point an extract from wheat digested at 40° C. for four hours was used. It was prepared as the other extracts used in this investigation. This was then titrated to the points P_H 7, P_H 8.3, and P_H 9.3, first without adding any formaldehyde, and second by adding the formaldehyde before starting the titration.

Corrections were made for the differences in volume of these two. These corrections were 0.1 and 0.2 cc. The following results were obtained (Table III).

TABLE III.—*Quantity of N/20 sodium hydroxid required for neutralization with and without formaldehyde*

Treatment	Quantity (in cubic centimeters) of *N/20* sodium hydroxid used to titrate to—		
	P_H 7	P_H 8.3	P_H 9.3
Adding the formaldehyde before starting the titration	7.6	14.5	18.0
Titrating without formaldehyde	4.9	11.2	14.6
Increase due to formaldehyde	2.7	3.3	3.4

One hundred-cc. portions of extract from the wheat prepared in the same way were then used to see what differences would be obtained if the formaldehyde was added after the titration had been made to the following concentrations: P_H 7, P_H 8.3, and P_H 9.3.

ADDING FORMALDEHYDE AT P_H 7

Total quantity (cc.) of *N/20* sodium hydroxid to neutralize to P_H 7.	4.9
Total quantity (cc.) of *N/20* sodium hydroxid to neutralize again to P_H 7 after adding formaldehyde	2.7

ADDING FORMALDEHYDE AT P_H 8.3

Total quantity (cc.) of *N/20* sodium hydroxid to neutralize to P_H 8.3	11.3
Total quantity (cc.) of *N/20* sodium hydroxid to neutralize again to P_H 8.3 after adding formaldehyde	3.3

ADDING FORMALDEHYDE AT P_H 9.3

Total quantity (cc.) of *N/20* sodium hydroxid to neutralize to P_H 9.3	14.8
Total quantity (cc.) of *N/20* sodium hydroxid to neutralize again to P_H 9.3 after adding formaldehyde	3.4

This shows that slightly higher results are obtained when the formaldehyde is added at the higher concentrations and the solution is titrated again to the same concentrations.

From data obtained in connection with some other work the following figures are added. The figures represent the number of cubic centimeters of *N/20* alkali needed to neutralize after the addition of formaldehyde, the titrations having first been made to the concentrations shown.

Concentration.	Flour A.	Flour B.	Flour C.
P_H 7	2.7	2.5	3.0
P_H 8.3	3.4	2.7	3.1
P_H 9.3	3.1	2.4	2.9

One question not settled by the data given in this paper is to what point should the titration be carried after the addition of the formaldehyde. This question is reserved for future work.

On the basis of the above discussion we may say that the results in Table I show the following in regard to amino nitrogen:

1. At 5° C. there is practically no increase in the amount of amino nitrogen as the time of digestion is increased.

2. At 20° C. the amount of amino nitrogen reaches the maximum shortly after 8 hours.

3. At 40° C. the amount of amino nitrogen reaches the maximum at 2 hours.

4. The calculated results in Table II show a gradual increase in the amount of amino nitrogen to the end of the 24 hours with one minor exception at 40° C. The results on amino nitrogen are graphically presented in figure 4.

PHOSPHORUS IN THE WHEAT EXTRACTS

As previously mentioned, determinations were made of total phosphorus and of phosphorus precipitated by magnesia mixture.

From each extraction mixture two portions of 50 cc. each, representing 5 gm. of ground wheat, were pipetted into beakers. Ten cc. of concentrated nitric acid were added and boiled until all the organic matter was destroyed, more nitric acid being added as needed. The residue was used for the determination of phosphorus in the usual way.

To two other 50-cc. portions of the extraction mixture there were added 40 cc. of magnesia mixture, and after standing for 15 minutes, 25 cc. of concentrated ammonia. After a thorough stirring the beakers

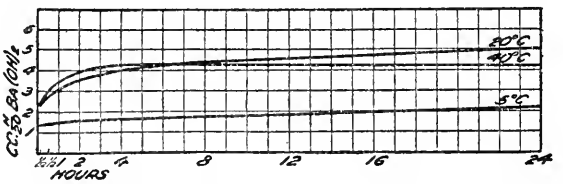

FIG. 4.—Graphs showing the production of amino nitrogen at different temperatures.

were allowed to stand overnight, and the contents were then filtered and the precipitate washed four times with 2 per cent ammonium hydroxid. The precipitate was then dissolved in 40 cc. of dilute (1:4) nitric acid and the filter washed with 100 cc. of hot water. The filtrate and washings were then boiled, in order to destroy organic matter, and the phosphorus determination was completed in the usual way. For convenience, this will be called inorganic phosphorus. Whether or not that is the case may be questioned, and the merits of this method of determination are not discussed here. We simply used this method as one best suited to our purpose, and the results obtained (Table IV) are used for their comparative value.

TABLE IV.—*Determination of phosphorus in wheat extract*

Temperature.	Duration of extraction.	Total percentage of phosphorus in the extract.	Percentage of inorganic phosphorus in the extract.
°C.			
5	5 minutes	0.018	0.019
5	30 minutes	.029	.020
5	1 hour	.027	.020
5	24 hours	.083	.070
20	5 minutes	.044	.031
20	30 minutes	.063	.031
20	1 hour	.067	.050
20	2 hours	.106	.062
20	4 hours	.159	.083
20	8 hours	.173	.092
20	16 hours	.208	.098
20	24 hours	.190	.113
40	5 minutes	.099	.032
40	30 minutes	.183	.116
40	1 hour	.210	.146
40	2 hours	.240	.177
40	4 hours	.253	.212
40	8 hours	.254	.223
40	16 hours	.257	.25
40	24 hours	.259	.24
50	5 minutes	.162	.109
50	30 minutes	.215	.151
50	1 hour	.236	.172
50	2 hours	.254	.220
50	4 hours	.258	.196

1. It will be noted that at 50° C. the total and inorganic phosphorus increases with the time of digestion.

2. At 20° C. the total and inorganic phosphorus increases with the time of digestion, but the total phosphorus does not show any increase after 16 hours. The ratio between the total and inorganic phosphorus varies considerably, but the latter averages a little more than one-half of the total.

3. At 40° C. the total and inorganic phosphorus increases with the time of digestion, but the maximum for both total and inorganic phosphorus is reached at about 4 hours. The proportion of inorganic phosphorus in relation to the total is much greater than at the lower temperature. After four hours the inorganic phosphorus is almost equal to the total.

4. At 50° C. the maximum of both total and inorganic phosphorus is reached in about two hours. The results are graphically presented in figures 5 and 6.

Why does hydrogen-ion concentration remain the same or show no increase with the increased time of digestion, while the amount of *N/20* barium hydroxid necessary to bring the solution to the concentration

P_H 7, P_H 8.3, or P_H 9.3 shows a constant increase corresponding with the time of digestion, and also an increase in the total and inorganic phosphorus? The only explanation that we have to offer at this time is that the extract of wheat contains a definite amount of the hydrogen ion,

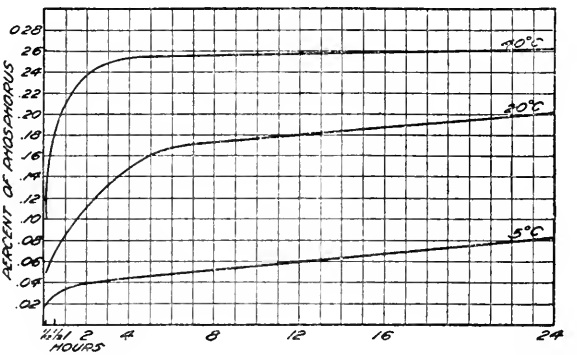

FIG. 5.—Graphs showing the total phosphorus in wheat extractat different periods of extraction and at different temperatures.

and that this amount is not increased during the hydrolysis of the wheat because the phosphates which are produced during digestion are of such a nature that they undergo very small ionization in the water. When, however, the hydroxid is added, they undergo ionization. The percentage of ionization in these phosphorus compounds must be very small.

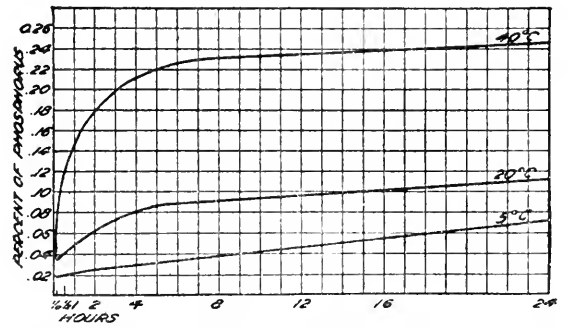

FIG. 6.—Graphs showing the inorganic phosphorus in wheat extract at different periods of extraction and at different temperatures.

SUMMARY

(1) This paper presents the results of a study in determining, by means of the hydrogen electrode, the different hydrogen-ion concentrations in the extract of ground wheat. The total phosphorus and the phosphorus precipitated with magnesia mixture were also determined in the extract.

(2) Extractions were made at the following temperatures: 5°, 20°, 40°, and 50° C.; and for the following periods: 5 and 30 minutes; 1, 2, 4, 8, 16, and 24 hours.

(3) The temperature at which the extraction was made was found to have but little influence upon the hydrogen-ion concentration. The higher temperatures give an extract of but slightly higher concentration.

(4) The duration of the digestion period did not influence the hydrogen-ion concentration. The average hydrogen-ion concentration when the extraction was made for 5 minutes, 30 minutes, and 1 hour was the same as when the extraction was made for 8, 16, and 24 hours.

(5) But while the hydrogen-ion concentration of the extract shows no increase with the duration of the digestion, the quantity of *N/20* barium hydroxid necessary to add in order to change the concentration to a definite point was greater in amount and within certain limits proportionate to the duration of the digestion.

(6) The substances produced when wheat is digested in water are not ionized until an alkali has been added. The amount of these substances produced bears a definite relation to the time and temperature used in digestion. A limit, however, is soon reached, and this limit is reached sooner at the highest temperature.

(7) The amino nitrogen as determined by the Sörensen formaldehyde method is all extracted in two hours at 40° C.

(8) At 20° C. the amount of phosphorus in the extract precipitated by magnesia mixture averages about half of the total. At 40° practically all of the total phosphorus is converted into forms that are precipitated by the magnesia mixture.

(9) The hydrogen-ion concentration of the water extract of wheat is definite in amount. This concentration is not changed during the extraction in proportion to the time. The reason for this is that the conditions for ionization are not present until an alkali is added. When, however, this is added, ionization takes place and the amount of standard alkali necessary to add in order to lower the hydrogen-ion concentration to a given point bears a proportionate relation to the temperature and duration of the digestion period.

ASH ABSORPTION BY SPINACH FROM CONCENTRATED SOIL SOLUTIONS

By RODNEY H. TRUE, *Physiologist in Charge*, OTIS F. BLACK, *Chemical Biologist*, and JAMES W. KELLY, *Laboratory Technician, Office of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

In 1915 when the authors were engaged in a study of the possible causes of spinach-blight, the theory was advanced that the disease was a form of "malnutrition" due, in a measure, to the accumulation of an excess of fertilizer salts in the soil solution.

FERTILIZER SUBSTANCES USED

In the hope of reproducing the symptoms seen, beds of spinach (*Spinacia oleracea*) grown on the grounds of the Virginia Truck Experiment Station,[1] in cooperation with which this work was carried on, were given large applications of the commoner fertilizer salts, singly and in the usual mixtures. The so-called "acid mixture" was that generally employed by the truck farmers of the Norfolk region for use in their spinach fields. The "basic mixture" was made up of substances which would likely be neutral or basic in the soil. The constituents of each were approximately as follows:

ACID MIXTURE.	Pounds.	BASIC MIXTURE.	Pounds.
Ammonium sulphate	340	Sodium nitrate	450
Acid phosphate	830	Basic slag from Birmingham, Ala.	720
Potassium muriate	170	Potassium sulphate	170
Dried blood	260	Dried blood	260
Tankage	400	Tankage	400

Single salts were supplied in two proportions: One was intended to be near the maximum; the other to cause clear injury. The substances applied are listed below in terms of pounds per acre:

SUBSTANCES.	QUANTITY USED.
Calcium carbonate	3 and 6 tons per acre.
Magnesium carbonate	1 and 2 tons per acre.
Potash	750 and 1,500 pounds per acre.
Sodium nitrate	Do.
Sodium chlorid	Do.
Sodium sulphate	Do.

[1] The authors are indebted to the Director of the Virginia Station and to his assistants for help in many ways. To Mr. J. A. McClintock, at that time Plant Pathologist of the Station, they owe an especial debt for careful notes made from time to time and for help rendered in other ways.

SUBSTANCES—continued.	QUANTITY USED.
Acid phosphate	1,000 and 2,000 pounds per acre.
Complete mixture:	
Acid	2,000 and 4,000 pounds per acre.
Basic	Do.
Stable manure	20 and 40 tons per acre.

Control plots alternated with those receiving treatment.

The land used for planting had not been used for spinach for many years previously and was in good condition. It had received excellent treatment for several years and seemed to be very uniform in all respects.

Each bed was 5 feet wide and 61 feet long, and received four rows of seed. The chemicals were applied on July 29, 1915, and immediately hoed into the soil. The land was kept free of weeds until September 11, when curled Savoy spinach seed was drilled in. In 10 days the stand could be reasonably well determined, and since the variation with the treatment was clearly seen, the result may be summarized here.

PROGRESS OF PLANTS IN THE FIELD

The "best" stand was seen in the beds receiving stable manure; "very good" in beds receiving magnesium carbonate; "good" in those with calcium carbonate, acid phosphate, sodium sulphate, and basic complete mixture; "scattering" only in those with potash, sodium nitrate, sodium chlorid, and complete acid mixture. The controls were in the class designated "Good," a few in "Very good."

Owing to the unsatisfactory stand in some of the beds, all were reseeded on September 23, and irrigated. On September 30 plants began to appear, a thick stand being seen by October 6. The usual cultivation and thinning took place about a fortnight later.

From notes taken on the beds as they appeared on October 27, certain outstanding features may be developed. On taking completeness of stand, growth, and color as criteria, the treated plots were distributed in the following groups:

Excellent: basic complete mixture.

Very good (equal or better than the best control plots): acid phosphate, sodium sulphate (heavier treatment), magnesium carbonate, manure.

Good (equal to the poorer control plots): calcium carbonate, sodium sulphate (lighter treatment).

Poor (poorer than controls): complete acid mixture, sodium chlorid, potash, sodium nitrate.

The poor plots were marked by a yellowish-green color, poor growth, and death of some of the seedlings, giving a bad stand. The greatest injury was seen in those parts of the plots receiving the heavier treatment,

Another review of the plots was made on December 1, 1915, with similar results.

The "best" plot in the whole experiment was that receiving the basic complete mixture.

Those having acid phosphate and sodium sulphate were "excellent."

"Good" would be said of plots receiving magnesium carbonate; somewhat less so, were those receiving calcium carbonate, which gave a very deep green color, and manure, especially the part of the bed receiving the lighter application.

The four plots found to be "poor" were those having sodium chlorid, sodium nitrate, and acid complete mixture. Poorest of all was potash. All poor plots were alike in having a crusted soil surface, with a suggestion of moisture.

At this stage samples were taken from several of the beds for ash analysis at Washington.

ASH DETERMINATIONS

The plants, after being divided into roots and tops, were ashed in an electric furnace at a low red heat of approximately 600° C. The total ash being determined, the chief constituents were worked out by the methods recommended by the Association of Official Agricultural Chemists.[1]

The results are given in Table I. In the first section the results are calculated as percentages of the air-dry weight of the plant material, while in the second section the individual constituents are calculated as percentages of the total ash.

TABLE I.—*Ash constituents of spinach*

CALCULATED AS PERCENTAGES OF DRY MATERIAL

Fertilizer.	Sodium nitrate.		Sodium chlorid.		Sodium sulphate.		Potassium chlorid.		Calcium carbonate.		Control.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
Total ash	19.94	8.87	21.81	8.58	20.70	8.30	17.67	7.40	21.39	9.40	20.68	8.01
Silica (SiO_2)	4.56	1.77	3.80	1.46	4.72	1.61	4.30	1.48	7.97	3.16	5.79	1.48
Manganous oxid (Mn_3O_4)	.035	.14	.02	.14	.02	.045	.03	.05	.03	.07	.025	.06
Lime (CaO)	1.11	.29	1.13	.45	.83	.30	.94	.30	1.26	.45	1.14	.30
Magnesia (MgO)	1.13	.47	1.44	.55	1.33	.41	1.68	.56	1.14	.49	1.38	.34
Potash (K_2O)	5.22	3.25	3.68	1.94	9.11	2.91	6.56	2.44	7.20	2.97	8.60	3.44
Soda (Na_2O)	3.50	.86	5.67	1.42	.80	1.28	1.53	.54	1.70	.45	.91	.33
Sulphur trioxid (SO_3)	.44	.21	.47	.23	.76	.32	.44	.15	.47	.23	.52	.33
Phosphorus pentoxid (P_2O_5)	1.03	.92	1.22	1.16	1.29	1.13	1.13	.98	.98	1.03	1.33	.80
Alumina (Al_2O_3)	.61	.30	.57	.20	.62	.24	.49	.18	.52	.45	.63	.26
Ferric oxid (Fe_2O_3)	.13	.07	.09	.06	.08	.06	.07	.05	.11	.07	.09	.06
Total	17.705	8.28	18.09	7.61	19.56	8.305	17.17	6.73	21.38	9.37	20.415	7.40

[1] WILEY, H. W., ED. OFFICIAL AND PROVISIONAL METHODS OF ANALYSIS, ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. U. S. Dept. Agr. Bur. Chem. Bul. 107 (rev.). 271 p., 13 fig. 1908.

TABLE I.—*Ash constituents of spinach*—Continued

CALCULATED AS PERCENTAGES OF DRY MATERIAL—continued

Fertilizer.	Acid phosphate.		Complete acid.		Complete basic.		Manure.		Average.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
Total ash	21.39	13.40	20.59	10.48	18.60	10.01	21.73	11.40	20.45	9.58
Silica	7.14	5.43	5.40	2.78	4.01	2.31	4.47	2.47	5.21	2.39
Manganous oxid	.04	.09	.05	.05	.03	.04	.016	.28	.029	.096
Lime	1.13	.38	.96	.43	.97	.40	.84	.49	1.031	.38
Magnesia	1.33	.49	1.33	.56	1.34	.75	1.59	.69	1.37	.53
Potash	6.40	1.76	8.69	3.36	8.70	3.97	9.76	4.56	7.39	3.06
Soda	2.15	.99	.78	1.58	.66	.40	.45	.70	1.82	.85
Sulphur trioxid	.53	.28	.58	.35	.53	.31	.48	.40	.52	.28
Phosphorus pentoxid	1.47	.84	1.12	1.26	1.32	1.49	1.08	1.36	1.20	1.10
Alumina	.70	.40	.65	.33	.45	.32	.51	.36	.58	.304
Ferric oxid	.09	.08	.10	.08	.10	.09	.11	.15	.097	.077
Total	20.98	10.74	19.66	10.78	18.11	10.08	19.306	11.46	19.247	9.067

INDIVIDUAL CONSTITUENTS CALCULATED AS PERCENTAGES OF THE TOTAL ASH

Fertilizer.	Sodium nitrate.		Sodium chlorid.		Sodium sulphate.		Potassium chlorid.		Calcium carbonate.		Control.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
Total ash	19.94	8.87	21.81	8.58	20.70	8.30	17.67	7.40	21.39	9.40	20.68	8.01
Silica	22.57	19.95	17.42	17.02	22.80	19.40	24.34	20.00	37.26	33.62	28.00	18.48
Manganous oxid	.18	1.58	.09	1.63	.10	.54	.17	.68	.14	.74	.12	.75
Lime	5.57	3.27	5.18	5.24	4.01	3.61	5.32	4.05	5.89	4.89	5.51	3.75
Magnesia	5.67	5.30	6.60	6.41	6.43	4.94	9.51	7.57	5.33	5.21	6.67	4.24
Potash	26.18	36.64	16.87	22.61	44.01	35.06	37.13	32.97	33.66	31.60	41.59	42.95
Soda	17.55	9.70	26.00	16.55	3.82	15.42	8.66	7.30	7.95	4.89	4.40	4.12
Sulphur trioxid	2.21	2.37	2.11	2.68	3.62	3.86	2.55	2.03	2.20	2.46	2.51	4.12
Phosphorus pentoxid	5.17	10.37	5.59	13.52	6.23	13.61	6.40	13.24	4.58	10.96	6.43	9.99
Alumina	3.06	3.38	2.61	2.33	3.00	2.89	2.77	2.43	2.43	4.89	3.05	2.25
Ferric oxid	.65	.80	.41	.70	.39	.72	.40	.68	.51	.74	.44	.75
Total	88.91	93.36	82.88	88.69	94.41	100.05	97.25	90.95	99.65	100.00	98.72	91.40

Fertilizer.	Acid phosphate.		Complete acid.		Complete basic.		Manure.		Average.	
	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.	Tops.	Roots.
Total ash	21.39	13.40	20.59	10.48	18.60	10.01	21.73	11.40	20.45	9.58
Silica	33.38	40.52	26.23	26.53	21.56	23.08	20.57	21.66	25.48	24.95
Manganous oxid	.19	.67	.24	.48	.16	.40	.07	2.46	.14	1.00
Lime	5.28	2.84	4.66	4.10	5.22	4.00	3.87	4.30	5.04	3.97
Magnesia	6.22	3.66	6.46	5.34	7.20	7.49	7.32	6.05	6.70	5.53
Potash	29.92	13.13	42.20	32.06	46.77	39.66	44.91	40.00	36.14	31.94
Soda	10.05	7.39	3.79	15.08	3.55	4.00	2.07	6.14	8.90	8.87
Sulphur trioxid	2.48	2.09	2.82	3.34	2.85	3.10	2.21	3.51	2.54	2.92
Phosphorus pentoxid	6.87	6.27	5.44	12.02	7.10	14.89	4.97	11.05	5.87	11.48
Alumina	3.27	2.99	3.16	3.15	2.42	3.20	2.35	3.16	2.84	3.17
Ferric oxid	.42	.60	.49	.76	.54	.90	.51	1.32	.47	.80
Total	98.08	80.16	95.49	102.86	97.37	100.72	88.85	99.65	94.12	94.63

In order to bring out more clearly the relationships involved in Table I, graphs have been prepared in which quantities are indicated on a uniform scale. In figure 1 appear the values found for the tops, and in figure 2 similar values for the roots. Each unit on the perpendicular axis represents 0.1 per cent of dry weight in every case.

TOTAL ASH

A casual inspection of these results reveals the fact that the total ash content of the tops calculated as percentage of dry weight, while showing considerable variation, is always greatly in excess of that of the roots, in obedience to the general rule.[1] As regards the influence of specific substances on the total ash absorption certain coincidences may be noted. The total ash reached its minimum in both roots and stems in the plot treated with potash. It is depressed in the tops nearly as much in the presence of the basic mixture, to a less degree in the roots. In general, the quantity of ash constituents is less for the roots in the plots treated with sodium salts and in the untreated control than in the other plots.

Of the single salts calcium carbonate alone goes with a total ash in the roots, approaching that seen in the mixtures of several salts. This depressing action of the sodium salts is not seen in the ash content of the tops. Acid phosphate goes with the highest total ash seen in any sample of roots; sodium chlorid and manure, with the highest totals seen in the tops.

INDIVIDUAL ASH CONSTITUENTS

In examining the quantities of the different ash constituents seen in Table I it will be noted that in some cases there is a great variation among the different plots; in others little difference is to be seen. Those showing great variation are silica, potash, and soda; those showing little change with the change in outside conditions are lime, magnesia, phosphorus pentoxid, sulphur trioxid, manganous oxid, alumina, and ferric oxid. It seems as though all plants were able to absorb these from the soil to a point of steady equilibrium without much regard to the substances offered. It appears that even when an excess of any of the ions present in this latter group of compounds is present no considerable increase in the absorption of these ions takes place.

The quantity of ions absorbed from the group of variable salts seems much more subject to influence from the added salts. In some cases the ions present in excess are themselves absorbed in greater quantity. This seems to be the case with sodium-nitrate and sodium-chlorid plots, in which considerably greater quantities of soda appear in the ash of the tops than in any other plots. Sodium sulphate, however, gives no

[1] PALLADIN, W. PFLANZENPHYSIOLOGIE. Bearb. aufgrund der 6. russischen aufl. p. 88. Berlin, 1911.

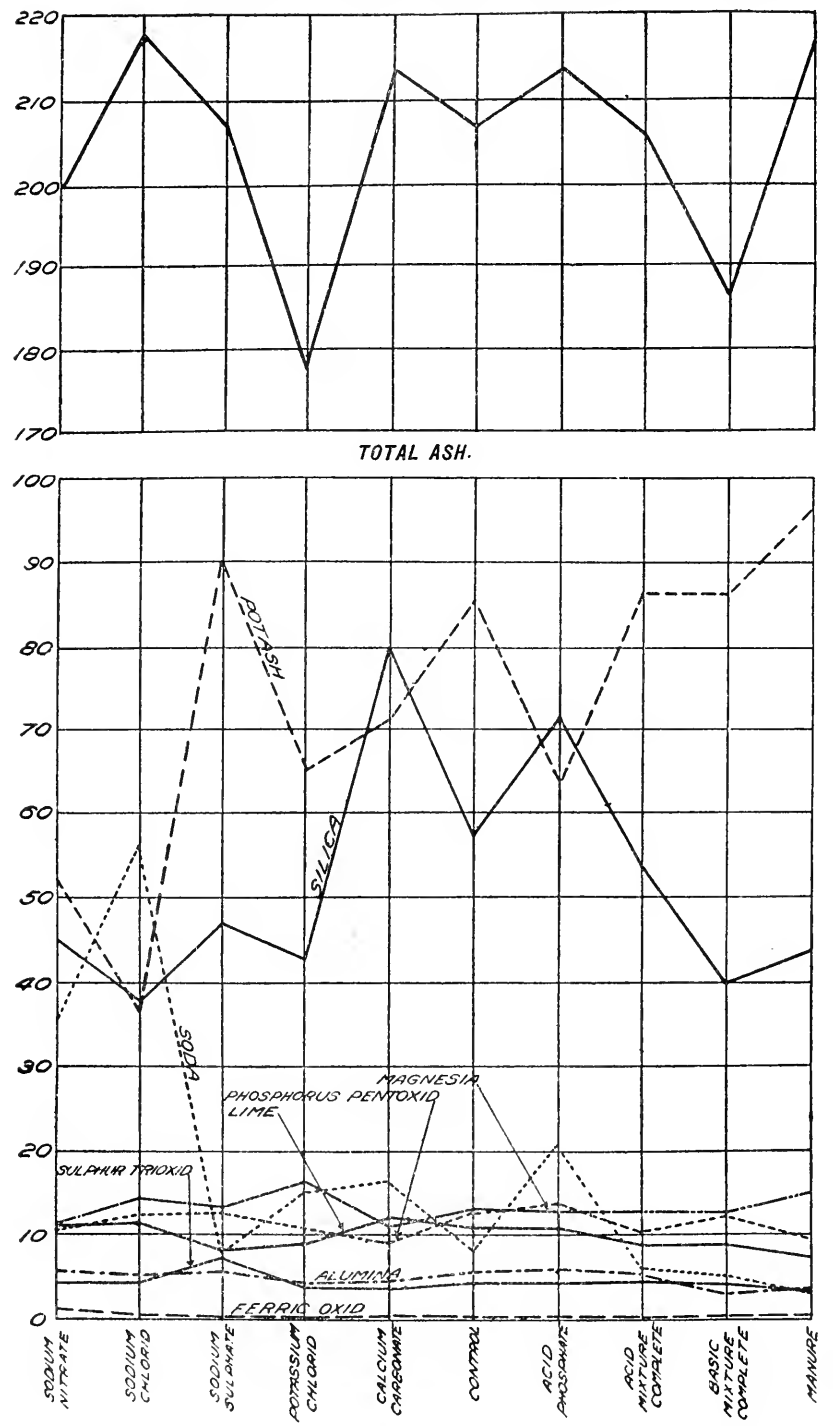

FIG. 1.—Graphs representing total ash and individual ash constituents found in spinach tops from plots receiving the substances indicated.

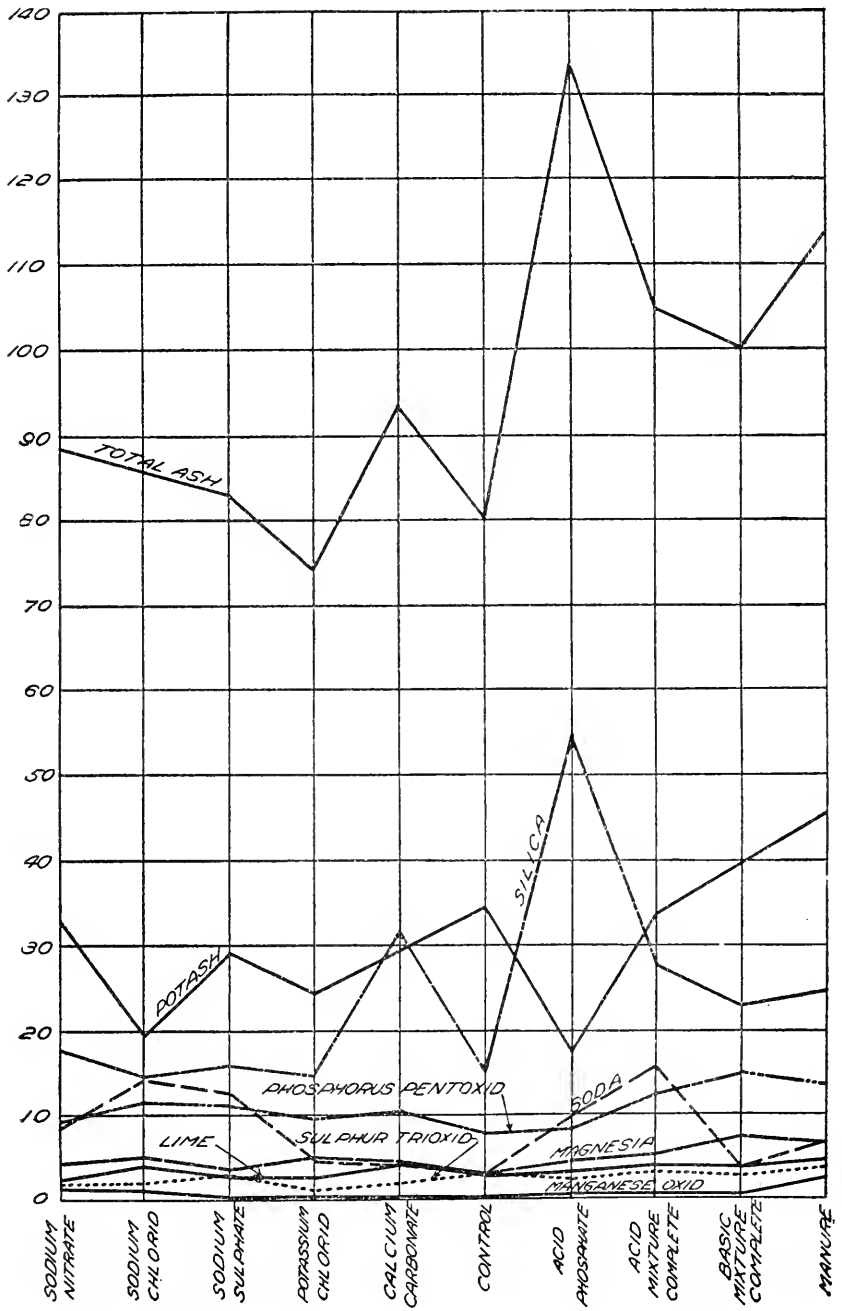

FIG. 2.—Graphs representing total ash and individual ash constituents in spinach roots from plots receiving the substances indicated.

such result. The greater absorption of sulphur trioxid occurs in the only plot treated with great quantities of sulphates, but the increase is not great.

In some cases a marked increase in a given constituent accompanies the presence in excess of some other ion. This is seen in the plot treated with sodium sulphate, in which potassium absorption in both root and top is high. A similar result is seen in the plots treated with stable manure and with both acid and basic complete mixtures. On the other hand, potassium absorption is decreased in the acid-phosphate plot in both tops and roots. Potassium chlorid in excess also accompanies a reduction of potash in the ash of both parts of the plant.

Silica is greatest in plots receiving calcium carbonate and acid phosphate, lowest in those dosed with sodium salts, and in the control plot.

It is interesting to note that, although the manganous oxid content when referred to dry weight is small in all cases, it is consistently higher in the roots than in the tops, acid complete mixture excepted, this being the only constituent which is not more abundant in the tops than in the roots.

RATIOS BETWEEN PAIRS OF CONSTITUENTS

In a number of cases there seemed to be some evidence that a roughly reciprocal relation exists between pairs of constituents. This tendency was most marked in the plots receiving mixtures of salts. Thus, in general, when silica was high, potash was low in both tops and roots.

The silica-potash ratios, as worked out for the different plots, are summarized in Table II.

TABLE II.—*Silica-potash ratio in ash of spinach plants. Silica (SiO_2)=1*

Fertilizer.	Potash (K_2O).	
	Tops.	Roots.
Sodium nitrate	1.16	1.84
Sodium sulphate	1.93	1.81
Sodium chlorid	.95	1.33
Potassium chlorid	1.53	1.65
Calcium carbonate	.90	.94
Acid phosphate	.90	.32
Complete acid	1.61	1.21
Basic complete	2.17	1.72
Manure	2.19	1.85
Control	1.49	2.32

It will be noted that, while both of these constituents belong to the group of the more variable ones, the ratio is less variable; silicia being unity, potash lies between 1.00 and 2.00 in a majority of cases. In plots receiving heavy doses of calcium-containing fertilizers (calcium carbonate and acid phosphate) as well as in the tops from the sodium-chlorid plot, the silica exceeds the potash. Again, potash has high

relative values, especially in the tops, in the plots to which the complete fertilizers were applied.

The relation of potash and soda is of particular interest in view of the halophytic nature of the spinach plant. It is perhaps worth noting in this connection that the sodium-potassium ratio varies over a wider range, something to be expected perhaps in view of the greater variability in the quantities of these constituents present. In Table III these ratios are given, soda being unity, the values of potash appearing in the appropriate columns.

TABLE III.—*Soda-potash ratio in spinach ash. Soda* (NaO_2)=1

Fertilizer.	Potash (K_2O).	
	Tops.	Roots.
Sodium nitrate	1.49	3.78
Sodium sulphate	11.53	2.27
Sodium chlorid	.65	1.37
Potassium chlorid	4.29	4.52
Calcium carbonate	4.24	6.46
Acid phosphate	2.98	1.78
Acid complete	11.14	2.13
Basic complete	13.18	9.92
Manure	21.70	6.51
Control	9.45	10.43

It will be seen that in a broad way, when the potash is high in the ash, soda is low, and vice versa. Potash, however, is always higher than soda, the excess being greater in the leaves than in the roots. High potash seen in the plot receiving sodium sulphate is accompanied by a very low soda content. The same is seen in the control as well as in both acid and basic complete mixtures and in stable manure. High soda is seen in the plots treated with sodium chlorid, in which potash reaches its minimum, and in that receiving sodium nitrate. The control culture and that receiving acid phosphate show the same less strikingly. Potash is in greatest relative excess in the plots receiving the complete fertilizers.

These results suggest the possibility that sodium may be able to perform some functions in the plant which are usually performed by potassium. It seems likely that by giving proper mixtures of the alkalis it might be possible without detriment to the plant to get along with less of the expensive potassium constituent, thus protecting the potassium in the soil.

In view of the fact that calcium is usually found in the ash of a great majority of plants in considerably greater quantity than magnesium[1] it is of interest to note the quantities found in these spinach plants.

[1] LOEW, Oscar. LIMING OF SOILS FROM A PHYSIOLOGICAL STANDPOINT. *In* U. S. Dept. Agr. Bur. Plant Indus. Bul. 1, p. 9-35. 1901.

It appears from data scattered through Wolff's [1] tables that in some cases the magnesium content exceeds that of calcium, especially in the beet family. In the leaves of these plants the calcium usually much exceeds magnesium, while the reverse holds for the roots. In the analysis given by Wolff [2] lime exceeds magnesia in spinach in an approximately 2 to 1 ratio. In Table IV the calcium-magnesium ratio is shown for each experimental plot.

TABLE IV.—*Calcium-magnesium ratio in ash of spinach plants. Lime (CaO)=1*

Fertilizer.	Magnesia (MgO).	
	Tops.	Roots.
Sodium nitrate	1.02	1.62
Sodium sulphate	1.60	1.37
Sodium chlorid	1.27	1.23
Potassium chlorid	1.79	1.87
Calcium carbonate	.90	1.07
Acid phosphate	1.18	1.29
Acid complete	1.39	1.30
Basic complete	1.40	1.87
Manure	1.89	1.41
Control	1.21	1.13

It will be noted that magnesia exceeds lime in every case, regardless of the nature of the substances applied, except in that of the tops in the plot receiving lime, in which the lime exceeds the magnesia. Spinach seems to be even a more pronounced user of magnesium than the sugar beet. The field notes show that the plot receiving magnesium carbonate was somewhat better than that receiving calcium carbonate. Unfortunately no sample from the magnesium-carbonate plot was ashed.

SUMMARY

Spinach plants grown on the grounds of the Virginia Truck Experiment Station at Norfolk in beds given heavy treatments of fertilizer salts, singly and in mixtures, gave best results in plots receiving a complete mixture having a basic or neutral character in the soil (sodium nitrate, basic slag, and potassium sulphate); next best with acid phosphate and with sodium sulphate; poor in plots receiving heavy treatments of sodium chlorid, sodium nitrate, and acid complete mixture (1 to 2 tons per acre); poorest with potassium chlorid.

A study of the ash showed the highest total ash in the tops in plots with sodium chlorid, calcium carbonate, acid phosphate, and manure; lowest with potassium chlorid and basic complete mixture. The highest ash was in roots accompanied with acid phosphate and manure, the lowest with potassium chlorid and sodium salts. General excellent condition of

[1] WOLFF, E. ASCHEN-ANALYSEN. T. 2, p. 42–50. Berlin. [2] WOLFF, E. OP. CIT, p. 128.

the crops does not parallel high ash absorption, the best and poorest plots having plants with low ash.

Ash constituents fall into two groups: (1) those present in quantities that show relatively little variation whatever be the chemicals added to the soil—lime, magnesia, phosphorous pentoxid, sulphur trioxid, manganous oxid, alumina, and ferric oxid; and (2) those which show great fluctuations in the quantity present—silica, potash, and soda.

In the first group the plants seemed to be able to get the required quantity of constituents mentioned from the soil of all plots studied whatever was offered in excess, and reached an equilibrium that was little affected by the varying conditions.

In the second group wide variations occur, sometimes with an increase of the ions offered in excess, as in sodium chlorid and sodium nitrate, sometimes by the absorption of something else, as increase in silica in plots receiving calcium carbonate and acid phosphate.

Manganous oxid is the only constituent regularly present in greater proportion in the roots than in the tops.

In some cases the high absorption of one constituent is accompanied by the low absorption of another, and vice versa. Such reciprocal pairs are silica and potash, soda and lime, and potash and magnesia. The silica-potash ratio is relatively steady. When silica equals 1, potash varies between 1.16 and 2.18 in the tops and between 1.33 and 2.32 in the roots, except when the substance added to the soil is high in calcium, when the value of potash becomes less than unity in both tops and roots.

The soda-potash ratio is much more variable, being always more than 1 in both tops and roots. When mixtures of salts are added to the soil, potash rises to very high relative values.

There is a suggestion that sodium may perform some functions also performed by potassium, indicating the possibility that sodium might in part replace potassium in fertilizers.

The calcium-magnesium ratio in spinach, both in leaves and in roots, is exceptional in having a value greater than unity. The only exception is seen in the tops of plants receiving a heavy treatment with calcium carbonate. This fact seems to suggest the practical importance of magnesium salts as fertilizers for spinach.

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Vol. XVI JANUARY 13, 1919 No. 2

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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., JANUARY 13, 1919 NO. 2

NITRATES, NITRIFICATION, AND BACTERIAL CONTENTS OF FIVE TYPICAL ACID SOILS AS AFFECTED BY LIME, FERTILIZER, CROPS, AND MOISTURE

By H. A. NOYES, *Research Associate in Horticultural Chemistry and Bacteriology*, and S. D. CONNER, *Associate Chemist in Soils and Crops, Purdue University Agricultural Experiment Station*

INTRODUCTION

The decay of organic matter and the transformation of nitrogen from one chemical combination to another were known and studied long before bacteria were isolated. These phenomena were attributed to purely chemical agencies until the discovery of the function of soil bacteria proved them to be almost entirely due to microorganic life. Most investigations in soil bacteriology have dealt with either the products of bacterial activities without reference to the number of organisms present or with only an enumeration of the bacteria present in the soil. This paper presents the results of an investigation taking into consideration both nitrates and bacterial numbers, as well as a correlation of the two, under certain specific conditions.

HISTORICAL REVIEW

NITRIFICATION

The difficulties attendant upon keeping an adequate supply of available nitrogen in the soil are so great that those bacterial activities which have to do with nitrate formation are important and have been extensively studied. As early as 1660 Digby (*2*)[1] mentioned the value of nitrates in agriculture. He attributed the growth of plants to the "nutritional and attractional" powers of a "nitrous salt." Many agricultural writers of the early part of the nineteenth century followed the lead of Liebig, who claimed that nitrogen was not needed as a soil amendment. In 1856 Boussingault and Ville (*8*) independently published experimental results which proved that nitrates are markedly beneficial to plant growth, but it was not until 21 years later that Schloesing and Müntz (*22*) demonstrated that nitrification in the soil was due to organized ferments and does not take place in the absence of these ferments.

[1] Reference is made by number (italic) to "Literature cited, p. 41–42."

Nitrification in soils is dependent upon several different factors, and chemists have not entirely agreed as to the conditions necessary for it to take place. It was early observed that calcareous material was necessary for the preparation of niter beds. Thouvenel (*4*) in 1787 found chalk and carbonate of lime to favor nitrification more than a number of earths and other chemicals. From the accumulated evidence that carbonate of lime increased nitrate formation and the fact that acid forest soils often contained no nitrates the conclusion was reached by many investigators that nitrification did not take place in an acid soil. In 1891 Warington (*25, p. 51*) said:

> A further condition of nitrification is the presence of a base with which the nitric acid when formed may combine. This condition is quite essential. Nitrification can only take place in a feebly alkaline medium.

A little later in 1894 Dehérain (*8*) (*p. 360*) made the following statements:

> The nitric ferment does not act in an acid medium . . . . . It is true that nitrification may go on in soil deficient in lime . . . . . Moreover, the application of carbonate of lime to such soils is very beneficial and increases the production of nitrates.

NITRATES IN ACID SOILS

Twenty-two years before Warington (*26*) stated that nitrification could only "take place in a feebly alkaline medium" Houzeau (*12*), in 1872, reported nitrification in an acid soil. In 1908 Hall, Miller, and Gimingham (*11*) found nitrates in an acid soil, but believing that nitrification could not take place in an acid medium, they atrributed the phenomena to the probable presence in the soil of small isolated particles of calcium carbonate. Since 1908 several workers have reported nitrification in acid soils. In 1913 Petit (*21*) found pronounced evidence of such a condition, while the same year Abbott, Conner, and Smalley (*1*) reported the presence of large amounts of nitrates in an excessively acid soil. The water extract of the soil was acid in reaction and contained considerable aluminium. The next year Temple (*23*) reported nitrification in acid or nonbasic soils. White (*26*) in 1915 from investigations on some unlimed and limed plots at the Pennsylvania Station found that nitrification was very active in many very acid areas. White remarks that—

> These results are entirely contrary to the general belief that nitrification ceases on very acid soils.

Since nitrification is the result of oxidation reactions and due to bacteria, it is affected by soil moisture and aeration. Schlösing (*8*), in 1868, found that rapid loss of nitrates occurred when a moist "humic soil" was kept in an atmosphere of nitrogen gas. Warington (*25*) in experiments at Rothamsted in 1880 found that saturating ordinary soil with water caused it to rapidly lose the nitrates it contained. Kellner (*14*) in 1891 and Kelley (*13*) in 1914 found that flooded rice fields contained little or no nitrates.

BACTERIAL NUMBERS

The conditions under which studies of the number of bacteria present in soils have been made have varied to such an extent that generalizations rather than specific correlations have resulted. Chester (*6*) was the first to note that applications of lime increased the bacterial content of soils. He concluded that the favorable action was not—

> due to any direct action of the lime, but due to the more favorable reaction which the lime gave the soil.

Later Fabricius and Feilitzen (*10*), Engberding (*9*), and Brown (*5*) reported increased bacterial numbers as the result of liming. Engberding showed that in most cases a lack of lime accounted for low bacterial counts.

Kossowicz (*16*) summarizes the results of investigations by Houston, Th. Remy, Fabricius, and Feilitzen and C. Hoffman, as follows:

> Manuring brings about an increased bacterial content and betters the conditions for the development of those organisms already present in the soil. The time of the year and weather conditions influence the bacterial content of the soil.—TRANSLATION.

Koch (*15*), Adametz (*18*) and others have shown that the majority of soil microflora consist principally of rod-shaped organisms. That anaerobic bacteria are present in great numbers has been shown by Ucke (*24*), who found over 13,000,000 anaerobes present in a garden soil.

Löhnis (*18*) states that the multiplication of soil organisms varies with different soil layers, and the number of bacteria present decreases with the depth, air and food being the first considerations.

PRESENT INVESTIGATIONS

Many uncontrolled conditions, such as variations in temperature, moisture, and aeration, are constantly occurring in field practice. The data reported in this paper were obtained in order to ascertain the differences in bacterial numbers, nitrates, and nitrification of five variously treated typical acid soils, after these soils had been kept for 10 months under the same temperatures and controlled moisture conditions in pots where nitrates could not be lost by leaching. The soils used were all very acid and varied widely in organic matter. They were: (1) A yellow silty clay containing 0.7 per cent of humus, 0.07 per cent of nitrogen; (2) a whitish silt loam containing 1.3 per cent of humus, 0.12 per cent of nitrogen; (3) a brown silt loam containing 3.1 per cent of humus, 0.22 per cent of nitrogen; (4) a black peaty sand containing 5 per cent of humus, 0.4 per cent of nitrogen; and (5) a dark-brown peat containing 52 per cent of humus, 2.04 per cent of nitrogen. More complete analyses of these soils and the changes in their acidities due to moisture changes are given by one of us in another paper (*7*).

PREPARATION OF SOILS

To obtain soils for the pot tests, quantities of field soil were taken from the surface 6 inches, sacked, transferred to the station where each soil was mixed over and over without drying, sieved, and potted. Equal weights of a soil were put in galvanized-iron paraffined culture pots 9.25 inches in diameter and 11 inches high. The soil was compacted to that of a good seed bed by dropping the pots a prescribed number of times onto the floor from a height of about 3 inches. The pots were kept in the greenhouse and maintained at the desired moisture contents by weighing two to three times a week and replenishing the evaporated moisture with pure distilled water through an open tube extending from above the surface of the soil to an arch at the bottom of the pot. The surface of the soils of all pots except those kept fully saturated with water was cultivated from time to time to give a very thin dust mulch. The wheat stubble and growing clover were in the pots when sampled. The samples were taken to represent the entire depth of soil in the pot by the use of Noyes' bacteriologists' soil samplers (*19*), and all determinations were made from these samples.[1]

NITRATES AND NITRIFICATION WITH LIME AND FERTILIZER TREATMENTS

The nitrates were determined by the phenoldisulphonic-acid method modified for the accurate determination of soil nitrates.[2] The results are held to be equally accurate for all the soils, since the modified method takes into consideration the obtaining of a clear solution, the presence of soluble salts and interfering organic matter. The nitrification tests were made by the beaker method. One hundred gm. of each soil except the peat, of which 50 gm. were used, were placed in half-pint jelly glasses. Five cc. of a 2 per cent ammonium-sulphate solution were added and the soil was incubated for six weeks at 20° to 21° C. The moisture content at the end of the period of incubation was in every case within 1 per cent of what it was when the soils were sampled. Table I gives the acidity, crop yields, and nitrate data for each soil with the different lime and fertilizer treatments.

The quantities of nitrates found in the untreated soils before incubation showed that nitrification had taken place in every one of the acid soils. The amounts of nitrate present in the untreated soils when sampled were in proportion to their total nitrogen contents rather than in any relation to their acidities. The presence of growing clover in some of the pots lowered the ratio of the nitrates before incubation to those after incubation. Those pots which contained large growths of clover when sampled and which had received applications of lime alone contained less nitrates than the unlimed pots, which contained little or no

[1] The pots used in this investigation were chosen from a series of different investigations on soil-acidity problems, and hence the lime and fertilizer treatments for each soil were not the same.

[2] NOYES, H. A. THE ACCURATE DETERMINATION OF SOIL NITRATES BY THE PHENOL DISULPHONIC-ACID METHOD. To be published in Jour. Indus. and Engin. Chem.

clover. This shows that the nitrates present in the soils were greatly influenced by the growing crop. The limed pot in the brown silt-loam series was no exception to this, as the untreated soil on this series grew good clover. With each soil the amounts of nitrates found after incubation were very much greater with lime than without lime, proving that calcium carbonate promotes nitrification in acid soils. As a rule, the less clover there was per pot the greater the ratio of nitrates before incubation to nitrates after incubation.

TABLE I.—*Effects of lime and fertilization on nitrates and nitrification of five typical acid soils*

Kind of soil and treatment per million pounds of soil.	Acidity.[a]		Crop yields.[b]		Nitrates.			
	Potassium nitrate.	Calcium acetate.	Wheat.	Clover.	Before incubation.	After incubation.	Increase on incubation.	Ratio before and after incubation.
YELLOW SILTY CLAY.			*Gm.*	*Gm.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	
No treatment	2,460	4,000	7	0	10	24	14	42
2 tons of calcium carbonate	20	750	10	14	Tr.	32	32	0
Nitrogen, phosphorus, potassium[c]	2,800	4,125	43	2	Tr.	Tr.	0	100
Nitrogen, phosphorus, potassium,[c] 2 tons of calcium carbonate	20	750	68	17	0	184	184	0
Nitrogen, phosphorus, potassium,[c] 6 tons of calcium carbonate	0	500	76	15	0	873	873	0
WHITISH SILT LOAM.								
No treatment	1,360	3,000	23	13	19	38	19	50
3 tons of calcium carbonate	20	500	40	37	17	879	862	2
500 pounds of acid phosphate	1,380	3,000	23	6	29	48	19	60
BROWN SILT LOAM.								
No treatment	460	3,750	19	20	23	92	69	25
3 tons of calcium carbonate	20	750	29	30	52	852	800	6
500 pounds of acid phosphate	460	3,750	20	31	19	119	100	16
BLACK PEATY SAND.								
No treatment	1,800	6,750	0.5	0	350	340	−10	103
2 tons of calcium carbonate	80	3,500	17	11	77	585	508	13
Nitrogen, phosphorus potassium[c]	1,760	6,750	2	1	305	473	168	64
Nitrogen, phosphorus, potassium,[c] 2 tons of calcium carbonate	40	3,000	35	13	52	913	861	6
Nitrogen, phosphorus, potassium,[c] 6 tons of calcium carbonate	10	750	52	14	233	1,280	1,047	18
DARK-BROWN PEAT.								
No treatment	2,040	35,000	0	0	710	710	0	100
2 tons of calcium carbonate	1,260	27,500	0.5	0	1,216	1,280	64	95
20 tons of calcium carbonate	100	9,250	48	14	154	4,736	4,582	3

[a] Acidity determinations were made by Hopkins potassium-nitrate method and C. H. Jones calcium-acetate methods, and expressed in calcium-carbonate requirement per million.

[b] Crop yields are given in grams per pot; average of two pots.

[c] Chemically pure salts: 91 pounds of ammonium nitrate, 72 pounds of ammonium phosphate, and 100 pounds of potassium phosphate on yellow silty clay. No ammonium nitrate was used on black peaty sand.

The yellow silty clay containing 0.07 per cent of nitrogen and the black peaty sand containing 0.40 per cent of nitrogen received the same lime and fertilizer treatments, but gave quite different crop yields, nitrates, and nitrification. These variations can not be entirely correlated with changes in soil acidity. On the yellow silty clay it took both lime and fertilizer to give a markedly increased nitrifying power, while on the black peaty sand, of higher initial nitrifying power, lime gave the large, increased nitrifying power.

The whitish silt loam containing 0.12 per cent of nitrogen received the same lime and acid-phosphate treatment as the brown silt loam containing 0.22 per cent of nitrogen. Lime increased nitrification on both these soils more than acid phosphate did.

SOIL MOISTURE IN RELATION TO NITRATES AND NITRIFICATION

In order to ascertain what effect keeping soils at different moisture contents without crop would have on the nitrates present in the soil and nitrification tests, samples were drawn from a series of pots where each of the five acid soils had been kept at different moisture contents. The nitrates present in the soils after standing 10 months with specified moisture contents are given in Table II.

TABLE II.—*Effects of variable moisture on nitrates and nitrification of five typical acid soils*

Kind of soil and moisture treatments.	Acidity.[a]		Nitrates.			
	Potassium nitrate.	Calcium acetate.	Before incubation.	After incubation.	Increase on incubation.	Ratio before and after incubation.
YELLOW SILTY CLAY.			*P. p. m.*	*P. p. m.*	*P. p. m.*	
One-half	3,075	4,500	24	19	−5	126
Full	1,740	3,125	0	0	0	.....
WHITISH SILT LOAM.						
One-fourth	1,550	3,125	136	82	−54	166
One-half	1,860	4,500	74	128	54	58
Full	888	2,750	0	0	0	.....
BROWN SILT LOAM.						
One-fourth	325	4,500	265	100	−165	265
One-half	487	5,000	319	122	−197	261
Full	225	2,500	0	0	0	.....
BLACK PEATY SAND.						
One-fourth	1,560	6,500	140	328	188	43
One-half	1,810	6,250	315	190	−125	166
Full	925	4,750	0	0	0	.....
DARK-BROWN PEAT.						
One-fourth	2,000	31,750	178	214	36	83
One-half	2,700	32,500	618	766	148	81
Full	3,360	34,750	0	0	0	.....

[a] Both methods are expressed in calcium-carbonate requirement per million.

The results given in Table II show that the amount of water present in a soil is concerned with its nitrification, and further, that soils fully saturated with moisture do not contain nitrates either before or after incubation with ammonium sulphate. This table shows even more strongly than Table I that nitrification takes place in an acid soil, for the nitrates contained in the soils when sampled varied directly with the organic matter content of the different soils, but did not increase with lower soil acidities. The many instances where the nitrates in the soil when sampled were greater than those after incubation show that the nitrates present in these uncropped soils were near the maximum that could be present under the conditions of the experiment.

METHOD OF OBTAINING COUNTS

Field conditions are variable, and the results of these variations are apparent in the soil processes, due to bacterial agencies. It was believed that bacterial counts properly made would show some correlations among these different acid soils, the lime and fertilizer treatments, and the variable moisture contents they were kept under. Not only the nitrifying organisms but all classes of organisms had been given 10 months to respond to the different treatments, and an enumeration of both aerobes and anaerobes should show the types of bacteria predominating under the different treatments.

Plate counts were made from plates of high bacterial dilutions of each treatment according to the technic of Noyes and Voigt(*20*). Unpublished work by one of us on aerobic and anaerobic soil bacteria has shown that the average of five plates of a bacterial dilution high enough so that all bacteria from 1 cc. of the dilution will have a chance to develop into colonies in 10 days, gives accordant results. The media used was Lipman and Brown(*17*) modified synthetic agar, which extensive tests have proved to be satisfactory for the development of soil microorganisms. The carbon dioxid and hydrogen incubations were carried out in an atmosphere of flowing hydrogen or carbon-dioxid gas.

AEROBIC AND ANAEROBIC COUNTS ON CROPPED, LIMED, AND FERTILIZED SOILS

The number of bacteria present under the different lime and fertilizer treatments are given in Table III.

Table III shows that large increases in bacterial numbers result from the use of lime. These increases are largely in the aerobic organisms, although with the soils that contain considerable partially decomposed organic matter the anaerobic count is also increased.

Representative aerobic plates obtained from the yellow silty clay are shown in Plate 1. The numbers of colonies per plate are small, allowing for maximum development;. yet no striking differences in kinds of microorganisms are apparent under the different treatments. Neither

lime nor complete fertilizer alone had any great influence on bacterial numbers, while complete fertilizer with 2 tons of lime more than doubled the bacterial index (sum of aerobes and anaerobes) of the soil. Six tons of lime with fertilizer did not increase the bacterial index as much as the 2 tons with fertilizer.

TABLE III.—*Effects of lime and fertilization on bacterial content of five typical acid soils*

Kind of soil and treatment per million pounds of soil.	Millions of bacteria per gram of dry soil.		Bacterial index.[a]	Increase of bacterial index due to—	
	Air incubation.	Hydrogen incubation.		Calcium carbonate.	Fertilizer.
YELLOW SILTY CLAY.					
No treatment	[b] 3.010	0.100	3.110	.....	.....
2 tons of calcium carbonate	3.046	.381	3.427	0.317	.....
Nitrogen, phosphorus, potassium [c]	3.027	.000	3.027	.....	−0.083
Nitrogen, phosphorus, potassium,[c] 2 tons of calcium carbonate	7.605	.000	7.605	4.578	4.178
Nitrogen, phosphorus, potassium,[c] 6 tons of calcium carbonate	5.244	.000	5.244	2.217	.....
WHITISH SILT LOAM.					
No treatment	5.021	1.545	6.566	.....	.....
3 tons of calcium carbonate	14.810	.898	15.708	9.142	.....
500 pounds of acid phosphate	5.531	.000	5.531	.....	−1.035
BROWN SILT LOAM.					
No treatment	9.904	.189	10.093	.....	.....
3 tons of calcium carbonate	23.921	2.556	26.477	16.384	.....
500 pounds of acid phosphate	11.164	2.714	13.878	.....	3.785
BLACK PEATY SAND.					
No treatment	3.146	1.154	4.300	.....	.....
2 tons of calcium carbonate	8.386	1.617	10.003	5.703	.....
Nitrogen, phosphorus, potassium [b]	2.813	2.907	5.720	.....	1.420
Nitrogen, phosphorus, potassium, 2 tons of calcium carbonate	10.583	.099	10.682	4.962	.679
Nitrogen, phosphorus, potassium, 6 tons of calcium carbonate	16.037	1.760	17.797	12.077	.....
DARK-BROWN PEAT.					
No treatment	1.554	.997	2.551	.....	.....
2 tons of calcium carbonate	3.420	1.440	4.860	2.309	.....
20 tons of calcium carbonate	91.846	11.752	103.598	101.047	.....
Average	12.109	1.585	13.694	15.874	.....

[a] Sum of air and hydrogen counts.
[b] Average of five plates. No count indicates no colonies on plates from 1:400,000 bacterial dilutions.
[c] Chemically pure salts: 91 pounds of ammonium nitrate, 72 pounds of ammonium phosphate, and 100 pounds of potassium phosphate on yellow silty clay. No ammonium nitrate was used on black peaty sand.

Lime more than doubled the bacterial indexes of the whitish silt and brown silt loams. Acid phosphate decreased the anaerobic counts of the whitish silt loam enough more than it increased the aerobic con-

tents so that the bacterial index was decreased. With the brown silt loam containing considerable undecayed organic matter the acid phosphate increased both the aerobic and anaerobic counts somewhat. Plate 2 shows representative petri plates from each treatment for the two soils. This plate shows a marked similarity between the colonies on the aerobic plates from the limed pots of both soils. The similarity of the appearances of the plates from the limed and the phosphated pots of the whitish silt loam, the similarity of all aerobic plates from the brown silt loam and the uniformity of colonies developing from the brown silt loam under anaerobic conditions are to be noted.

The black peaty sand containing six times as much nitrogen as the yellow silty clay, received the same lime and fertilizer treatments as the yellow clay, but gives entirely different results. Lime and fertilizer both alone and in combination give increased bacterial indexes. While the aerobic organisms are increased by lime, the organic matter of the black peaty sand must be in an advanced stage of decay since the counts are lower than they should be if the organic matter was good food for bacteria. Plate 3 shows representative culture plates from this soil. These illustrations emphasize the effect of lime on bacterial numbers and the small proportion of the bacteria which are anaerobic.

The dark-brown peat shows an increase of over 100,000,000 in bacterial index as the result of liming. Peats *in situ* are generally low in bacterial content. Working them over after drainage generally causes enormous increases in their bacterial content. This peat, even when aerated, had only 1½ times as many aerobic as anaerobic bacteria, but adequate liming increased the aerobes more than 60 times and the anaerobes over 11 times. The increase in anaerobes is believed to be associated with the large amount of organic matter present in the soil. Plate 4 shows representative petri plates of the colonies developing in air and hydrogen. Attention is called to the small variation in colony types on the anaerobic plates as compared to the aerobic. The aerobic culture plates from the heavily limed soil showed many chromogenic differences between colonies not observable in the photographs.

SOIL MOISTURE IN RELATION TO BACTERIAL COUNTS

In addition to the incubations in air and hydrogen another set of plates was incubated in an atmosphere of flowing carbon-dioxid gas for 10 days. No colonies developed on this set of plates while they were in carbon-dioxid gas. The counts given were computed from colonies developing in 10 days in air after the plates had been removed from the carbon dioxid.[1] Table IV gives the counts under the different conditions of incubation and the various soil-moisture contents.

[1] These organisms, as far as tested, have been found to be spore formers.

TABLE IV.—*Effects of variable moisture on bacterial content of five typical acid soils*

Kind of soil and degree of moisture saturation.	Millions of bacteria per gram of dry soil.			Bacterial index.[b]	Ratios to bacterial index as 100.		
	Air.	Hydrogen.	Carbon-dioxid-surviving.[a]		Air.	Hydrogen.	Carbon-dioxid-surviving.
YELLOW SILTY CLAY.							
One-half	[c] 1.556	0.101	0.131	1.657	94	6	8
Full	.184	.032	.075	.216	85	15	35
WHITISH SILT LOAM.							
One-fourth	2.792	.367	.282	3.159	88	12	9
One-half	3.688	1.171	.216	4.859	76	24	4
Full	3.179	.353	.246	3.532	90	10	7
BROWN SILT LOAM.							
One-fourth	4.920	1.879	.533	6.799	72	28	8
One-half	4.513	1.640	.847	6.153	73	27	14
Full	7.854	2.864	.575	10.718	73	27	5
BLACK PEATY SAND.							
One-fourth	1.641	.453	.286	2.094	78	22	14
One-half	2.363	1.270	.463	3.633	65	35	13
Full	3.316	.129	1.071	3.445	96	4	31
DARK-BROWN PEAT.							
One-fourth	2.425	1.988	1.101	4.413	55	45	25
One-half	1.796	1.914	1.204	3.710	48	52	33
Full	2.257	.735	.499	2.992	75	25	17
Averages	3.034	1.064	.538	4.098	74	26	13

[a] Incubated 10 days in carbon dioxid; then 10 days in air.
[b] Sum of air and hydrogen incubation.
[c] All figures were computed from 5 plates.

The bacterial content, as well as the proportions of aerobes to anaerobes, was changed by the degree of saturation of the soil, but the nature of the soil had a greater effect than the moisture content on bacterial numbers. The proportions of anaerobes to the aerobes which survived carbon-dioxid incubation increased with soil organic matter when the soils were held under optimum moisture conditions.

Plates 5 to 9 show representative petri plates from the 1 to 40,000 bacterial dilution of these soils. Figures A_1, H_1, and C_1 in each plate show representative petri plates after air (A), hydrogen (H), and carbon-dioxid, then air (C) incubations of bacterial dilutions of samples from pots of soils kept one-fourth saturated with water. Figures A_2, H_2, and C_2 are from samples from pots of soils kept half saturated, while A_3, H_3, and C_3 are from pots of soils kept fully saturated with water.

Plates 5 to 9 show that the bacterial flora of each soil is different from that of every other soil. The soils kept one-fourth saturated with water

contained the largest numbers of microorganisms developing moldlike colonies, and the fully saturated soils gave culture plates containing the smallest numbers of spreading moldlike colonies.

NITRATES AND NITRIFICATION IN RELATION TO BACTERIAL COUNTS

Lime increased both nitrification and bacterial counts. A study of Plates 1 to 4 shows that the increases in bacterial numbers can be asso-

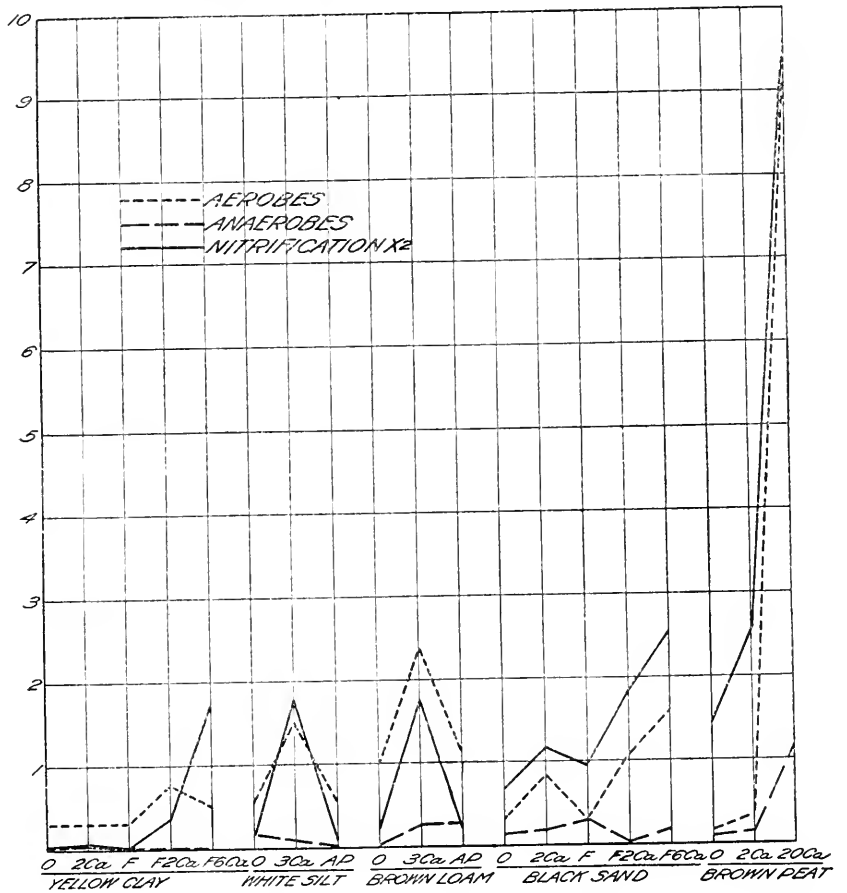

FIG. 1.—Graphs showing the relation of aerobes and anaerobes to nitrification of five acid soils with and without lime and fertilizer treatments.

ciated principally with the aerobic small, round, entire colonies on the petri plates. Figure 1 shows the relations between aerobic and anaerobic bacteria and the nitrates after incubation for the cropped, limed, and fertilized soils kept at optimum moisture content. The nitrates after incubation varied directly with the aerobic bacteria. The aerobic count and nitrates after incubation show that it is the increased number of aerobic organisms that are to be associated with increased nitrification.

Figure 2 gives soil nitrates, aerobic, and anaerobic bacterial numbers for the series of soils where moisture was the variable. These graphs shows that lack of aeration which changed the proportions of aerobes to anaerobes prevented a correlation between nitrates and aerobic counts.

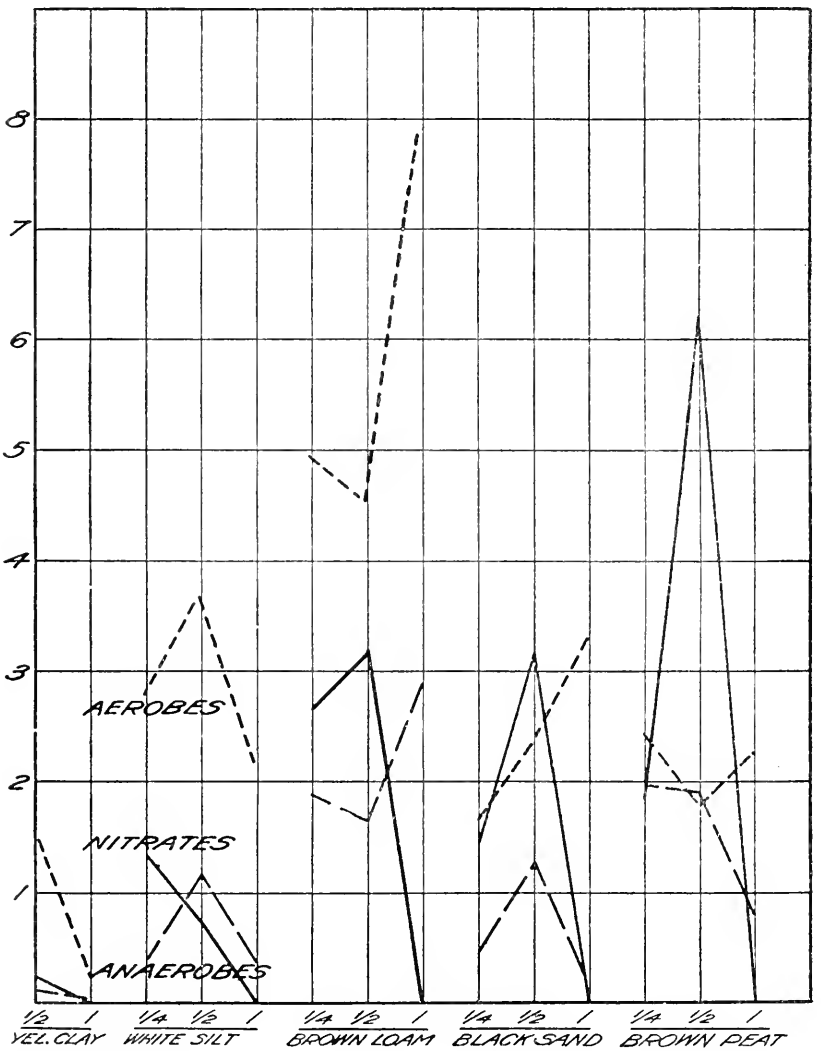

FIG. 2.—Graphs showing the relation of aerobes and anaerobes to nitrates in five acid soils kept at different moisture contents.

GENERAL DISCUSSION

After conducting bacteriological investigations on acid soils to ascertain "if it might be desirable to consider more carefully the possibilities of a system of acid agriculture," Bear (3) concluded that—

> the supply of nitrogen in acid soils may be maintained by growing acid-resistant legumes, of which the soy bean is one. Undoubtedly the use of acid phosphate aids

materially in the nitrogen-fixation processes of acid soils. Small applications of calcium carbonate are, as a rule, relatively more effective than large applications as a means of increasing the bacterial activities in acid soils.

The problem of maintaining soil fertility resolves itself into maintaining and increasing the available supply of organic matter and nitrogen in the soil and the replenishing of the mineral elements. One system now generally recommended and used is to apply lime and phosphates, then to grow legumes, and to plow them under. This system of soil maintenance and improvement is in accordance with the important rôle of soil bacteria in plant nutrition, and the results obtained in the controlled investigations reported here illustrate some good reasons for such a method of soil management.

When the soil was limed, the aerobic bacteria concerned with oxidation reactions increased in numbers. This is illustrated by the increased bacterial numbers and nitrification wherever the soils were limed.

Plenty of organic matter is necessary for high bacterial numbers, a condition which is well illustrated by the low bacterial content and nitrate results with the limed yellow silty clay (low in organic matter) compared with the high bacterial contents and nitrates on the limed brown silt loam and dark-brown peat (high in organic matter).

Mineral fertilizers serve as food for larger crops and larger crops in turn leave more residues in roots and stubble for bacterial food.

The number of bacteria in an arable soil can be correlated with crop yield to about the same degree that soil moisture can be. Soil moisture is conceded to be the most vital single factor influencing crop yields; yet because of so many other variable conditions it is not always possible to correlate soil moisture and crops any more than it is possible to always correlate bacterial numbers and crops. Below a certain minimum in moisture or bacterial numbers field soils will not produce crops; above that minimum, everything else being equal, crops may be in general correlated with bacterial numbers as well as with moisture.

Changes in bacterial numbers, especially differences in the proportions of aerobes to anaerobes, are of prime importance in soil-biology studies. The results here reported under controlled conditions make it evident that soil-fertility investigations should include both chemical and biological examinations of the soil.

SUMMARY

(1) Controlled greenhouse investigations were conducted on five typical acid soils. In part of the experiments the soils were fertilized with calcium carbonate, acid phosphate, and complete fertilizer, cropped to wheat and clover, and kept at optimum moisture content, while in another series the soils were unfertilized, uncropped, and kept one-fourth, one-half, and fully saturated with water.

(2) The results reported include crop yields, soil-acidity determinations, nitrates in the soil when sampled and after incubation with ammo-

nium sulphate, and also the numbers of aerobic, anaerobic, and carbon-dioxid surviving microorganisms present in the soils.

(3) All the untreated soils were quite acid and contained nitrates when sampled, showing that nitrification takes place in acid soils.

(4) The amounts of nitrates present and the nitrifying power of the untreated acid soils varied with the organic matter and total nitrogen rather than with the soil acidity.

(5) Calcium-carbonate additions markedly increased the nitrification of all five soils.

(6) Fertilization tended to increase nitrification, but not so much as calcium carbonate did.

(7) Regardless of treatments the presence of growing clover kept down nitrate contents of the soils.

(8) The degree of saturation of the soils affected the nitrates present. As a rule, more nitrate were found in soil kept one-half saturated with water than in soil kept one-fourth saturated.

(9) The soils that had been kept fully saturated with water for the 10 months contained no nitrates and formed no nitrates when incubated with ammonium sulphate.

(10) The relation of nitrates present in the uncropped soils before incubation to the nitrates present after incubation shows that the nitrate contents of these acid soils tend to reach an equilibrium, above which no increase is obtained without additional treatment.

(11) The bacterial flora of each soil was different from that of every other soil.

(12) No bacteria developed into colonies visible to the eye as long as plates were incubated in an atmosphere of flowing carbon-dioxid gas.

(13) Calcium-carbonate additions increased the bacterial contents of the soils. This increase was largely in the aerobic organisms.

(14) Small increases in bacterial content resulted from the use of fertilizer.

(15) The degree of saturation at which the soil was kept changed the proportions between the aerobic, anaerobic, and carbon-dioxid-surviving bacteria.

(16) Cultures from samples that had been kept one-fourth saturated with water contained the largest proportions of organisms forming moldlike colonies.

(17) Under optimum moisture conditions both without and with lime and fertilizer treatments the nitrates after incubation varied directly with the aerobic counts.

(18) In general, the greater the aerobic bacterial content and the nitrifying power of the soil the larger the crop yields.

(19) These investigations show many reasons why a system of soil improvement which includes the addition of lime, phosphate, and organic matter is worth while.

(20) It is evident that soil fertility investigations should include both chemical and biological examinations of the soil.

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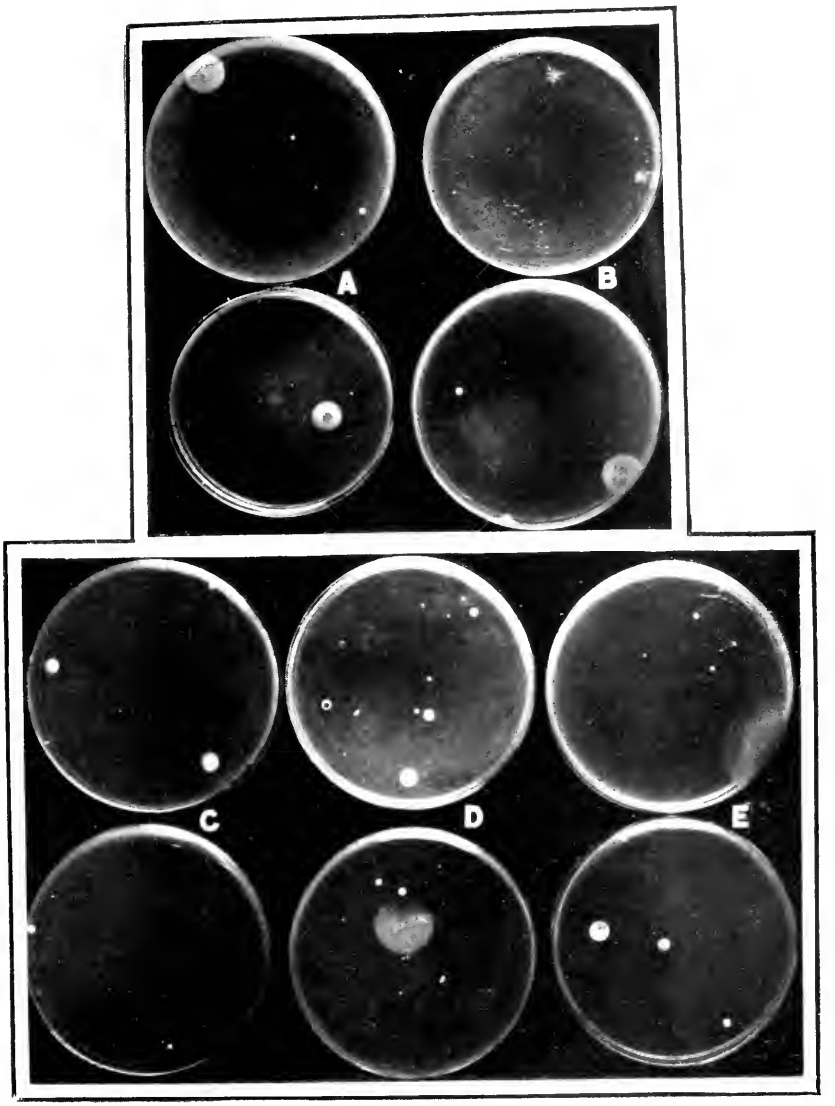
PLATE 1

Representative plates from 1 to 400,000 bacterial dilution of acid yellow silty clay, cropped and held under optimum moisture conditions:

A.—Aerobic plates, untreated.
B.—Aerobic plates, treated with 2 tons of calcium carbonate.
C.—Aerobic plates, treated with complete fertilizer.
D.—Aerobic plates, treated with complete fertilizer and 2 tons of calcium carbonate.
E.—Aerobic plates, treated with complete fertilizer and 6 tons of calcium carbonate.


A
B
C
D
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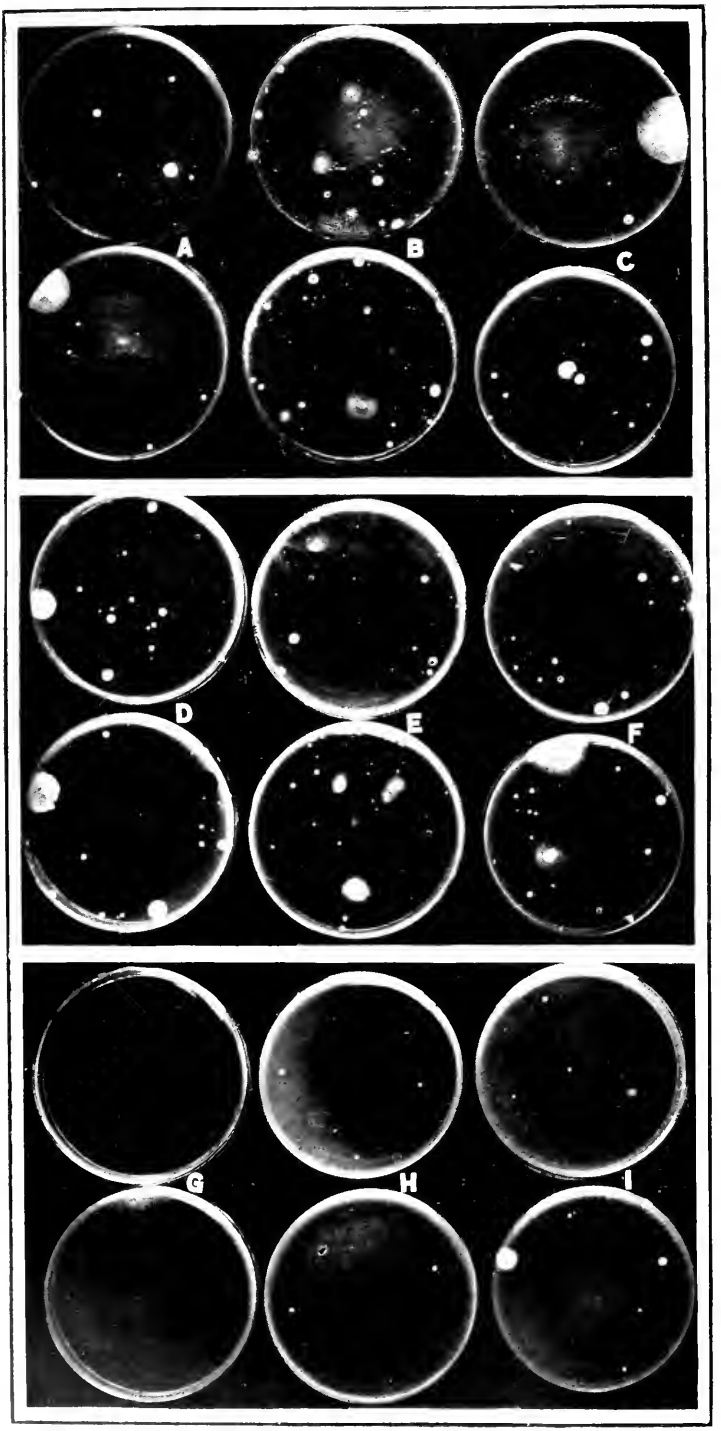
A
B
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PLATE 2

Representative plates from 1 to 400,000 bacterial dilution of acid whitish silt loam and acid brown silt loam cropped and held under optimum moisture conditions:

A.—Aerobic plates, acid whitish silt loam, untreated.

B.—Aerobic plates, acid whitish silt loam, treated with 3 tons of calcium carbonate.

C.—Aerobic plates, acid whitish silt loam, treated with 500 pounds of acid phosphate.

D.—Aerobic plates, acid brown silt loam, untreated.

E.—Aerobic plates, acid brown silt loam, treated with 3 tons of calcium carbonate.

F.—Aerobic plates, acid brown silt loam, treated with 500 pounds of acid phosphate.

G.—Anaerobic plates, acid brown silt loam, untreated.

H.—Anaerobic plates, acid brown silt loam, treated with 3 tons of calcium carbonate.

I.—Anaerobic plates, acid brown silt loam, treated with 500 pounds of acid phosphate.

PLATE 3

Representative plates from 1 to 400,000 bacterial dilution of acid black peaty sand, cropped and held under optimum moisture conditions:

A.—Aerobic plates, untreated.

B.—Aerobic plates, treated with 2 tons of calcium carbonate.

C.—Aerobic plates, treated with complete fertilizer.

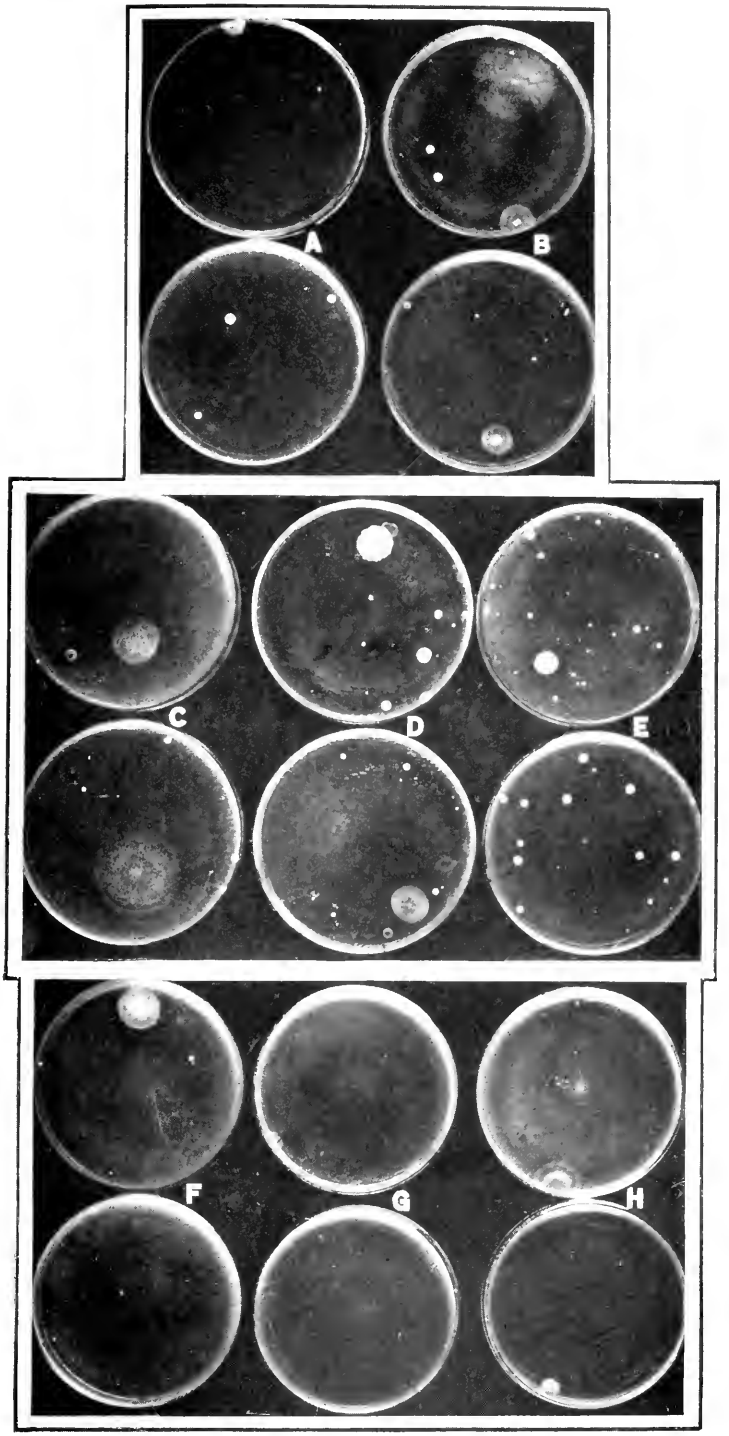
D.—Aerobic plates, treated with complete fertilizer and 2 tons of calcium carbonate.

E.—Aerobic plates, treated with complete fertilizer and 6 tons of calcium carbonate.

F.—Anaerobic plates, treated with complete fertilizer.

G.—Anaerobic plates, treated with complete fertilizer and 2 tons of calcium carbonate.

H.—Anaerobic plates, treated with complete fertilizer and 6 tons of calcium carbonate.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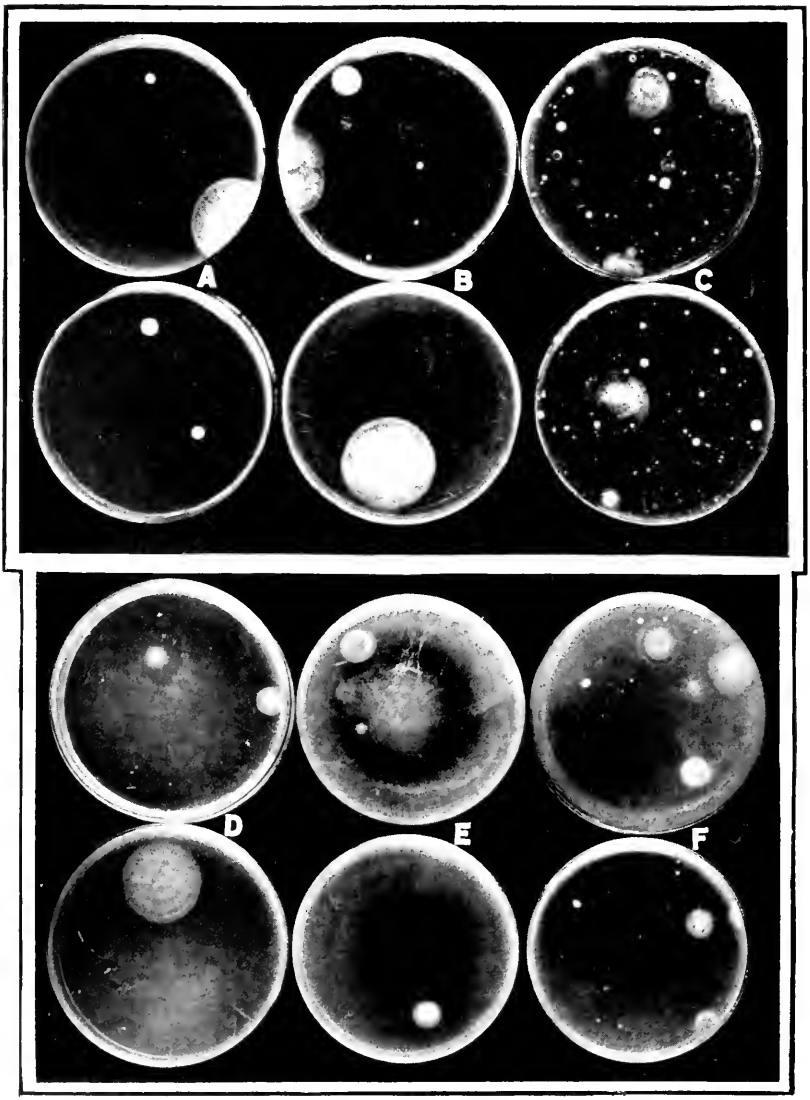

A
B
C
D
E
F

PLATE 4

Representative plates from 1 to 400,000 bacterial dilution of acid dark-brown peat, cropped and held under optimum moisture conditions:

A.—Aerobic plates, untreated.
B.—Aerobic plates, treated with 2 tons of calcium carbonate.
C.—Aerobic plates, treated with 20 tons of calcium carbonate.
D.—Anaerobic plates, untreated.
E.—Anaerobic plates, treated with 2 tons of calcium carbonate.
F.—Anaerobic plates, treated with 20 tons of calcium carbonate.

PLATE 5

Representative plates from 1 to 40,000 bacterial dilution of acid yellow silty clay kept at different moisture contents:

A_2.—Aerobic plates, from soil kept one-half saturated.

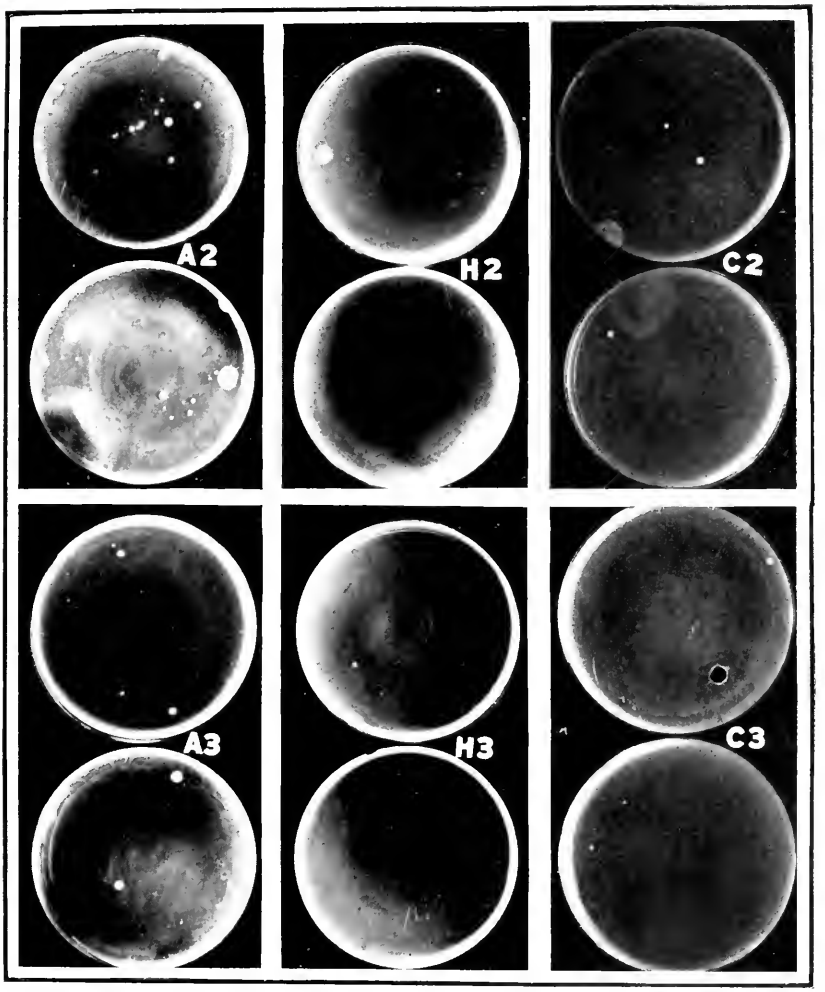
H_2.—Anaerobic plates, from soil kept one-half saturated.

C_2.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-half saturated.

A_3.—Aerobic plates from soil kept fully saturated.

H_3.—Anaerobic plates from soil kept fully saturated.

C_3.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept fully saturated.


A2
H2
C2
A3
H3
C3

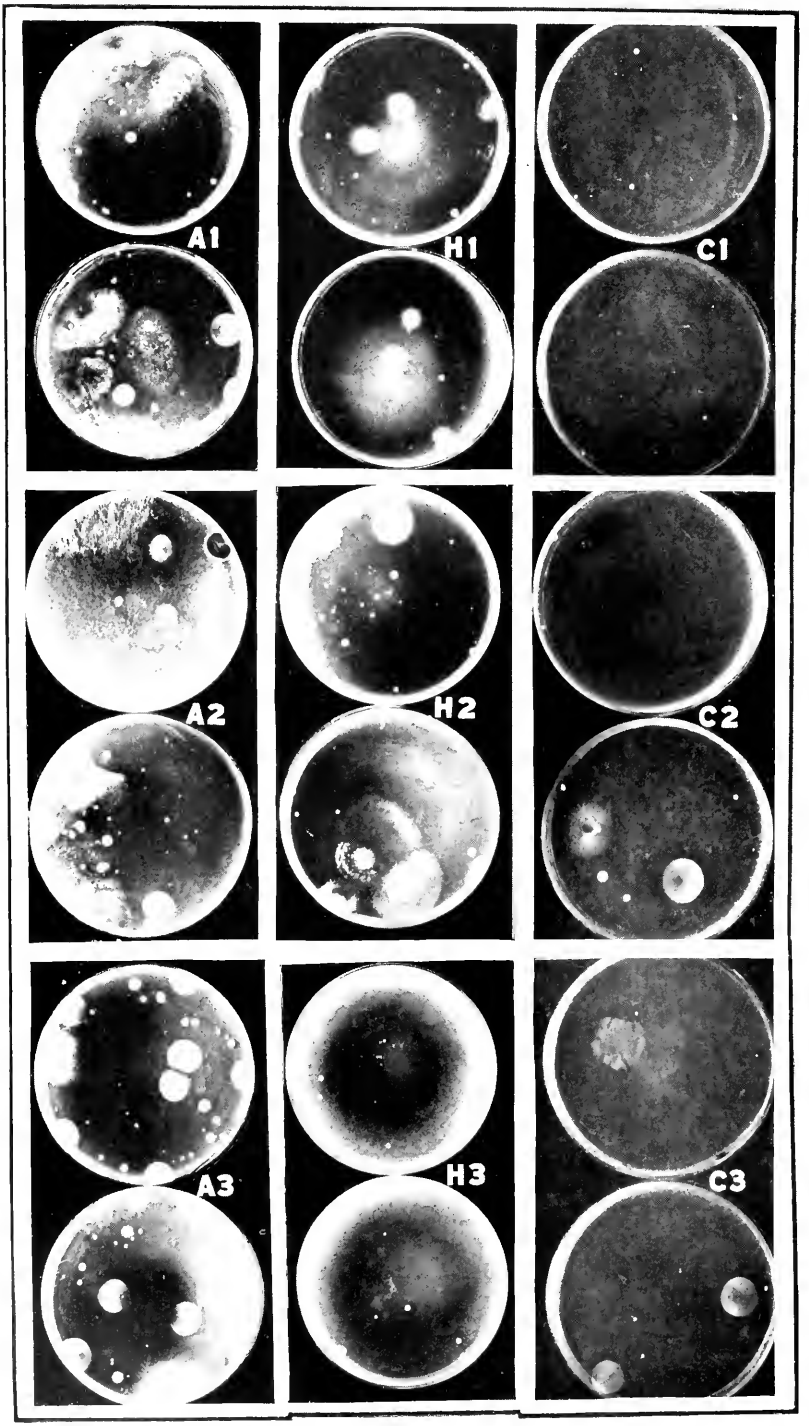

A1
H1
C1
A2
H2
C2
A3
H3
C3

PLATE 6

Representative plates from 1 to 40,000 bacterial dilution of acid whitish silt loam kept at different moisture contents:

A_1.—Aerobic plates from soil kept one-fourth saturated.

H_1.—Anaerobic plates from soil kept one-fourth saturated.

C_1.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-fourth saturated.

A_2.—Aerobic plates from soil kept one-half saturated.

H_2.—Anaerobic plates from soil kept one-half saturated.

C_2.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-half saturated.

A_3.—Aerobic plates from soil kept fully saturated.

H_3.—Anaerobic plates from soil kept fully saturated.

C_3.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept fully saturated.

PLATE 7

Representative plates from 1 to 40,000 bacterial dilution of acid brown silt loam kept at different moisture contents:

A_1.—Aerobic plates from soil kept one-fourth saturated.

H_1.—Anaerobic plates from soil kept one-fourth saturated.

C_1.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-fourth saturated.

A_2.—Aerobic plates from soil kept one-half saturated.

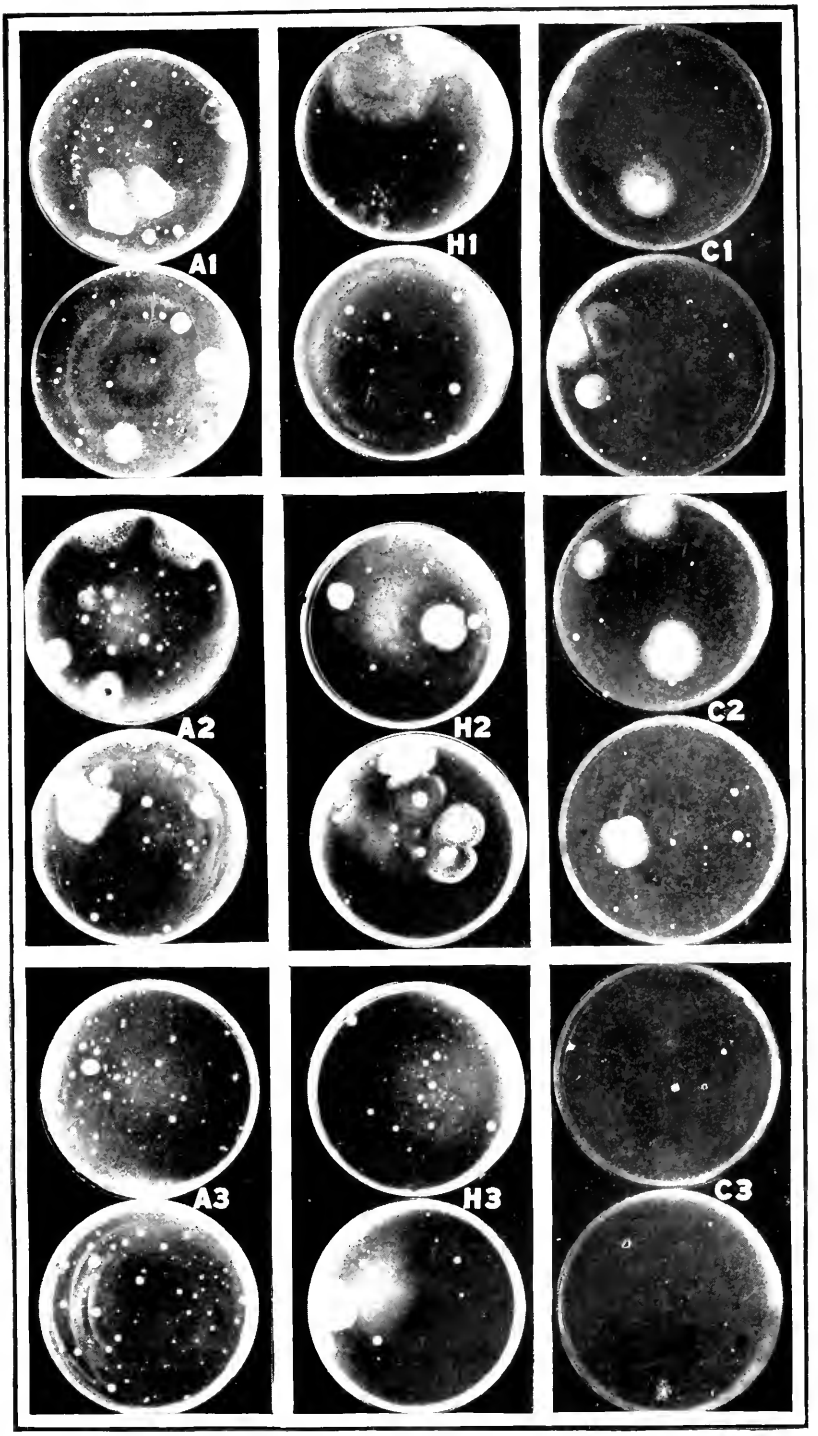
H_2.—Anaerobic plates from soil kept one-half saturated.

C_2.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept half saturated.

A_3.—Aerobic plates from soil kept fully saturated.

H_3.—Anaerobic plates from soil kept fully saturated.

C_3.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept fully saturated.


A1
H1
C1
A2
H2
C2
A3
H3
C3

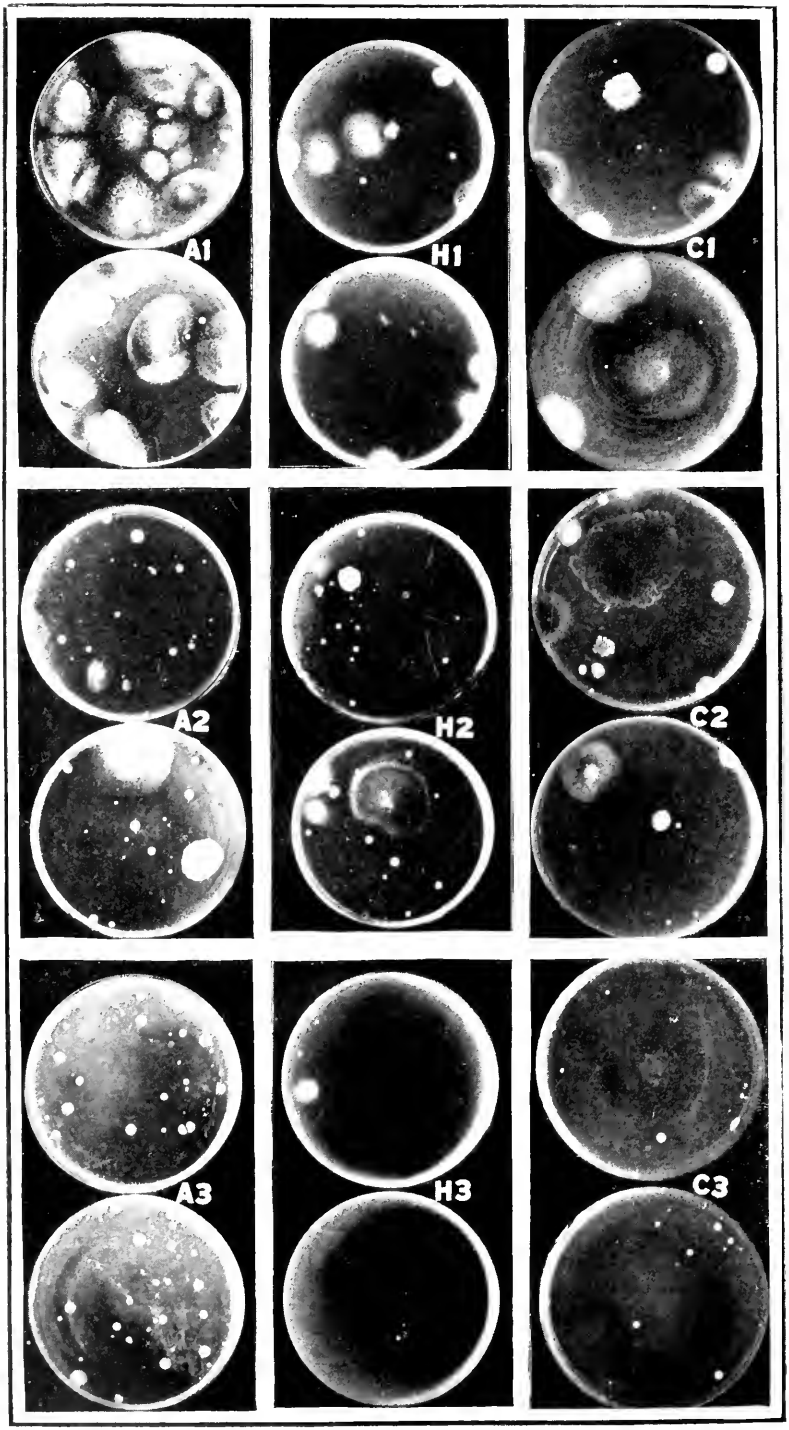

A1
H1
C1
A2
H2
C2
A3
H3
C3

PLATE 8

Representative plates from 1 to 40,000 bacterial dilution of acid black peaty sand kept at different moisture contents:

A_1.—Aerobic plates from soil kept one-fourth saturated.

H_1.—Anaerobic plates from soil kept one-fourth saturated.

C_1.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-fourth saturated.

A_2.—Aerobic plates from soil kept one-half saturated.

H_2.—Anaerobic plates from soil kept one-half saturated.

C_2.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-half saturated.

A_3.—Aerobic plates from soil kept fully saturated.

H_3.—Anaerobic plates from soil kept fully saturated.

C_3.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept fully saturated.

PLATE 9

Representative plates from 1 to 40,000 bacterial dilution of acid dark-brown peat kept at different moisture contents:

A_1.—Aerobic plates from soil kept one-fourth saturated.

H_1.—Anaerobic plates from soil kept one-fourth saturated.

C_1.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-fourth saturated.

A_2.—Aerobic plates from soil kept one-half saturated.

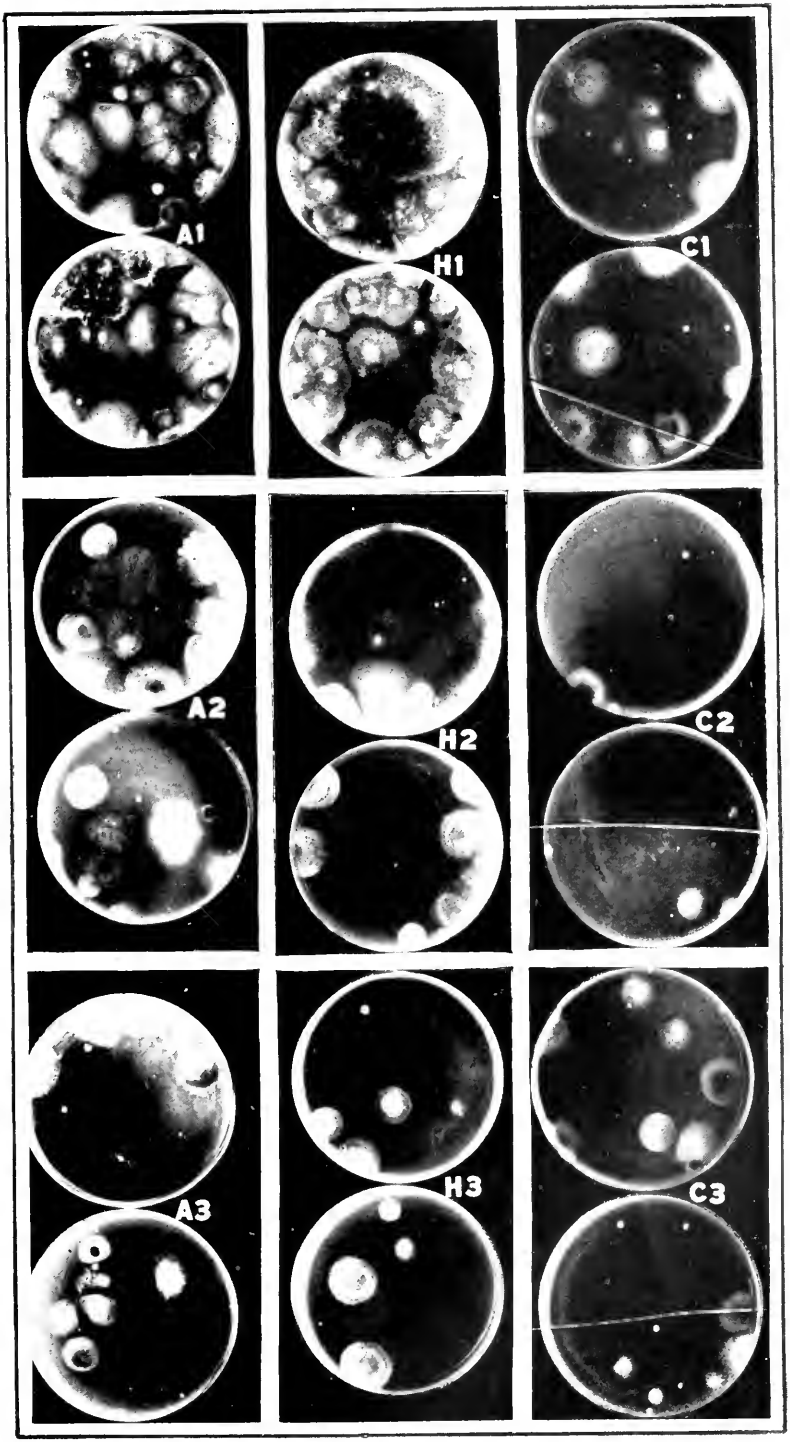
H_2.—Anaerobic plates from soil kept one-half saturated.

C_2.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept one-half saturated.

A_3.—Aerobic plates from soil kept fully saturated.

H_3.—Anaerobic plates from soil kept fully saturated.

C_3.—Aerobic plates of carbon-dioxid-surviving organisms from soil kept fully saturated.


A1
H1
C1
A2
H2
C2
A3
H3
C3

EFFECT OF CERTAIN ECOLOGICAL FACTORS ON THE MORPHOLOGY OF THE UREDINIOSPORES OF PUCCINIA GRAMINIS [1]

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COOPERATIVE INVESTIGATIONS BETWEEN THE AGRICULTURAL EXPERIMENT STATION OF THE UNIVERSITY OF MINNESOTA AND THE BUREAU OF PLANT INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE

INTRODUCTION

Extensive studies have been made of biologic forms of *Puccinia graminis* Pers., but these studies have been mainly on the physiological rather than on the morphological phase of the problem. The effect of host plants and other factors on the parasitic capabilities of biologic forms has been quite thoroughly investigated. Some work has also been done on the effect of host plants on the morphology of the fungus, but hardly as much as the importance of the problem warrants.

The question whether biologic forms change readily in response to environmental conditions is important practically and scientifically. The measure of plasticity has usually been the parasitic performance of the rust. But if there is a tendency for biologic forms to change rather quickly, it is reasonable to expect that the morphology might change also. The object of this work, therefore, was to determine the effect of hosts and of physical factors such as heat, light, and humidity on the morphology or urediniospores. It would be desirable to include a study of teliospores and æciospores also, but the difficulties are obvious.

Since the effect of physical factors on the morphology of the urediniospores may be indirect—by affecting the vigor of the rust—the virulence of the rust under different conditions was also studied.

Although it has been generally believed that the various biologic forms of *P. graminis* differ only functionally, yet as early as 1902, Ward (*15, p. 236*) [2] suggested that each specialized form—

> is in course of becoming a species and may during the lapse of time actually become a species of Puccinia, which will eventually show morphological differences in addition to the physiological ones it already shows.

Freeman and Johnson (*3, p. 14*) expressed a similar opinion in 1911. Stakman also (*11*) obtained some evidence that long association with

[1] Published, with the approval of the Director, as Paper No. 143 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 77.

Journal of Agricultural Research, Washington, D. C.
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Vol. XVI. No. 2
Jan. 13. 1919
Key No. Minn.-35

a given host might change the urediniospore dimensions of a biologic form.

Recently Long (*8*) working with *Puccinia ellisiana* Thuem. and *P. andropogonis* Schw., whose æcial hosts are certain species of Viola and Pentstemon, respectively, found he could change the morphological characters of the urediniospores of these rusts by reversing their æcial hosts. Thus *P. ellisiana* after passing through *Pentstemon* as the æcial host acquired the morphological characteristics of the urediniospores of *P. andropogonis;* likewise, *P. andropogonis* assumed the characters of *P. ellisiana* after passing through *Viola* sp. as the æcial host.

Attention has been called several times to the fact that when a biologic form of stemrust develops weakly on a partly resistant host the urediniospores usually are appreciably smaller than they normally are. It seems, therefore, that physical factors might also influence the spore size by affecting the vigor of the rust. But, although a great deal of work has been done on the effect of environmental factors on the severity of rust attacks, the possible correlation between the degree of vigor of the rust fungus and the size of the spores has not been investigated thoroughly.

Ward (*15, p. 274*) noted tnat differences of temperature, illumination, drouth, etc., affecting the transpiration, assimilation, and other processes of the seedlings, also affect the period of germination, incubation and maturation of the rusts. Fromme (*4, p. 507–509*) has tabulated a number of recorded observations of this nature.

Johnson (*7, p. 47*) found the cardinal temperature of *Puccinia graminis* on wheat, barley, and oats to vary from about 35 to 90° F. Butler and Hayman (*1, p. 11*) have not succeeded in producing rust artificially on plants grown in the open in the hot weather, in India, and they doubted—

> whether the spores have power to infect when exposed to temperatures exceeding 100° F.

Christman (*2, p. 106*)—

> found by experiment that [in Wisconsin] in the cooler weather of spring the incubation period following inoculation with uredospores is usually lengthened to between three and four weeks.

Although there was considerable evidence on the effect of these environmental factors on rust, their effect was investigated again, especially for the purpose of getting evidence of the effect on spore morphology.

EXPERIMENTAL METHODS

The methods employed in these experiments were essentially the same as those described by Stakman and Piemeisel (*14*). But it was thought advisable to obtain additional data on the following points:

(1) Quantity of inoculum to be used; (2) optimum length of incubation; (3) condition of urediniospores necessary to insure uniform measurements; (4) number of measurements to be made of a given strain; and (5) method of computation to be employed.

In order to determine the amount of inoculum to be used, eight sets of inoculations were made with very heavy, with moderate, and with exceedingly light applications of inoculum. The very heavily inoculated plants produced 132 successful infections out of 142 inoculations, or 93 per cent; 130 out of 142, or 91 per cent, of the leaves inoculated with a moderate amount became infected; and 104 out of 118, or 88 per cent, of those which had received a small amount of inoculum became infected. Whenever infection resulted, there was no perceptible difference in the size of either the uredinia or the urediniospores, or in the virulence of attack in general.

A liberal amount of inoculum was used whenever possible in all subsequent experiments.

Jaczewski (*5, p. 330*) found that the germination of urediniospores begins two or three hours after placing them in water or on the surface of the plant blades, after which it progresses very rapidly, provided the spores are fresh. Fromme (*4, p. 513*) points out that in order to obtain a successful infection on plants a saturated atmosphere is necessary.

To determine the optimum length of the incubation period, wheat plants in 10 pots, each containing 10 wheat seedlings 6 days old, were inoculated with an equal and liberal amount of viable urediniospore material of *P. graminis tritici* and placed in two pans containing a small amount of water and then placed under glass bell jars. Equal amounts of water were put in both pans, and all other conditions were kept uniform.

At the end of 12 hours two pots were removed from under the bell jars and set out on the bench; after 24 hours the second pair of pots were set out; and the rest were taken out from the pans in pairs every 24 hours following the second pair—that is, 48, 72, and 96 hours after inoculation.

The first observation was made 80 hours after inoculation, and no signs of infection could be detected on the plants incubated for 12, 24, or 48 hours; but 6 plants of those which had been under for 72 hours and 12 of those that were still under the bell jar showed very indistinct, but apparently typical, rust flecks. These were later found not to be infection flecks, but the result of supersensibility, due to the long confinement in the moist chambers. At 128 hours after inoculation clearly defined rust flecks appeared on all plants which had been incubated for 48, 72, and 96 hours. Of the 20 plants that had been under the bell jar for 24 hours, 18 plants were flecked, while only a single fleck showed on one plant of those that had been under only 12 hours.

The first uredinia began to burst through the epidermis 144 hours after inoculation, except on those plants which had been under the bell jar for 12 hours. On these plants the first and only uredinium appeared 10 days after inoculation. At this time the rust was well developed on all the plants that had been under for 48 hours, whereas those that were kept under 24, 72, and 96 hours showed the maximum infection only two days later.

Although all the fully developed uredinia were in every case approximately the same size, color, and shape, the virulence of the attack varied considerably. The plants kept under the bell jar for 48 hours produced the greatest number of uredinia per leaf; those which had been under 24 and 72 hours, respectively, somewhat fewer, and those which had been under 96 hours, still fewer; while only one uredinium appeared on the single infected leaf of the plants kept under for 12 hours. In the present work, therefore, all inoculated plants were kept for 48 hours in the moist chamber, then removed to their respective places on the greenhouse benches.

It was found that the superficial layer of each uredinium contains larger spores, and when this layer is removed, the remaining spores are considerably smaller. But if the uredinium is allowed to produce a new crop of spores, those on the surface again attain the same dimensions as the original ones. For this reason precaution was taken to measure spores from uredinia in the same stage of development.

TABLE I.—*Results of measuring varying numbers of urediniospores of Puccinia graminis tritici and P. graminis avenae*

Experiment. No.	Source of urediniospores.	Number of spores measured.	Spore dimensions.		
			Range of length.	Range of width.	Modes.
			μ	μ	μ
1	*Triticum aestivum*	25	26. 88–38. 72	17. 92–22. 08	[a]32. 00×19. 52
2	do	50	26. 88–40. 32	17. 92–23. 04	[b]33. 60×19. 84
3	do	100	26. 56–40. 32	16. 96–23. 36	32. 96×19. 84
4	do	200	25. 60–40. 32	16. 32–23. 36	32. 96×19. 84
5	do	400	23. 68–40. 32	16. 32–23. 36	32. 96×19. 84
6	*Avena sativa*	25	23. 36–35. 20	17. 28–22. 08	[c]
7	do	50	23. 36–35. 20	17. 28–22. 08	[b]27. 52×19. 52
8	do	100	23. 04–35. 20	16. 96–22. 08	29. 12×19. 52
9	do	200	21. 12–36. 48	16. 32–23. 04	29. 12×19. 52

[a] Modes doubtful but showing tendency to form as indicated.
[b] Mode of length doubtful but that of width definitely established.
[c] Modes indeterminate.

As to the number of spores to be measured from a given group, it was found that 100 gave equally as good results as 200 or 400, while when less than 100 were used the results were not always representative or conclusive. Table I gives the results of measuring different numbers of

spores from the same plant taken on the same day from uredinia on the same leaves. Stemrust of both wheat and oats was tried with similar results. As noted from Table I, the modes of a population of 100, 200, or 400 spores are the same, but the limits of variation are less in a population of 100 than in those of 200 or 400. It will also be seen from the table that at least 100 spores should be used. In the present investigation 200 spore measurements were made for each experimental group until January 1, 1916, which constituted about half of all measurements made. Beginning with this date 100 spore measurements for length and 100 for width were made, instead of 200 for each.

As a comparative basis of dimensions in this work, the biometric mode is used in preference to the arithmetic mean. The mode represents the group containing the largest number of individuals of a certain size, thus indicating that this size is the prevailing one in a given spore population. Comparative calculations made show that, as a general rule, arithmetic means usually fluctuate around the biometric modes, as seen from Table II; and consequently there is, on the whole, but little difference between the two bases of recording. It will be seen that in most cases the figures are almost identical; in two cases they are the same; and only in one case is there a difference of 0.24 μ, which may be considered negligible, since two consecutive measurements of the same group of spores may give even greater variation. In this experiment 100 spores were measured in each case.

TABLE II.—*Correlation of biometric modes with arithemetic means of urediniospore dimensions of Puccinia graminis tritici on wheat*

Number of generations rust was confined to wheat.	Biometric modes.	Arithmetic means.
	μ	μ
1	33. 28×19. 84	33. 36×19. 69
7	32. 00×19. 84	31. 58×19. 84
10	32. 64×20. 16	32. 64×20. 02

The apparatus used for meteorological observations is fully described in the discussions of the particular experiments performed. General notes on the behavior of the various cultures were taken at the close of each urediniospore generation before transfers were made to new plants, on the average every two or three weeks. The preliminary spore measurements were made of the original rusts found on the grasses in the field, the subsequent measurements were made on the first following generation and once or twice more during the period the rust was kept in culture. For color determination Ridgway's[1] chromotaxia was used. The Zeiss screw micrometer was used for measuring the urediniospores.

[1] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C.

MORPHOLOGY OF BIOLOGIC FORMS STUDIED

The following biologic forms were investigated: *Puccinia graminis tritici* Erikss. and Henn., *P. graminis tritici-compacti* Stak. and Piem., *P. graminis secalis* Erikss. and Henn., *P. graminis avenae* Erikss. and Henn., *P. graminis phleipratensis* (Erikss. and Henn.) Stak. and Piem. and *P. graminis agrostis* Erikss.

It has been stated by Stakman and Piemeisel (*14, p. 484*) that—

> In general, the size and shape of urediniospores of different biologic forms of *Puccinia graminis* are similar. If, however, large numbers of spores are measured and the arithmetical mean or biometrical mode is determined, it becomes quite apparent that there are appreciable and fairly constant differences, provided the spores measured be taken from congenial hosts.

This was substantiated by the writers by many thousands of spore measurements and careful computations. It is necessary, however, to maintain uniform cultural conditions, since the range of variability in size of urediniospores under different conditions is sufficiently great to cause overlapping in some cases. A summary of the outstanding morphological features of the urediniospores of the biologic forms studied is given below.

P. graminis tritici.—The urediniospores are quite constant in size, shape, and color. They are the longest of all the biologic forms of *P. graminis*, but in width they exceed only slightly those of *P. graminis avenae*. Their shape is elliptic to ovoid, color light cadmium-yellow.

P. graminis tritici-compacti.—The urediniospores are very similar to those of *P. graminis tritici*, but are slightly shorter, and consequently are inclined to be ellipsoid and oval. In color they are somewhat duller.

P. graminis secalis.—The spores are uniform in size, color, and shape. The color is dull, ashy yellowish to grayish; in length they are somewhat shorter than those of *P. graminis* on oats, width approaching that of spores of *P. graminis phleipratensis;* in shape cylindric-elliptic.

P. graminis avenae.—The size and shape of the urediniospores are very variable. The shape ranges from ellipsoid to ovoid to pyriform to subplobose, even when grown on its type host, *Avena sativa*. Their color is similar to that of spores of *P. graminis tritici*.

P. graminis phleipratensis.—The spore shape is predominantly pyriform; they are very short and fairly uniform; their color is even duller and more grayish than that of spores of *P. graminis secalis*.

P. graminis agrostis.—The spores are remarkably constant in size, but are smaller than those of any other form. In color and shape they resemble spores of *P. graminis phleipratensis*, but possibly are not quite so pyriform.

The spore dimensions for the above biologic forms are given in Table III, in order to facilitate ready comparison. The "size limits" in this table show the extreme variations of all of the urediniospore dimensions

of a given form studied. The "mode averages" were obtained by finding the arithmetic mean of all modes of a given biologic form cultured on various congenial hosts.

TABLE III.—*Comparative sizes of urediniospores of biologic forms of Puccinia graminis*

Biologic form.	Size limits.	Mode averages.
	μ	μ
P. graminis tritici	23. 04–41. 92×15. 04–24. 96	32. 36×19. 82
P. graminis tritici-compacti	23. 68–40. 00×14. 40–25. 28	31. 72×19. 48
P. graminis secalis	17. 92–38. 72×13. 44–21. 44	27. 14×17. 26
P. graminis avenae	19. 20–37. 12×13. 76–25. 60	28. 48×19. 46
P. graminis phleipratensis	16. 00–32. 00×11. 84–21. 12	23. 04×17. 24
P. graminis agrostis	15. 04–31. 68×12. 16–20. 48	22. 48×15. 95

Table III shows distinctly the considerable variation in the size of spores of the different biologic forms. The uredinospores of *P. graminis tritici* are the largest of all, while those of *P. graminis tritici-compacti* are less than 1μ shorter and only a fraction of a micron narrower. The other forms vary more perceptibly. The spores of *P. graminis secalis* approach those of *P. graminis avenae* in length, the latter resembling those of *P. graminis tritici* in width. The spores of *P. graminis phleipratensis* are similar in width to those of *P. graminis secalis*, but considerably shorter; while *P. graminis agrostis* has smaller uredinospores than any other biologic form of *P. graminis* studied.

Relative to shape, the six biologic forms discussed in this paper could be classified in two principal groups; the ellipsoid-cylindric group, consisting of *P. graminis tritici*, *P. graminis tritici-compacti*, and *P. graminis secalis;* and the ovoid-subglobose group, including *P. graminis avenae*, *P. graminis phleipratensis*, and *P. graminis agrostis*. Stakman and Piemeisel (*14*) made an identical classification of these forms on the basis of their parasitism.

It is interesting to note that the morphological differences between the individual biologic forms of *Puccinia graminis* are fully as great and distinct as those between many generally recognized species of fungi. Because of similar morphological variation in certain biologic forms of *Erysiphe graminis*, expressed by distinctive characteristics in the color of the conidia and in some cases also in their size, Salmon (*9*) concluded that those forms were "incipient morphological species." The same may be true of the biologic forms of *Puccinia graminis*.

INFLUENCE OF HOST

If biologic forms of *Puccinia graminis* are incipient species, they are probably evolving gradually. If the change is sudden and accidental, finding the evidence may be merely a matter of chance. If, however,

the change is a gradual one, it is reasonable to hope that some evidence of this change may be obtained by the methods used in the present work.

Two lines of work were pursued: (1) Attempts were made to develop a number of morphological strains of a given biologic form by culturing it for fairly long periods of time on several different hosts, and (2) attempts were made to unify spore sizes of different biologic forms by growing them on the same hosts. For instance, *P. graminis tritici* develops well on common wheat, barley, and on various species of Agropyron, Hordeum, and Elymus. The writers tried to ascertain whether these hosts exerted an appreciable effect on the rust when it had been confined to them for considerable periods of time. Again the *secalis*, *tritici*, and *tritici-compacti* forms grow about equally well on barley. Theoretically, therefore, it could be assumed that they ought to become morphologically similar if grown on barley long enough. In fact, all of the biologic forms discussed in this paper develop at least weakly on barley. It could be assumed that if they could all be grown on barley long enough, they would eventually become similar morphologically. The results of the effect of hosts are given on Tables IV to XII.

KEY TO TABLES IV TO XII

In Tables IV to XII the host from which the rust was originally cultured is given in the second column. Intermediate hosts refer to the hosts on which the rust had been grown up to the time the plant was inoculated. The term "intermediate host" is not used here in the sense of bridging. W, O, B, and R refer to wheat, oats, barley, and rye, respectively. Other symbols are explained when used. The number of "urediniospore generations" (successive transfers) on the host is indicated by the figure immediately following the symbol for that host. Thus, $R_2B_4R_1W_2B_5$ indicates that the rust was transferred to rye twice, then to barley four times, followed by one transfer to rye, two to wheat, and five to barley. The degree of infection is self-explanatory. The result of inoculation is given in the usual manner in the form of a fraction, the denominator showing the number of plants inoculated and the numerator the number which became infected.

ATTEMPTS TO DEVELOP MORPHOLOGIC STRAINS OF BIOLOGIC FORMS BY CULTURING ON DIFFERENT HOSTS

To determine whether or not a given biologic form of *P. graminis* has a tendency to split up into a number of different morphological strains on account of confinement for fairly long periods of time to a number of different hosts, a series of experiments was conducted with the six biologic forms indicated above. The host plants employed were very frequently of distant taxonomic relationship, but, unless they were equally congenial to the parasitic attack of the fungus, their effect was not considered when the final conclusions were drawn. The result of this phase of the work, which extended over a period of two years, is given in Tables IV to IX.

Many inoculations were made with *P. graminis tritici* on wheat, barley, rye, and *Hordeum jubatum* (Table IV). All except rye are very susceptible. On congenial hosts the spores remained quite constant in shape, size, and color, irrespective of their origin and subsequent history. On rye, however, an uncongenial host, both the uredinia and the urediniospores became appreciably smaller, especially in length.

These results are not in accordance with those of Freeman and Johnson (*3, p. 28*), who say:

> The host-plant exercises a strong influence, not only on the physiological and biological relationship, but in some cases even on the morphology of the uredospores.

The difference in results might possibly be explained by supposing that Freeman and Johnson worked with a mixed strain, or that they did not measure enough spores. Their rust, however, may actually have changed. It will be readily seen from Table IV that the writers were not able to change the dimensions more than about 1 μ, which is within the range of experimental error.

The color of the urediniospores of *P. graminis triciti* is pale cadmium-yellow; their shape predominantly elliptic to ovoid; size limits 23 to 42 by 15 to 25 μ, and average modes 32.36 by 19.82 μ.

Results obtained by Stakman and Piemeisel (*14*) showed that the biologic form of rust, *P. graminis tritici-compacti*, discovered west of the Rocky Mountains on several different grasses and on club wheat, varied parasitically from *P. graminis tritici*, found east of the Rockies. Many common *aestivum* wheats, such as Haynes Bluestem and Fife, are resistant to this biologic form, while barley is fairly tolerant and the club wheats inoculated and Pacific Bluestem are very susceptible. Spore measurements of over a dozen strains (Table V) indicate that, whereas the spore sizes on the susceptible hosts vary but little (less than 1 μ) from those of *P. graminis tritici*, yet they are on the average nearly 2 μ shorter on the tolerant hosts and almost 4 μ shorter on the resistant ones. The width of the spores does not seem to be influenced by the host.

Identical results were obtained with *P. graminis tritici-compacti*, found in the summer of 1917 in Louisiana and Alabama, on several soft wheats. The constancy of size is remarkable, and, like the western strain, the southern strain, too, exhibits a special affinity for club wheats, while the most of the hard wheats are resistant.

The color of the urediniospores of *P. graminis tritici-compacti* is practically the same as that of *P. graminis tritici*. The spores are slightly shorter, and ovoid to ellipsoid in shape.

TABLE IV.—*Effect of various hosts on the morphology of urediniospores of Puccinia graminis tritici*

[Hj=Hordeum jubatum. x=Long time association with host; number of urediniospore generations indefinite]

Experiment No.	Original host.	Intermediate host.	Plant inoculated.	Degree of infection.	Result.	Spore dimensions.	
						Size limits.	Modes.
						μ	μ
1	*Hordeum jubatum*	None	*Triticum aestivum*	Heavy	16/16	23. 04–40. 32×15. 68–24. 96	31. 68×19. 84
2	do	do	do	do	2/31	23. 04–40. 32×15. 04–23. 04	32. 32×19. 52
3	do	W_4	do	do	33/33	23. 04–38. 08×16. 00–22. 40	32. 00×19. 52
4	do	W_7	do	do	26/27	25. 60–40. 96×16. 96–23. 36	32. 96×20. 16
5	*Agropyron smithii*	$W_2B_{14}Hj_1$	do	do	19/19	25. 60–39. 68×16. 96–22. 72	32. 64×19. 84
6	*Triticum aestivum*	W_x	do	do	13/14	25. 60–40. 32×16. 32–23. 36	32. 96×19. 84
7	*Hordeum jubatum*	W_2	*Hordeum vulgare*	do	22/22	24. 92–40. 00×16. 00–24. 96	32. 00×20. 48
8	do	W_2B_5	do	Moderate	23/23	26. 24–38. 72×15. 36–23. 04	31. 36×19. 20
9	do	W_2B_8	do	Heavy	31/31	25. 60–38. 72×16. 32–22. 72	32. 00×19. 52
10	do	W_5	do	do	16/18	24. 00–40. 64×16. 64–23. 68	32. 32×20. 16
11	do	W_5B_2	do	do	25/25	24. 32–40. 96×16. 32–23. 36	32. 64×19. 84
12	do	B_{35}	do	do	27/27	25. 60–38. 72×16. 64–23. 04	32. 32×19. 84
13	do	$R_2B_4R_1W_2B_5$	do	do	28/28	25. 28–40. 64×16. 64–23. 04	32. 96×19. 84
14	*Agropyron tenerum*	B_{14}	do	do	26/26	25. 60–38. 40×15. 04–23. 36	32. 00×19. 20

15	*A. smithii*	W_2B_{14}	do	do	$\frac{30}{30}$	23. 68–41. 92×17. 28–22. 72	32. 64×19. 84
16	*Hordeum jubatum*	W_5	*Hordeum jubatum*	do	$\frac{23}{26}$	24. 96–40. 32×17. 28–23. 36	32. 64×20. 16
17	do	W_5H_{j2}	do	Moderate	$\frac{19}{19}$	24. 64–40. 64×17. 60–23. 04	32. 64×20. 16
18	*Agropyron smithii*	$W_2B_{14}H_{j1}$	*Secale cereale*	Weak	$\frac{2}{14}$	23. 04–36. 48×16. 96–23. 04	30. 08×19. 84

TABLE V.—*Effect of various hosts on the morphology of urediniospores of Puccinia graminis tritici-compacti*

[C=Club wheat. M=Marquis wheat]

Experiment No.	Original host.	Place of collection.	Intermediate hosts.	Plant inoculated.	Degree of infection.	Result.	Spore dimensions.	
							Size limits.	Modes.
							μ	μ
1	*Triticum compactum*	Pullman, Wash.	B_3C_1	Pacific Bluestem wheat.	Heavy	10/11	26.24–37.44×16.96–22.08	31.68×19.52
2	do	do	B_3C_1	*Triticum compactum.*	do	26/30	25.60–38.08×14.40–24.00	32.00×19.20
3	*Elymus condensatus*	do	W_1C_3	do	do	28/31	25.28–37.76×14.40–25.28	31.68×19.52
4	do	Ritzville, Wash.	W_2	do	do	57/58	24.96–37.76×16.64–22.72	31.68×19.84
5	*Hordeum vulgare*	Corvallis, Ore.	C_2	do	do	9/10	24.64–37.76×16.00–22.40	31.68×19.20
6	*Secale cereale*	Baton Rouge, La.	B_4C_3	do	Moderate	3/5	24.96–37.76×15.68–23.36	31.68×19.52
7	*Triticum aestivum*	Brundidge, Ala.	$B_2W_1C_3$	do	Heavy	10/18	24.00–38.72×16.64–22.40	31.36×19.52
8	*Elymus glaucus*	Ellensburg, Wash.	$W_1B_2M_1B_1$	*Hordeum vulgare*	do	24/24	24.00–36.16×16.96–21.76	30.08×19.52
9	*Hordeum jubatum*	Pullman, Wash.	None	do	do	31/31	24.00–37.76×16.00–22.72	30.72×19.52
10	*Elymus glaucus*	Ellensburg, Wash.	do	*Triticum aestivum*	do	16/17	21.44–36.48×15.68–22.08	26.56×18.88
11	do	do	W_1B_1	do	do	30/36	19.84–34.56×17.28–21.76	27.84×19.52
12	*Triticum compactum*	do	$B_2W_1C_1$	do	do	13/15	22.40–33.92×16.00–23.36	28.16×19.52
13	*Triticum aestivum*	Brundidge, Ala.	$B_2W_1C_3$	do	do	10/16	21.44–33.92×16.96–22.72	28.16×19.52

14	*Triticum compactum*	Pullman, Wash...	B_3C_1.....	Turkey (Minn.,829)	...do.....	$\frac{9}{10}$	23. 04–33. 60×17. 28–22. 72	28. 48×19. 84
15	.....do.....	.....do.....	B_3C_1.....	*Triticum dicoccum*..	...do.....	$\frac{7}{11}$	23. 36–34. 24×16. 32–23. 04	28. 80×19. 52
16	*Elymus glaucus*.....	.....do.....	None.....	*Secale cereale*.....	...do.....	$\frac{4}{27}$	21. 12–36. 16×16. 32–22. 40	28. 16×19. 20

TABLE VI.—*Effect of various hosts on the morphology of urediniospores of Puccinia graminis secalis*

[Ar=*Agropyron repens*; At=*A. tenerum*; Ec=*Elymus canadensis*; Er=*E. robustus*; Ev=*E. virginicus*; Hj=*Hordeum jubatum*; x=Long-time association with host; number of urediniospore generations indefinite]

Experiment No.	Original host.	Intermediate hosts.	Plant inoculated.	Degree of infection.	Result.	Spore dimensions.	
						Size limits.	Modes.
						μ	μ
1	*Agropyron repens*	None	*Secale cereale*	Heavy	21/23	20. 16–38. 72×13. 44–21. 44	28. 16×17. 28
2	do	R_4	do	Moderate	26/42	17. 92–32. 00×13. 76–20. 16	25. 60×17. 28
3	do	R_7	do	Heavy	27/29	21. 12–33. 28×14. 72–20. 16	26. 88×17. 28
4	*Hordeum jubatum*	None	do	do	24/27	19. 84–36. 48×14. 08–20. 80	27. 20×16. 96
5	do	R_4	do	Weak	22/28	21. 12–34. 24×13. 44–21. 12	26. 56×17. 28
6	do	R_7	do	Heavy	27/29	19. 20–34. 88×14. 40–20. 16	27. 20×17. 28
7	*Secale cereale*	R_x	do	Moderate	24/25	20. 48–33. 92×14. 08–19. 52	27. 52×16. 64
8	*Agropyron repens*	None	*Hordeum vulgare*	Weak	13/33	19. 20–34. 88×15. 04–19. 84	26. 24×17. 28
9	do	R_5	do	Moderate	18/20	19. 52–35. 20×14. 72–19. 84	27. 52×17. 28
10	do	R_5B_2	do	do	26/29	23. 04–33. 28×13. 76–20. 48	28. 16×16. 96
11	*Hordeum jubatum*	R_5	do	Weak	16/18	19. 20–34. 88×14. 40–20. 16	27. 20×17 28
12	do	R_5B_2	do	Moderate	25/25	21. 76–33. 92×14. 72–19. 52	27. 52×16. 96
13	*Agropyron repens*	B_{13}	do	do	12/12	20. 48–37. 44×15. 36–19. 20	28. 80×17. 28

14	*Agropyron smithii*.....	$R_2B_4R_2B_3$.....	.....do.....	...do.....	$\frac{15}{16}$	21. 44–35. 52×14. 40–19. 84	28. 16×16. 96
15	*Hordeum jubatum*.....	$R_1W_1R_6B_6$.....	.....do.....	...do.....	$\frac{16}{21}$	22. 72–32. 96×14. 40–19. 84	27. 84×17. 28
16	*Hystrix patula*.....	$R_2B_4R_2B_6$.....	.....do.....	...do.....	$\frac{13}{17}$	21. 76–32. 32×15. 04–20. 16	27. 20×17. 60
17	.....do.....	$R_2B_4R_2B_3Ar_1B_1$..... $Ev_1At_4B_5$.....	....do.....	Weak.....	$\frac{38}{43}$	21. 44–32. 32×15. 04–20. 16	27. 20×17. 60
18	.....do.....	$R_2B_4R_2B_3Ar_1B_1$..... $Ev_1At_4B_7$.....	....do.....	Moderate...	$\frac{18}{25}$	22. 08–33. 60×13. 44–21. 44	27. 20×17. 60
19	*Hordeum jubatum*.....	None.....	*Hordeum jubatum*.....	...do.....	$\frac{9}{11}$	20. 48–35. 52×13. 76–20. 16	26. 56×16. 96
20	.....do.....	R_5.....	.....do.....	...do.....	$\frac{9}{18}$	18. 88–35. 20×14. 08–20. 48	27. 20×17. 28
21	.....do.....	R_5Hj_2.....	.....do.....	...do.....	$\frac{22}{22}$	20. 48–33. 60×14. 08–20. 16	27. 52×16. 96
22	.....do.....	Hj_4.....	.....do.....	...do.....	$\frac{14}{18}$	19. 84–32. 00×13. 44–21. 12	25. 92×16. 96
23	*Agropyron repens*.....	None.....	*Agropyron repens*.....	Weak.....	$\frac{3}{13}$	17. 92–32. 96×14. 08–20. 48	25. 92×17. 28
24	.....do.....	Ar_4.....	.....do.....	Moderate...	$\frac{23}{29}$	19. 52–33. 92×15. 04–20. 16	25. 60×17. 60
25	.....do.....	Ar_7.....	.....do.....	Heavy.....	$\frac{23}{23}$	19. 52–35. 52×14. 40–20. 16	27. 52×17. 28
26	.....do.....	$B_1R_1B_7Er_{12}Ec_5Ev_5$....	*Elymus virginicus*.....	...do.....	$\frac{67}{67}$	21. 76–33. 28×15. 04–20. 16	27. 52×17. 60
27	*Hystrix patula*.....	$R_2B_4R_2B_3Ar_1B_1Ev_1$....	*Hystrix patula*.....	...do.....	$\frac{22}{22}$	21. 12–32. 96×15. 04–20. 48	27. 20×17. 60
28	.....do.....	$R_2B_4R_2B_3Ar_1B_1$..... Ev_1At_{10}.....	*Agropyron tenerum*....	Weak.....	$\frac{60}{63}$	18. 88–34. 24×14. 72–20. 48	26. 88×17. 60

TABLE VII.—*Effect of various hosts on the morphology of urediniospores of Puccinia graminis avenae*

[Pp=*Phleum pratense;* Dg=*Dactylis glomerata;* Bt=*Bromus tectorum;* X=Long-time association with host; number of urediniospore generations indefinite]

Experiment No.	Original host.	Intermediate hosts.	Plant inoculated.	Degree of infection.	Result.	Spore dimensions.	
						Size limits.	Modes.
						μ	μ
1	*Dactylis glomerata*	None	*Avena sativa*	Heavy	21/21	20. 80–37. 12×16. 00–25. 60	27. 84×19. 84
2	do	do	do	Moderate	4/35	19. 20–35. 20×13. 76–23. 04	26. 88×18. 56
3	do	O_5	do	Heavy	19/19	22. 08–34. 88×16. 00–22. 40	28. 48×19. 20
4	do	O_8	do	do	25/25	21. 12–36. 48×16. 32–23. 04	29. 12×19. 52
5	do	O_{22}	do	do	23/23	19. 52–36. 48×16. 00–23. 36	28. 48×20. 16
6	do	$O_{33}Pp_1$	do	1 uredinium.	1/5	22. 72–36. 16×16. 00–22. 72	29. 44×19. 52
7	*Avena sativa*	O_x	do	Heavy	22/23	23. 04–35. 20×16. 96–22. 08	29. 12×19. 52
8	*Dactylis glomerata*	None	*Dactylis glomerata*	do	18/18	19. 20–32. 96×16. 00–24. 00	25. 92×19. 84
9	do	do	do	do	11/15	18. 56–35. 20×15. 36–23. 36	26. 24×19. 52
10	do	O_6	do	do	11/12	19. 20–32. 00×16. 32–22. 40	25. 60×19. 52
11	do	O_6Dg_2	do	do	19/20	20. 16–31. 68×16. 64–22. 08	25. 92×19. 52
12	do	O_6	*Bromus tectorum*	Weak	10/10	18. 56–29. 12×16. 64–21. 12	23. 68×18. 88
13	do	O_6–Bt_2	do	do	5/12	19. 20–28. 80×16. 32–21. 12	23. 68×18. 88

14	.....do.....	O_{31}.....	*Phleum pratense*.....	...do.....	$\frac{5}{50}$	20. 16–32. 64× (a).....	25. 60× (a)
15	.....do.....	O_{33}.....	*Hordeum vulgare*.....	...do.....	$\frac{3}{15}$	20. 80–32. 96× (a).....	25. 60× (a)
16	.....do.....	O_{36}.....	*Alopecurus pratensis*..	Heavy.....	$\frac{60}{60}$	19. 84–33. 60×16. 96–22. 72..	26. 56×19. 84
17	.....do.....	O_{36}.....	*Lolium temulentum*...	Moderate..	$\frac{4}{20}$	19. 52–33. 60×16. 64–22. 40..	26. 56×19. 52
18	.....do.....	O_{36}.....	*Hordeum pusillum*....	Weak.....	$\frac{4}{28}$	19. 20–30. 40×17. 28–21. 76..	24. 32×19. 52

[a] Widths of the urediniospores were not measured.

TABLE VIII.—*Effect of various hosts on the morphology of urediniospores of Puccinia graminis phleipratensis*

[Dg=*Dactylis glomerata;* Pp=*Phleum pratense;* x=Long association with host; number of urediniospore generations indefinite]

Experiment No.	Original host.	Intermediate hosts.	Plant inoculated.	Degree of infection.	Result.	Spore dimensions.	
						Size limits.	Modes.
1	*Phleum pratense*	None	*Phleum pratense*	Heavy	25/25	17. 92–29. 44×13. 12–21. 12 μ	23. 68×17. 28 μ
2	do	Pp_5	do	Moderate	18/24	16. 00–28. 80×13. 44–20. 16	21. 76×16. 96
3	do	Pp_8	do	Heavy	23/23	16. 32–29. 76×14. 40–19. 84	23. 04×17. 28
4	*Dactylis glomerata*	None	do	Weak	4/35	16. 64–28. 80×12. 80–18. 88	22. 08×16. 96
5	do	Pp_5	do	Heavy	13/13	16. 32–29. 12×13. 76–19. 84	22. 08×17. 28
6	do	Pp_8	do	do	20/21	18. 24–28. 48×14. 40–19. 20	23. 04×16. 96
7	*Phleum pratense*	Pp_x	do	do	18/18	17. 60–29. 44×13. 12–20. 48	24. 00×16. 96
8	do	None	*Dactylis glomerata*	Moderate	22/22	16. 64–29. 12×13. 76–20. 80	22. 08×17. 28
9	do	Pp_6	do	do	4/8	16. 32–28. 48×14. 40–20. 16	22. 40×17. 28
10	do	Pp_6Dg_2	do	do	18/20	19. 20–28. 48×14. 08–19. 84	24. 00×16. 96
11	*Dactylis glomerata*	Pp_6	do	do	9/12	16. 96–29. 44×13. 76–20. 48	23. 36×17. 28
12	do	Pp_6Dg_2	do	do	17/19	18. 56–28. 80×13. 44–19. 84	23. 68×16. 64

13	*Phleum pratense*	None	*Avena sativa*	Weak	$\frac{14}{53}$	17. 60–30. 08×14. 72–20. 48	22. 72×17. 60
14	....do	Pp_1	....do	....do	$\frac{6}{12}$	16. 96–32. 00×13. 76–20. 48	23. 68×17. 28
15	....do	Pp_8	....do	....do	$\frac{8}{21}$	17. 92–28. 16×15. 04–20. 80	23. 04×17. 92
16	*Dactylis glomerata*	Pp_6	....do	....do	$\frac{2}{13}$	17. 60–31. 36×11. 84–21. 12	23. 68×17. 28
17	....do	Pp_6O_2	....do	....do	$\frac{1}{8}$	18. 56–28. 48×14. 72–20. 48	23. 36×17. 92
18	*Phleum pratense*	B_2	*Hordeum vulgare*	....do	$\frac{1}{1}$	14. 08–27. 20×11. 20–17. 60	21. 76×14. 08
19	....do	B_2	....do	....do	$\frac{1}{9}$	14. 08–25. 92×11. 52–17. 28	20. 80×14. 40

A rather distinct and consistent specificity in shape and color was exhibited by the rye rust urediniospores regardless of the host on which they developed, length of time confined to it, or of origin and subsequent history. As seen from Table VI, the spore modes average more than 5 μ shorter and about 2.5 μ narrower than those of the spores of *P. graminis tritici;* the shape is cylindric-elliptic, and the color dull, ashy-yellowish to grayish. The size of the spores is perceptibly affected by cultural conditions, but not by the host plants. Under the same circumstances the spores were in each case distinctly and consistently smaller than those of *P. graminis tritici.* During the cloudy and cold weather of midwinter and the excessive heat of the summer months the spores grew only to their minimum size and again attained their maximum size in spring and fall.

P. graminis avenae (Table VII) was thought to be an especially interesting form for study in the attempt to determine the amount of variation which could be induced by growing it on different congenial hosts, because it appeared from inoculation results that this biologic form might possibly be considered as plastic. In contrast to *P. graminis tritici, P. graminis tritici-compacti,* and *P. graminis secalis,* the urediniospores of which are, as a rule, of a definite specific size and shape when grown on a congenial host, those of *P. graminis avenae* are very variable even when parasitizing oats. They may be ellipsoid, ovoid, pyriform, or subglobose in shape. In size they are somewhat longer than those of *P. graminis secalis,* and in width approach those of *P. graminis tritici.* The spore color is bright cadmium-yellow.

On *Dactylis glomerata* the spores show a tendency to shorten, the width remaining practically the same as on oats. On *Bromus tectorum,* which is only a tolerant host, the spores decreased both in length and width, becoming nearly globose. The presence of equatorial germ pores, however, showed clearly that the rust was *P. graminis.* Both barley and timothy are very uncongenial hosts for *P. graminis avenae* and both uredinia and spores are very small (*13*). This is similar to the behavior of *P. graminis tritici* on resistant varieties of wheat on which Stakman (*11, p. 31*) found the spores to be smaller than on the susceptible wheat varieties. Similar results were obtained by the writers with *P. graminis tritici-compacti* (see Table V).

It will thus be seen that the size and shape of *P. graminis avenae* are quite easily influenced by the host on which they grow; the color, however, remains constant. The size of uredinia is directly proportional to the size of the spores, and vice versa. This morphological variation is interesting because the rust is also versatile parasitically.

TABLE IX.—*Results showing the effect of various hosts on the morphology of urediniospores of Puccinia graminis agrostis*

[Aa=*Agrostis alba*]

Experiment No.	Original host.	Place of collection.	Intermediate host.	Plant inoculated.	Degree of infection.	Result.	Spore dimensions.	
							Size limits.	Modes.
							μ	μ
1	*Agrostis alba*....	Crookston, Minn..	Aa_1.....	*Agrostis alba*.....	Heavy.....	$\frac{50}{50}$	18. 88–26. 56×13. 76–18. 24	22. 72×16. 00
2	.....do.....	.....do.....	Aa_7.....	.....do.....	.....do.....	100%	17. 60–28. 48×14. 08–18. 88	22. 72×16. 32
3	.....do.....	.....do.....	Aa_7.....	*Alopecurus pratensis*.....	Moderate to heavy.	$\frac{20}{30}$	16. 32–28. 80×13. 12–18. 24	22. 40×15. 68
4	.....do.....	.....do.....	Aa_7.....	*Holcus lanatus*.....	Moderate..	$\frac{35}{45}$	17. 92–27. 20×13. 44–17. 92	22. 40×15. 68
5	.....do.....	.....	None.....	*Hordeum vulgare*.....	Weak.....	$\frac{7}{26}$	15. 04–30. 08×13. 12–18. 56	22. 40×16. 00
6	.....do.....	.....	...do.....	*Secale cereale*.....	.....do.....	$\frac{2}{17}$	18. 24–25. 92×13. 44–17. 28	21. 44×16. 00
7	.....do.....	Long Beach, Cal..	...do.....	*Avena sativa*.....	.....do.....	$\frac{4}{36}$	15. 68–28. 48×12. 16–20. 16	22. 08×16. 00

TABLE X.—*Results of using Hordeum vulgare as a common host to unify the size of uredinisopores of Puccinia graminis tritici and Puccinia graminis secalis*

[Hj=*Hordeum jubatum*]

Experiment No.	Biologic form.	Original host.	Subsequent hosts.	Degree of infection.	Result.	Spore dimensions.	
						Size limits.	Modes.
						μ	μ
1	*P. graminis tritici*	*Hordeum jubatum*	W_2B_1	Heavy	22/22	25. 92–40. 00×16. 00–24. 96	32. 00×20. 48
2	do	do	W_2B_6	Moderate	23/23	26. 24–38. 72×15. 36–23. 04	31. 36×19. 20
3	do	do	W_2B_9	do	31/31	25. 60–38. 72×16. 32–22. 72	32. 00×19. 52
4	do	do	Hj_5B_1	do	16/18	24. 00–40. 64×16. 64–23. 68	32. 32×20. 16
5	do	do	Hj_5B_3	do	25/25	24. 32–40. 96×16. 32–23. 36	32. 64×19. 84
6	do	do	B_{36}	Heavy	27/27	25. 60–38. 72×16. 64–23. 04	32. 32×19. 84
7	do	do	$R_2B_4R_1W_2B_6$	do	28/28	25. 28–40. 64×16. 64–23. 04	32. 96×19. 84
8	do	*Agropyron tenerum*	B_{15}	do	26/26	25. 60–38. 40×15. 04–23. 36	32. 00×19. 20
9	do	*Agropyron smithii*	W_2B_{15}	do	30/30	23. 68–41. 92×17. 28–22. 72	32. 64×19. 84
10	*P. graminis secalis*	*Agropyron repens*	None	Weak	13/33	19. 20–34. 88×15. 04–19. 84	26. 24×17. 28
11	do	do	R_5B_1	Moderate	18/20	19. 52–35. 20×14. 72–19. 84	27. 52×17. 28
12	do	do	R_5B_3	do	26/29	23. 04–33. 28×13. 76–20. 48	28. 16×16. 96
13	do	do	B_{14}	do	12/12	20. 48–37. 44×15. 36–19. 20	28. 80×17. 28

14	.....do.....	*Hordeum jubatum*.....	R_5B_1.....	Weak.....	$\frac{16}{18}$	19. 20–34. 88×14. 40–20. 16	27. 20×17. 28
15	.....do.....	.....do.....	R_5B_3.....	Moderate...	$\frac{25}{25}$	21. 76–33. 92×14. 72–19. 52	27. 52×16. 96
16	.....do.....	.....do.....	$R_1W_1R_6B_7$.....	.....do.....	$\frac{16}{21}$	22. 72–32. 96×14. 40–19. 84	27. 84×17. 28
17	.....do.....	*Hystrix patula*.....	$R_2B_4R_2B_7$.....	.....do.....	$\frac{13}{17}$	21. 76–32. 32×15. 04–20. 16	27. 20×17. 60
18	.....do.....	*Agropyron smithii*....	$R_3B_4R_2B_4$.....	.....do.....	$\frac{15}{16}$	21. 44–35. 52×14. 40–19. 84	28. 16×16. 96

Johnson (*6, p. 8*) gives the urediniospore dimensions of timothy-rust as 18 to 27 μ in length and 15 to 19 μ in width. Stakman and Jensen (*12, p. 214*) found that on timothy the spores ranged from 17 to 31 μ in length and from 14.5 to 23 μ in width, the modes falling at about 26 and 18 μ. On barley they found them to be smaller than those produced on any other host, ranging from 18.5 to 28.3 μ in length and from 13 to 20 μ in width, with modes at about 23 and 17 μ. In the present work it was found that all the modes, except those of spores cultured on barley, fluctuated about those of a strain of *P. graminis phleipratensis*, which had been confined to timothy for more than a year. The modes fluctuated about 24 and 17 μ, varying perceptibly with the existing climatic and edaphic conditions.

That barley is not a congenial host for timothy-rust is shown by the slight virulence of infection (Table VIII), very small uredinia, and considerably decreased size of the urediniospores, with average mode of 21.28 by 14.24 μ. The results of inoculation with *P. graminis phleipratensis*, obtained from two different sources—timothy (*Phleum pratense*) and orchard-grass (*Dactylis glomerata*)—and grown on three different hosts (timothy, orchard-grass, and oats), show (Table VIII) that the urediniospores retain their characteristic size, except for small negligible variations, whether parasitizing very congenial or merely tolerant hosts. The spore shape is predominantly pyriform, and the color is dull, dirty yellow to grayish.

The infection capabilities of *P. graminis agrostis* are similar to those of *P. graminis phleipratensis* and *P. graminis avenae* The urediniospores of this rust are the smallest of all the biologic forms of *P. graminis*, especially in width. In shape and color they resemble very closely timothy-rust spores although not quite so dominantly pyriform. The spore dimensions are given in Table IX.

The results given in Table IX show clearly that the influence of host on the size of the spores was negligible.

Attempts to Unify Spore Sizes of Different Biologic Forms by Culturing Them on the Same Host

P. graminis tritici has a number of hosts in common with *P. graminis secalis*. *P. graminis avenae* and *P. graminis phleipratensis* also have several hosts in common. The work was directed toward an attempt to determine whether the spore morphology of these biologic forms could be made identical by the use of common hosts. Table X gives the results of using *Hordeum vulgare* as a common host to unify *P. graminis tritici* and *P. graminis secalis*, and in Table XI are given the results of using *Avena sativa* as a common host for *P. graminis avenae* and *P. graminis phleipratensis*. A condensed tabulated summary of these results is given in Table XII.

TABLE XI.—*Results of using Avena sativa as a common host to unify the size of urediniospores of Puccinia graminis avenae and Puccinia graminis phleipratensis*

[*Pp*=*Phleum pratense*]

Experiment No.	Biologic form.	Original host.	Subsequent hosts.[1]	Degree of infection.	Result.	Spore dimensions.	
						Size limits.	Modes.
						μ	μ
1	*P. graminis avenae*	*Dactylis glomerata*	O_1	Heavy	21/21	20. 80–37. 12×16. 00–25. 60	27. 84×19. 84
2	do	do	O_1	Moderate	4/35	19. 20–35. 20×13. 76–23. 04	26. 88×18. 56
3	do	do	O_6	Heavy	19/19	22. 08–34. 88×16. 00–22. 40	28. 48×19. 20
4	do	do	O_8	do	25/25	21. 12–36. 48×16. 32–23. 04	29. 12×19. 52
5	do	do	O_{22}	do	32/32	19. 52–36. 48×16. 00–23. 36	28. 48×20. 16
6	*P. graminis phleipratensis*	*Phleum pratense*	O_1	Weak	14/53	17. 60–30. 08×14. 72–20. 48	22. 72×17. 60
7	do	do	Pp_1O_1	do	6/12	16. 96–32. 00×13. 76–20. 48	23. 68×17. 28
8	do	do	Pp_8O_1	do	8/21	17. 92–28. 16×15. 04–20. 80	23. 04×17. 92
9	do	*Dactylis glomerata*	Pp_6O_1	do	2/13	17. 60–31. 36×11. 84–21. 12	23. 68×17. 28
10	do	do	Pp_6O_3	do	1/8	17. 60–28. 48×14. 72–20. 48	23. 36×17. 92

TABLE XII.—*Comparative sizes of urediniospores of Puccinia graminis as affected by common hosts. Summary of Tables X and XI*

Biologic form.	Host.	Size limits.	Mode averages.
		μ	μ
P. graminis tritici	*Hordeum vulgare*	24. 00–41. 92×15. 04–24. 96	32. 25×19. 77
P. graminis secalis	do	19. 20–37. 44×13. 76–20. 48	27. 63×17. 21
P. graminis avenae	*Avena sativa*	19. 20–37. 12×13. 76–25. 60	28. 16×19. 46
P. graminis phleibratensis.	do	16. 96–32. 00×11. 84–21. 12	23. 29×17. 60

It is evident from Table XII that barley can not change or unify the wheat and rye forms; neither can oats do so with the oats and timothy forms. This substantiates the writers' results on the constancy and stability of the biologic forms of *P. graminis* in general. Only uncongenial hosts appear to have the property of changing the morphology of urediniospores as expressed by size or shape. And even in such cases the urediniospores resume their original size and shape when grown again on congenial hosts.

EFFECT OF PHYSICAL FACTORS

The attempts to change the spore morphology by means of physical factors are given in Tables XIII to XVII. While some of the observations on the effect of these factors on the development of the rust are repetitions of those previously made by other investigators and while some of the others may seem perfectly obvious, they are made to show the correlation, if any exists, between the vigor of the fungus and the morphological characters of the spores. At the same time some of the results on the development of the rust under various conditions are valuable in themselves.

EFFECT OF TEMPERATURE

In the present experiment on temperature wheat seedlings were inoculated with fresh urediniospores of *P. graminis tritici* in the usual manner, given normal germination conditions, and then exposed to various temperatures. The high temperature was obtained by means of an electric heater put under a glass bell jar where the plants were kept continuously. For low temperature the plants under a bell jar, as in the above case, were kept either in an unheated greenhouse or outside, according to existing conditions. The temperatures were recorded by thermographs from which the records were then computed. A set of control plants was kept under normal greenhouse conditions (Table XIII).

TABLE XIII.—*Results showing the effect of temperature on the physiology and morphology of urediniospores of Puccinia graminis tritici on wheat*

Experiment No.	Temperature. Daily mean. Maximum.	Daily mean. Minimum.	Average for generation.	Degree of infection.	Result.	Spore dimensions. Size limits.	Spore dimensions. Modes.
	°F.	°F.	°F.			μ	μ
1	92.3	76.4	81.8	Moderate.....	13/13	23.04–36.48×16.32–24.00...	29.44×19.84
2	103.5	71.1	79.5	.....do.....	21/21	23.04–40.00×16.32–24.96...	30.72×20.16
3	78.0	61.0	69.7	Heavy.....	24/24	25.92–40.00×16.96–23.36...	32.64×20.16
4	79.4	63.2	69.3	.....do.....	18/18	22.40–40.32×16.32–23.68...	32.00×19.84
5	89.7	52.3	66.4	.....do.....	16/18	25.60–40.00×16.00–23.04...	33.28×19.84
6	72.0	40.8	55.8	.....do.....	16/16	25.28–40.00×18.24–22.72...	32.64×20.48
7	62.9	49.3	55.8	.....do.....	12/13	26.24–40.00×16.96–22.72...	31.36×19.84

It was found that the most favorable temperature for shortening the incubation period, hastening the maturity and obtaining a vigorous infection, appeared to be between 66.5° and 70° F. Fromme's tabulation (*4, p. 507–509*) shows that this is in accordance with the results obtained by Wüthrich on *P. graminis* and by Ward on *P. dispersa*. This temperature is also the optimum for the production of the largest urediniospores. The reason the spores in No. 6, Table XIII, became so large is on account of the high maximum temperature.

At this temperature (66.5°–70° F.) rust flecks appeared in from five to seven days and uredinia developed within another day or two. At a higher temperature the development of the uredinia was retarded at the rate of one day for every 10 degrees of rise of temperature, but rust developed at as high temperatures as the host endured, although the size of the spores was considerably decreased. At low temperatures the development of the uredinia was retarded at the rate of one day for every 5 degrees of fall in temperature. Infection resulted at as low temperatures as the host could possibly stand. The spores were rather small, but the difference was not as great as in the case of high temperatures, with moderate temperature as a basis for comparison.

The uredinia produced under high temperatures were darker in color than those produced under moderate temperatures, while those produced at low temperatures were lighter than those produced at moderate temperatures. The color of the uredinia developed at high temperatures varied from Brussels-brown to argus-brown; at moderate temperature it varied from Sudan-brown or antique-brown to Brussels-brown, while at low temperature from amber-brown to Sanford's-brown.

Effect of Humidity

Plants for this experiment on humidity (Table XIV) were placed under two glass bell jars, under one of which there were exposed three beakers filled with water to secure a humid atmosphere; under the other bell jar three plates with calcium chlorid were placed to absorb the moisture in the air and that of transpiration by the plants. To prevent the evaporation of moisture from the soil in one case and the absorption of moisture by the soil in the other case, the surface of the pots was covered with a paraffin layer before they were placed under the jar. A third set of plants was kept as a control under normal greenhouse conditions. For the purpose of determining the relative humidity, hygrometers were employed for each set, and readings were taken daily; at the same time barometric readings in another end of the same building were being taken.

Table XIV.—*Results showing the effect of humidity on the physiology and morphology of urediniospores of Puccinia graminis tritici on wheat*

Experiment No.	Humidity.			Degree of infection.	Result.	Spore dimensions.	
	Daily limits.		Total average.			Size limits.	Modes.
	Maximum.	Minimum.					
	Per ct.	*Per ct.*	*Per ct.*			μ	μ
1	92.5	76.5	85.3	Moderate	22/23	23.36–40.32×16.64–23.04	32.96×19.84
2	67.5	47.0	60.4	Heavy	16/18	25.60–40.00×16.00–23.04	32.28×19.84
3	54.0	50.5	52.3	Weak	23/25	24.96–40.32×15.36–24.32	32.00×20.16
4	97.0	91.0	94.6	Moderate	5/6	24.96–40.32×15.68–24.96	32.32×20.80
5	83.5	73.0	78.5	.....do	18/18	22.40–40.32×16.32–23.68	32.00×19.84
6	66.5	59.5	62.2	.....do	7/7	22.72–39.36×15.68–24.64	30.40×19.84
7	95.0	90.5	93.4	Heavy	12/12	24.00–40.00×16.64–24.00	32.00×20.16
8	80.0	68.5	74.3	.....do	20/20	25.28–40.32×16.96–23.04	32.64×19.84
9	76.0	67.5	71.0	.....do	12/12	23.68–39.04×15.36–24.00	31.36×19.52

From the results shown in Table XIV, it would seem as though either excessively high or excessively low humidity causes a decrease in size of spores, but the difference, however, is neither very pronounced nor consistent. This can be explained by the fact that the inoculated plants were placed under the restricted conditions only subsequent to the usual confinement of 48 hours in the moist chambers. The germination period would thus appear to constitute the critical period, the difference in humidity thereafter being apparently of lesser importance.

The uredinia were generally larger on the control plants and smaller on those grown in the dry air. The color of the uredinia grown in humid atmosphere was commonly antique-brown or amber-brown, those produced in the arid atmosphere were amber to argus-brown, while on the control plants they were Sudan to Brussels-brown; in other words, they were darkest in the dry air and lightest in the moist air.

Effect of Soil Moisture

In this experiment on soil moisture three series of plants were employed, one of which was very heavily watered, the second moderately, and the third received only enough water to prevent the plants from wilting. Otherwise, the usual methods of innoculation, germination, and incubation were used. The water content of the soil was determined on the basis of the oven-dried method.

The plants in the wet soil were more severely attacked, and the urediniospores developed on them were larger than those in either the control or the dry series. The series that suffered from drouth produced the smallest spores. There was no apparent difference in the color of the uredinia or spores. Table XV gives the detailed results.

Table XV.—*Results showing the effect of soil moisture on the physiology and morphology of urediniospores of Puccinia graminis tritici on wheat*

Experiment No.	Soil moisture.		Degree of infection.	Result.	Spore dimensions.	
	Water applied.	Water content.			Size limits.	Modes.
	Cc.	*Per cent.*			μ	μ
1	900	32.14	Heavy	20/20	22.72–43.84×16.96–22.40	33.28×19.84
2	850	31.65	do	16/16	26.24–40.96×17.60–22.72	33.28×20.16
3	400	17.12	do	20/20	25.28–40.32×16.96–23.04	32.64×19.84
4	250	16.28	do	24/24	25.92–40.00×16.96–23.36	32.64×20.16
5	150	9.24	Moderate	18/18	24.96–38.72×16.32–23.36	32.32×19.84
6	100	5.38	do	20/20	22.40–38.08×16.64–21.76	30.40×19.20

Effect of Illumination

In testing the effect of illumination two series of plants were employed, one of which was kept beneath a double-layer muslin cage, while the other one was exposed to the direct sunlight in the greenhouse (Table XVI). As the experiment was conducted during the winter months the light was at no time exceptionally bright. The cultural conditions, except for the variation in light intensity, were maintained the same for both series. The light readings were taken daily, sometimes two and three times a day, with the Clements photometer charged with a

printing-out photographic paper. The percentage of intensity was determined by means of a standard print made at noon of a bright sunny day in the fall of 1915.

TABLE XVI.—*Results showing the effect of illumination on the physiology and morphology of urediniospores of Puccinia graminis tritici on wheat*

Experiment No.	Intensity.			Degree of infection.	Result.	Spore dimensions	
	Daily limits.		Total average.			Size limits.	Modes.
	Maximum.	Minimum.					
						μ	μ
1	35.0	7.5	16.9	Heavy	20/20	25.28–40.32×16.96–23.68...	32.64×19.84
2	28.7	3.5	15.4	.....do	16/18	25.60–40.00×16.00–23.04...	33.28×19.84
3	46.7	6.6	14.0	.....do	18/18	22.40–40.32×16.32–32.68...	32.00×19.84
4	12.5	1.7	6.6	Weak	6/7	22.72–35.84×16.96–22.08...	29.76×19.52
5	10.0	2.0	3.1	.....do	20/20	21.76–36.48×17.28–22.72...	29.12×19.84
6	2.0	0.9	1.3	Moderate	20/23	23.04–40.64×14.72–22.72...	29.76×18.88

The rust consistently developed better in fairly high intensities than in the lower ones. The size of the urediniospores, as given in Table XVI, responded in similar manner. The color of the uredinia in the shade varied from antique-brown to Sudan-brown, while of those in the light ranged from Sudan-brown to argus-brown—that is, somewhat lighter in shade than in the open. It appears that in as much as the photosynthetic activities of the host plant are affected by the light intensity in so much does the function and structure of the rust fungus depend on the same factor.

EFFECT OF EXCESSIVE NITROGENOUS FERTILIZATION

The preliminary results obtained by the writers seem to indicate that an excessive amount of sodium nitrate, inhibiting the growth of the host, also inhibits the development of the rust and dimimishes very perceptibly the size of the urediniospores, as is shown in Table XVII. This is in accord with Sheldon's (*10*) carnation rust experiments which showed that the kind of soil that favored the growth of the host also favored the attack of the rust, and that, as a rule, the period of incubation of the rust was inversely proportional to the vigor of the host. The plants were considerably shriveled by the chemical and badly dried two weeks after application. The rust, however, had made a fair start on one blade out of the eight inoculated and developed uredinia of moderate size and extent. The uredinia were darker in color than those developed under normal conditions.

TABLE XVII.—*Effect of excessive nitrogenous fertilization on the physiology and morphology of urediniospores of Puccinia graminis tritici on wheat*

Fertilizer.	Condition of host.	Degree of infection.	Result.	Spore dimensions.	
				Size limits.	Modes.
Sodium nitrate....	Shriveled and dried up	Moderate	$\frac{1}{8}$	20.80–36.80×16.00–23.04	28.48×19.52
Control.....	Healthy and thrifty...	Heavy...	$\frac{20}{20}$	25.28–40.32×16.96–23.68	32.64×19.84

EXPERIMENTS ON CULTURAL METHODS

It was thought well worth while conducting a few experiments to ascertain the effect of the age of the host plant at the time of inoculation on the rust growth and on the size of the urediniospores, also to find out the length of time during which urediniospores retain their vitality and what is the relation of the age of the fungus to the morphology of the urediniospores. The results obtained (Table XVIII) show that the susceptibility of the host is little dependent on its age, and that urediniospores retain their vitality for a considerable length of time with no perceptible variation in size.

TABLE XVIII.—*Results showing the effect of age of host plant on the morphology of urediniospores of Puccinia graminis avenae*

Experiment No.	Host plant.	Age of plants inoculated.	Spore dimensions.	
			Size limits.	Modes.
		Days.	μ	μ
1.....	*Avena sativa*.....	7	24.00–35.52×16.00–22.08	29.44×19.20
2.....	.....do.....	7	23.04–35.52×16.96–22.08	29.44×19.52
3.....	.....do.....	14	24.96–34.88×14.72–22.72	29.44×19.20
4.....	.....do.....	21	24.96–35.84×16.00–22.40	29.44×19.20
5.....	.....do.....	21	21.76–37.12×16.64–21.76	29.44×19.20
6.....	.....do.....	35	22.72–35.84×16.00–22.40	29.12×19.20

EFFECT OF THE AGE OF HOST PLANTS ON THE DEVELOPMENT OF THE RUST FUNGUS AND SIZE OF THE UREDINIOSPORES

Oats were here used as host plants, because they were found to thrive better under greenhouse conditions than wheat, barley, or rye. Inoculations were made when the plants were 7, 14, 21, and 35 days old, counting the age from date of sowing. The 7 and 21 days series were later duplicated. In all cases the plants were inoculated with a uniform amount of fresh urediniospore material of *P. graminis avenae* and cultured under similar and normal conditions.

The plants 1 week old were slightly more vigorously affected (Table XVIII) at first, but at the end of 10 days the infection was heavier on the older plants, and especially so on those that were three weeks old at the time of inoculation. From Table XVIII it will be seen that the

size of the urediniospores was remarkably uniform regardless of the age of the host; nor was there any difference in the shape and color of the spores.

The junior author has also obtained very successful infection on mature plants of more than a hundred different varieties of wheat, grown in the greenhouse and artificially inoculated with *P. graminis tritici*. This latter work was conducted at the Kansas Agricultural Experiment Station in cooperation with the United States Department of Agriculture.

EFFECT OF THE AGE OF THE RUST FUNGUS ON THE VITALITY AND MORPHOLOGY OF THE UREDINIOSPORES

Fromme (*4*, p. 504) states that De Bary found the length of time during which urediniospores of *P. graminis* retain their vitality to vary between one and two months, and that Bolley obtained a 5 per cent germination with urediniospores of *P. graminis* after exposure to air and sunlight during the month of August. The object of this experiment was to determine the vitality of the urediniospores after different periods of association with their respective hosts and effect of the length of association on the rust morphology.

For the determination of the first phase of the experiment inoculations were made (Table XIX) with rust material at different stages of the development of the uredinia. Transfers were made when the uredinia were merely beginning to break through the epidermis and two and four weeks afterwards. There was no apparent difference in the degree of infection produced by these methods of inoculation.

TABLE XIX.—*Results showing the effect of age of the fungus on the morphology of urediniospores of Puccinia graminis*

Experiment No.	Biologic form.	Age of spores measured.	Spore dimensions.	
			Size limits.	Modes.
		Days.	μ	μ
1	*P. graminis tritici*.....	8	25. 60–39. 68×16. 96–22. 72	32. 64×19. 84
2	.....do.....	14	25. 60–40. 32×16. 32–23. 36	32. 96×19. 84
3	.....do.....	22	23. 36–39. 04×15. 68–22. 72	30. 72×19. 20
4	.....do.....	63	24. 32–40. 96×16. 64–23. 04	32. 64×19. 52
5	*P. graminis avenae*.....	7	24. 96–35. 52×16. 96–23. 36	29. 44×20. 16
6	.....do.....	20	23. 04–35. 20×16. 96–22. 08	29. 12×19. 52
7	.....do.....	40	23. 04–35. 52×16. 96–22. 08	29. 44×19. 52
8	*P. graminis secalis*.....	8	22. 40–34. 24×13. 76–19. 84	28. 48×16. 96
9	.....do.....	14	20. 48–33. 92×14. 08–19. 52	27. 52×16. 64

In determining the effect of the age of the fungus on the morphology of the spores, measurements were made at different stages of the development of uredinia, beginning 7 days after inoculation and ending with 63 days. Three biologic forms of *P. graminis* were used in this experi-

ment and, as shown in Table XIX, there seemed to be no appreciable effect on the size of the spores, although the size of the uredinia gradually and persistently became larger, which was due to the additional shedding of spores and coalescence of adjacent uredinia. The color of the uredinia became darker with age and the spores lost their coherent floccose consistency and by the least disturbance were separated from the uredinia.

GENERAL DISCUSSION

The data presented in this paper provide ample evidence to show that the morphology of biologic forms is but slightly and only temporarily changed in response to biotic and physical factors. Resistant host plants and unfavorable cultural conditions affecting the normal development and vigor of the rust fungus may also affect the size of its urediniospores. But as soon as the unfavorable factors are removed the fungus resumes its normal functions and regains its original structure.

No host which is congenial to a given biologic form can, under favorable cultural conditions, exert any perceptible influence on the morphology of the rust spores. *P. graminis avenae* appears to deviate from this rule in so far as shape and size of urediniospores are concerned. The shape varies considerably on any host to which the rust may be confined, while the spore sizes appear to be peculiar of the particular host the rust parasitizes.

The attempts to split up the various biologic forms of *P. graminis* studied into a number of different morphological strains by culturing them for long periods of time on several different but definitely congenial hosts have utterly failed. The attempts to unify the spore sizes of different biologic forms by culturing them continuously for a considerable length of time on the same host were also unsuccessful.

Adverse environmental conditions, such as resistant host varieties, affect the virulence and spore size of the rust fungus. Excessive heat is more injurious to the rust growth and affects the size of urediniospores more effectively than does very low temperature. High humidity during the incubation period appears to be an indispensable condition; the difference in humidity later is probably of lesser importance. Deficiency of soil moisture and sunlight, and other ecological factors affecting the host plant unfavorably, appear to be equally unfavorable to the rust parasite.

The results show that *P. graminis* is quite stable and can not be expected to change rapidly. This is true both of its parasitic capabilities and of its morphologic characters. The facts presented in this paper give additional support to the rapidly accumulating body of data which show that the biologic forms studied are fairly constant. Whether this will apply equally well to the large number of forms recently found on varieties of wheat is a question which can be answered only by future investigation.

SUMMARY

(1) The amount of spore material used for inoculation has no perceptible effect on the result of infection or size of spores, except in so far as a more extensive area of and a greater certainty for successful infection may be secured.

(2) The optimum length of incubation period in the moist chamber is 48 hours, thereby securing all certainty of infection without causing a tendency to supersensibility.

(3) The superficial layer of each uredinium contains larger spores, and when this layer is removed the remaining spores are considerably smaller. But if the uredinium is allowed to produce a new crop of spores those on the surface again attain the same dimensions as the original ones.

(4) In spore measurements 100 spores, obtained from a number of uredinia, are representative of their group. Modes are a practical basis for comparison.

(5) Biologic forms are constant not only parasitically but also morphologically. As a general rule the morphologic differences between the various biologic forms are fully as great and distinct as between many established species of fungi. The morphologic stability of a biologic form is exhibited in the constancy of size, shape, and color of the urediniospores of the particular form. The stemrust of oats (caused by *P. graminis avenae*) is an exception to this rule in so far as the shape and size of urediniospores are concerned, these being very plastic.

(6) Common hosts which are congenial to different biologic forms lack the ability to unify them, as they are unable to exert any influence on the spore morphology. Uncongenial hosts, on the other hand, almost invariably tend to decrease the size of uredinia and spores.

(7) In computing data and comparing results it is necessary to take into consideration the ecological conditions under which the rust had been cultured—that is, cultural conditions should be kept as far as possible uniform; or proper allowances should be made for any variation before final conclusions are drawn.

(8) Adverse environmental conditions unfavorable for the host are also unfavorable for the parasite, affecting the virulence and spore size of the latter.

(9) The optimum atmospheric temperature for the development of the rusts studied appears to range between 66.5° and 70° F. Sufficiency of water and plentiful light are indispensable for the best growth of the rust.

(10) The age of the host seedlings, provided they are healthy at the time of inoculation, has no determining affect on the virulence of infection or size of the urediniospores.

(11) The length of association of a rust with its host, after the first uredinia have burst the epidermis until teliospores are formed, does not impair the viability of the urediniospores, nor does it exhibit any marked and consistent effect on their size.

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Vol. XVI JANUARY 20, 1919 No. 3

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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from State Experiment Stations should be addressed to H. P. Armsby, Institute of Animal Nutrition, State College, Pa.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., JANUARY 20, 1919 NO. 3

VARIATIONS AND MODE OF SECRETION OF MILK SOLIDS[1]

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INTRODUCTION

The chief contributors to the subject of the physiology of milk formation in the mammary gland have chosen as the foundation for their theories the grounds of analogy with the mode of formation of the secretion in the two types of glands, sebaceous and salivary, and as positive evidence have cited histological studies of the mammary tissue. On such grounds the conclusions are likely to be weak. As yet there has been little attempt to use the store of accurate mathematical data on the composition and variation of the milk constituents for the analysis of this problem.

The investigation reported in this paper is an attempt to analyze the variations and associations of the constituents of Holstein-Friesian milk to furnish definite mathematical evidence bearing on the problem of the kind of mechanism liberating these constituents to form the fluid known as milk. The specific problems and the viewpoints taken in this paper may be best understood by considering the natural divisions into which milk secretion falls. The mammary functions may be divided into two main divisions. The first of these has to do with the formation of the materials of milk before the constituents are finally brought together as milk. The second has to do with the release of the complete product, milk. The first of these needs only concern us. This problem may be again narrowed to exclude the genetic differences which most certainly exist between individual cows. Given the constituents of cow's milk, our problem is thus limited to the manner in which these constituents are released into the milk ducts. Is it through the secretory action of the gland cells or is it through the destruction of the whole or a part of these cells? Toward the analysis of this problem and related problems the data on the variations and associations of the constituents of cow's milk have been collected. The special problems bearing on the question will be discussed in connection with the data of the later sections of the paper.

[1] Papers from the Biological Laboratory of the Maine Agricultural Station No. 121. This paper is the fourth of a series of studies on milk now being conducted in the Biological Laboratory of the Maine Station.

MATERIAL AND METHODS

The Holstein-Friesian Association (*15*)[1] has, as part of its semiofficial advanced registry work, collected a considerable amount of data on the yearly production of Holstein-Friesian cows. The majority of these records contain the following data: The name, advanced registry number, and herd-book number are given, together with the volume where the last entry record was made, as the requirements of the semiofficial test include the making of the 7-day official test. The rest of the animal's record includes age at calving, length of record, weight of milk, percentage of butter fat and total weight of butter. Some of these records, fewer than we could desire but still far more ample than those of any other known breed, give the total solids for the milk produced. These records will furnish the material for the analyses of the problems previously indicated.

The objection may be raised that these data are not accurate, since they are taken from this type of record. It is realized that there may be some justice in the criticism; yet the inaccuracy should not be magnified. Any advanced registry system must be subject to all the criticism that may be brought against these particular records. Criticism can not be made on the ground of conscious inaccuracy due to poor management on the part of the association officials, as every record is carefully checked for inaccuracies. Each record is under oath as to its accuracy by both parties, the tester, and the owner. The only justifiable criticisms which can be brought are those of the personal equation type, errors from the variation in the values as read by two different men. These errors can not at any time be very great. They constitute that group of errors which are as likely to go one way as another—that is, they should counterbalance.

The methods used are, in general, those of any adequate statistical treatment of a quantitative subject. The constants for the distributions, means, standard deviations, and coefficients of variations are calculated by the usual formula for grouped distributions and without the use of Sheppard (*27*) correction. The correlations are calculated from the correlation surfaces by the usual Bravais formula. The necessity of correcting for the effect of age and quantity of milk in the comparison with the amount of butter fat and solids-not-fat have made necessary the use of partial correlation coefficient to measure such association for a constant value of the disturbing variables. These constants have been calculated from the ordinary correlations by the method devised by Pearson (*21*).

VARIATION OF MILK, BUTTER FAT, AND SOLIDS-NOT-FAT

Some study of the variation of the milk and butter fat have been made, notably those by Gavin (*11*), Vigor (*34*), and Pearl (*20*), where definite variation constants have been determined. These studies in-

[1] Reference is made by number (italic) to "Literature cited", p. 99-102.

clude chiefly the determination of the relation of this milk and butter fat as it varies within itself and as it varies with age. They do not consider the relation of the variation of the other solids; in fact, the available material has been wanting. Such variation constants are highly desirable; in reality, indispensable for the succeeding studies that are to follow.

These Holstein-Friesian data are exceptional in that they are accurate data taken from one breed under the best of conditions. The material as tabled is taken from volumes 18 to 28 of the Advanced Registry of the Holstein-Friesian Association (*15*) for their semiofficial year records. All of the records for milk, total solids, butter fat, and solids-not-fat are for 365 days. The constants are calculated from the grouped frequencies of the formed correlation tables (IV to IX). It is evident from the correlation table for age and percentage of solids-not-fat that one error probably exists in the records either from a typographical cause or from an error in the determination. I shall therefore give the constants both for this animal included and for the distribution where it is omitted. Table I shows the means, standard deviations, and coefficients of variation of the variates. The two values of some of the constants in this table are, first, those for the whole population of the Holstein-Friesian milch cows, and second, those for the population which also records the values for the total solids.

TABLE I.—*Fundamental constants for Holstein-Friesian mean production of milk*

Character.	Number of individuals.	Mean.	Standard deviation.	Coefficient of variation.
Amount of milk.....pounds..	1,387	15,417.44 ± 67.7667	3,741.59 ±47.9111	24.268±0.3285
Do.....do....	334	15,149.70 ±111.3272	3,016.18 ±78.7223	19.909± .5398
Age.....years..	1,387	4.05 ± .0369	2.04 ± .0261	50.371± .7919
Do.....do....	334	4.49 ± .0838	2.27 ± .0592	50.556±1.6218
Butter fat.....pounds..	335	515.37 ± 3.9956	108.43 ± 2.8256	21.039± .5720
Do.....do....	1,387	528.01 ± 2.4334	134.36 ± 1.7204	25.446± .3334
Solids-not-fat.....do....	335	1,302.86 ± 9.5828	260.05 ± 6.7769	19.959± .5404
Do.....do....	334	1,301.20 ± 9.5489	258.71 ± 6.7523	19.882± .5390
Total solids.....do....	335	1,814.78 ± 12.9929	352.59 ± 9.1884	19.429± .5250
Do.....do....	334	1,812.92 ± 12.9723	351.46 ± 9.1731	19.386± .5246
Butter fat.....per cent..	1,387	3.441± .0058	.318± .0040	9.228± .1191
Solids-not-fat.....do....	335	8.612± .0134	.364± .0094	4.221± .1101
Do.....do....	334	8.604± .0124	.338± .0088	3.923± .1025
Total solids.....do....	335	12.02 ± .0206	.560± .0146	4.657± .1215
Do.....do....	334	12.014± .0202	.547± .0143	4.549± .1188

The following facts are easily deducted from Table I: The Holstein-Friesian cows making the semiofficial record have a mean milk production of slightly over 15,000 pounds of milk, containing about 520 pounds of butter fat and 1,300 pounds of solids-not-fat. The ratio of the solids-not-fat to the butter fat is approximately 2.5 to 1. Taken in the form of percentages, the Holstein-Friesian milk contains slightly over 12 per cent of total solids, composed of 3.43 per cent of fat and 8.60 per cent of solids-not-fat. The mean age of the group is slightly more than 4 years. The animals who have total-solids records are, on the average, about ½ year

older than those without such records. This is no doubt due to the progressive tendency to test young animals and to select animals for high production. The animals whose total-solid records were determined are found in the earlier herd books.

It is interesting to compare these values with the milk of other breeds. To facilitate this, Table II has been drawn up. The data for this table have been gathered from many sources, chief among which are the papers of the Agricultural Experiment Stations and the analyses of public chemists. Each tabulated value is, in general, the mean of a considerable number of observations and may be considered close to the true value. Unfortunately it is not possible to obtain the original data so that the other variation constants could be obtained.

TABLE II.—*Mean milk constituents of the different breeds* [a]

Breed.	Total solids.	Fat.	Solids-not-fat.	Ratio of solids-not-fat to butter fat.
	Per cent.	*Per cent.*	*Per cent.*	
Molltaler	13. 22	3. 86	9. 39	2. 43 : 1
Blondvich	12. 75	3. 67	9. 09	2. 48 : 1
Angler	12. 51	3. 51	9. 00	2. 56 : 1
Jeverland	11. 86	3. 09	8. 77	2. 83 : 1
Holland	11. 54	3. 05	8. 04	2. 63 : 1
East Friesian	11. 80	3. 09	8. 71	2. 81 : 1
Lova Rhine	12. 12	3. 31	8. 81	2. 66 : 1
Breitenburg	12. 34	3. 36	8. 98	2. 67 : 1
Red Holstein	12. 07	3. 27	8. 80	2. 69 : 1
Wesermarsch	11. 85	3. 24	8. 61	2. 65 : 1
Schwyz	12. 76	3. 60	9. 16	2. 52 : 1
Simmental	13. 27	4. 05	9. 22	2. 28 : 1
Westerwold	12. 99	3. 79	9 20	2. 42 : 1
Glan	13. 57	4. 16	9. 41	2. 26 : 1
Alderney	13. 60	3. 81	9. 79	2. 57 : 1
Jersey	14. 39	5. 12	9. 27	1. 81 : 1
Guernsey	13. 61	4. 53	9. 08	2. 00 : 1
Holstein-Friesian	11. 78	3. 32	8. 46	2. 55 : 1
Ayrshire	12. 46	3. 62	8. 84	2. 44 : 1
Shorthorn	12. 61	3. 70	8. 91	2. 41 : 1
Polled Jersey	13. 93	4. 67	9. 26	1. 98 : 1
French Canadian	13. 32	4. 00	9. 32	2. 33 : 1
Dutch Belted	12. 31	3. 40	8. 91	2. 62 : 1
Brown Swiss	12. 61	3. 62	8. 99	2. 48 : 1
Bed Polled	12. 66	3. 67	8. 99	2. 45 : 1
South Devon	12. 93	3. 72	9. 21	2. 47 : 1
Kerries	13. 10	4. 02	9. 08	2. 26 : 1
Dexter	12. 58	3. 46	9. 11	2. 63 : 1
Holstein-Friesian	12. 02	3. 44	8. 60	2. 50 : 1

[a] The references to the data combined in this table will be found in the following numbered papers of the "Literature Cited": 1, 7, 10, 12, 14, 19, 24, 30, 35, 36, 39, 40.

These data are not entirely satisfactory, representing, as they do, data collected under a great variety of conditions. This heterogeneity is unfortunate. Two errors are easily discernible: The fat percentage of the Holstein-Friesian is about 0.1 per cent too low, and the fat percentage of the Guernsey from unpublished data on 4,900 animals for a year's test is 4.9 instead of the 4.53 of the above list. However, this is the only

material available where any number of animals are tested for their total solids and percentage of fat. The belief is held that even with these discrepancies the table will give a fair comparative view of the average composition of the milk of the various breeds included. Taken at their face value, the data show that the butter fat percentage in the different breeds varies between 3.05 and 5.12 per cent. Similarly, the total solids are shown to vary between 11.54 and 14.39 and the solids-not-fat between 8.04 and 9.79. This would make the average composition of the Holstein-Friesian breed rather lower than most of the other breeds, both in the percentage of butter fat and in the total solids. If we consider now the ratio of the solids-not-fat to the butter fat when the milk is constant, the values of the ratios run between 1.8 and 2.81. This means that the breed considered in the data has a high proportion of solids-not-fat. There appears to be an association between the percentage of fat characteristic of the breed and the content of the solids-not-fat carried in the milk—that is, the Jerseys, with their high fat percentage, also have an increased amount of the solids-not-fat over the other breeds, and the Holland, one of the lowest breeds, also has the lowest amount of solids-not-fat. This increase does not go up in direct proportion to the amount of fat present in the milk, as a glance at the proportion of the two will show. It will remain for a later section to show how these constituents vary within the Holstein-Friesian race.

Data have been tabulated to show the differences in the milk of different species of animals (Table III).

TABLE III.—*Mean milk constituents of different species of animals* [a]

Species.	Total solids.	Fat.	Solids-not-fat.	Ratio of solids-not-fat to butter fat.
	Per cent.	*Per cent.*	*Per cent.*	
Sow	18.51	6.60	11.91	1.8 : 1
Goat	11.80	3.54	8.26	2.3 : 1
Ewe	18.52	7.17	11.35	1.6 : 1
Indian buffalo	16.24	6.77	9.47	1.4 : 1
Bitch	15.89	5.65	10.24	1.8 : 1
Ass	9.72	.90	8.82	9.8 : 1
Mare	10.92	.99	8.93	9.0 : 1
Man	11.78	3.28	8.50	2.6 : 1
Cow	12.01	3.44	8.61	2.5 : 1
Colostrum of cow	22.88	2.30	20.58	8.9 : 1
Colostrum of man	12.91	2.60	10.31	4.0 : 1

[a] The references to the data named in this table will be found in the following numbers of the "Literature cited:" 5, 6, 8, 9, 18, 22, 25, 26, 29, 32, 33, 38.

The data given above are open to the same criticism as that in Table II, and are to be taken with the same limitations.

The milk of the different species varies considerably both in its butter-fat content and in its solids-not-fat. The lowest percentage of fat produced is 0.90 per cent, found in the milk of the ass. The milk of the mare corresponds closely to this, 0.99 per cent. The highest percentage of

butter fat is 7.17 per cent, contained in the milk of the ewe, closely followed by that of the Indian buffalo and the sow. The milk of the dairy cow is about intermediate between these two extremes. The colostrum is lower in its fat content than is either of the normal milks of the same species. The solids-not-fat content varies from 8.61 per cent for the cow to 11.91 per cent for the normal milk of the species included in the table. It reaches its highest value in the colostrum, where the cow's milk includes as much as 20.58 per cent. The same general association is also seen in the milk of the different species that are present in the milk of the different breeds—that is, the milk of species containing a low percentage of fat contains proportionately more solids-not-fat than does the species which contains the higher percentage of fat. The species containing a low percentage of fat contains less actual solids-not-fat per hundred pounds of milk than does the species containing a higher percentage of fat.

A survey of Table I shows that the most variable character in the Holstein-Friesians, is the age included in the tests. This has a very high coefficient of variation, the highest shown by any of the measured characters, emphasizing the need of a proper age correction when the amount of milk is to be studied for its hereditary behavior.

The next highest variation coefficient is that for milk (24.3). This is closely followed by the variation coefficients for butter fat, 21.0; solids-not-fat, 19.9; and total solids, 19.4 These last four coefficients may be considered as dependent variables and owe part of their variation to variations in other characters. Thus, the large constant of variation for the amount of milk is due in some part to the age differences in the animals included, for, as has already been shown, milk production rises in a logarithmic curve with increasing age. Again, as will be shown later, the relation of the milk constituents to the amount of milk is so close that a large part of their variation may be explained by variations in the amount of milk. As a matter of fact, these data are not needed here, for when the coefficients of variation for the percentages of butter fat, solids-not-fat, and total solids are considered, it is seen that the coefficients are reduced to 9.2, 3.9, and 4.5, respectively, or coefficients which compare rather favorably with physical variables. The high coefficients of the milk solids are thus shown to be due to the variations in amount of milk and not to variations in percentage contents of these constituents.

FACTORS AFFECTING THE COMPOSITION OF MILK

Already some work has been done on factors affecting the composition of milk by Wilson (*37*) and by Pearson (*21-23*). In his studies Wilson attempts to show that the percentage of butter fat is not dependent on the amount of milk. The methods used to draw this conclusion are, according to Pearson, open to criticism on the following grounds: The tail frequencies are clubbed together so that the real correlation can not be

determined, and the means of the separate distributions lead us to a correlation ratio, which, while small, is highly significant in showing the dependence of butter-fat concentration on amount of milk.

These studies limit themselves to the relation of butter fat to the milk. In dealing with this question the association of the percentage solids-not-fat with the quantity of milk produced will also be studied. Tables IV and V show the correlation surfaces for weight of milk and percentage of butter fat and the age of the cow and the percentage of butter fat.

TABLE IV.—*Correlation surface for amount of milk and percentage of butter fat as deduced from individual year records of Holstein-Friesian cows*

Weight of milk (pounds).	Percentage of butter fat.											Total.
	2.4–2.6	2.6–2.8	2.8–3.0	3.0–3.2	3.2–3.4	3.4–3.6	3.6–3.8	3.8–4.0	4.0–4.2	4.2–4.4	4.4–4.6	4.6–4.8	
7,000– 9,000	....	....	....	3	5	6	5	3	1	....	1	....	24
9,000–11,000	....	....	1	17	32	25	25	15	4	....	....	....	119
11,000–13,000	....	2	17	42	66	90	58	17	11	3	1	....	307
13,000–15,000	....	4	12	59	96	117	67	30	9	5	1	....	400
15,000–17,000	1	3	9	66	107	92	49	31	8	5	....	....	371
17,000–19,000	2	4	20	55	79	66	48	14	4	1	1	1	295
19,000–21,000	....	3	7	16	49	50	13	8	3	2	3	....	154
21,000–23,000	....	3	7	16	25	24	8	7	2	2	2	....	96
23,000–25,000	....	2	3	6	8	4	6	1	....	....	2	....	32
25,000–27,000	....	1	4	5	4	3	3	3	2	....	....	....	25
27,000–29,000	....	....	2	5	1	2	2	1	1	1	....	....	15
29,000–31,000	....	....	....	5	....	....	....	....	....	....	....	....	5
Total	3	22	82	295	472	479	284	130	45	19	11	1	1,843

TABLE V.—*Correlation surface for age at the beginning of test and percentage of butter fat contained in the milk for the individual records of Holstein-Friesian cows*

Age at test.				Percentage of butter fat.											Total.
				2.4–2.6	2.6–2.8	2.8–3.0	3.0–3.2	3.2–3.4	3.4–3.6	3.6–3.8	3.8–4.0	4.0–4.2	4.2–4.4	4.4–4.6	4.6–4.8	
Yr.	*mo.*	*Yr.*	*mo.*												
1	6–	2	0.	....	....	1	7	12	16	10	3	3	1	....	....	53
2	0–	2	6.	2	4	11	66	84	91	66	21	6	1	2	....	354
2	6–	3	0.	....	2	7	23	38	59	38	21	8	4	....	....	200
3	0–	3	6.	....	1	4	15	29	25	19	13	1	2	1	1	111
3	6–	4	0.	....	2	8	20	32	28	20	9	2	2	....	....	123
4	0–	4	6.	....	1	5	15	17	12	18	7	4	....	2	....	81
4	6–	5	0.	1	2	6	7	20	23	8	8	1	....	1	....	77
5	0–	5	6.	....	....	5	10	16	20	8	8	2	2	1	....	72
5	6–	6	0.	....	2	2	14	22	23	13	2	2	....	....	....	80
6	0–	6	6.	....	....	....	10	19	13	6	3	....	....	....	....	51
6	6–	7	0.	....	....	1	6	17	18	4	....	3	....	....	....	49
7	0–	7	6.	....	1	1	11	5	17	3	....	1	1	....	....	40
7	6–	8	0.	....	....	....	4	5	8	4	2	....	....	....	....	23
8	0–	8	6.	....	....	....	3	8	5	2	....	....	....	....	....	18
8	6–	9	0.	....	....	1	3	5	6	....	1	....	1	....	....	17
9	0–	9	6.	....	1	1	2	5	....	4	2	....	....	....	....	15
9	6–	11	0.	....	....	2	2	3	6	2	1	1	....	....	....	17
11	0–	15	0.	....	....	....	2	2	1	....	....	1	....	....	....	6
Total				3	16	55	220	339	371	225	101	35	14	7	1	1,387

A glance at these tables shows that there is little correlation between butter fat percentage and the weight of milk or age at test for the Holstein-Friesian cows. It seems well before tabulating these coefficients that we consider the effect of increased production in Holstein-Friesian cattle on the percentage of solids-not-fat contained in the milk. Two tables similar to those above are necessary for this comparison. These data are given in Tables VI and VII.

TABLE VI.—*Correlation surface for the amount of milk produced in one year and the percentage of solids not fat contained in the milk of Holstein-Friesian cows*

Weight of milk (pounds).	Percentage of solids-not-fat.																				
	7.0–7.2	7.2–7.4	7.4–7.6	7.6–7.8	7.8–8.0	8.0–8.2	8.2–8.4	8.4–8.6	8.6–8.8	8.8–9.0	9.0–9.2	9.2–9.4	9.4–9.6	9.6–9.8	9.8–10.0	10.0–10.2	10.2–10.4	10.4–10.6	10.6–10.8	10.8–11.0	11.0–11.2	Total.
7,000–9,000	...	...	...	...	...	...	...	...	1	....	1	...	...	...	...	...	...	...	...	...	...	2
9,000–11,000	...	...	...	1	1	....	2	5	6	4	....	1	...	...	...	...	...	...	...	...	...	20
11,000–13,000	...	...	...	...	1	7	5	10	13	17	9	1	...	...	...	...	...	...	...	...	...	63
13,000–15,000	1	...	...	...	4	7	10	16	24	15	6	2	...	...	...	...	...	...	...	...	...	85
15,000–17,000	...	...	1	...	1	7	10	19	21	10	4	...	...	...	...	...	...	...	...	...	(1)	74
17,000–19,000	...	...	...	...	3	5	4	18	13	9	4	2	...	1	...	...	...	...	...	...	...	59
19,000–21,000	...	...	...	...	....	4	2	5	6	3	3	...	...	...	...	...	...	...	...	...	...	23
21,000–23,000	...	...	...	...	...	1	1	2	....	1	1	...	...	...	...	...	...	...	...	...	...	6
23,000–25,000	...	...	...	...	...	....	....	1	....	....	....	...	...	...	...	...	...	...	...	...	...	1
25,000–27,000	...	...	...	...	...	....	....	2	....	....	....	...	...	...	...	...	...	...	...	...	...	2
Total	1	...	1	1	10	31	34	78	84	59	28	6	...	1	...	...	...	...	...	...	1	335

TABLE VII.—*Correlation surface for the variables age at test and percentage of solids not fat for the semiofficial year records of Holstein-Freisian cows*

Age at test.	Percentage of solids-not-fat.																				
	7.0–7.2	7.2–7.4	7.4–7.6	7.6–7.8	7.8–8.0	8.0–8.2	8.2–8.4	8.4–8.6	8.6–8.8	8.8–9.0	9.0–9.2	9.2–9.4	9.4–9.6	9.6–9.8	9.8–10.0	10.0–10.2	10.2–10.4	10.4–10.6	10.6–10.8	10.8–11.0	11.0–11.2	Total.
Yr.mo.yr.mo.																						
1 6–2 6	1	...	...	1	....	2	7	15	22	18	12	4	...	...	...	...	...	...	...	...	...	82
2 6–3 6	...	...	...	...	3	6	1	14	18	15	3	...	...	1	...	...	...	...	...	...	...	61
3 6–4 6	...	...	...	...	....	4	9	15	14	7	6	1	...	...	...	...	...	...	...	...	...	56
4 6–5 6	...	...	1	...	1	4	6	11	8	10	1	1	...	...	...	...	...	...	...	...	...	43
5 6–6 6	...	...	...	...	2	5	3	10	9	4	1	...	...	...	...	...	...	...	...	...	...	34
6 6–7 6	...	...	...	...	1	5	4	5	4	....	3	...	...	...	...	...	...	...	...	...	...	22
7 6–8 6	...	...	...	...	....	2	1	4	1	2	...	...	...	...	...	...	...	...	...	...	...	10
8 6–9 6	...	...	...	...	1	2	1	3	5	2	1	...	...	...	...	...	...	...	...	...	(1)	16
9 6–10 6	...	...	...	...	1	1	....	1	2	....	1	...	...	...	...	...	...	...	...	...	...	6
10 6–11 6	...	...	...	...	1	...	...	...	....	1	...	...	...	...	...	...	...	...	...	...	...	2
11 6–12 6	...	...	...	...	....	....	2	....	1	....	....	...	...	...	...	...	...	...	...	...	...	3
Total	1	...	1	1	10	31	34	78	84	59	28	6	...	1	...	...	...	...	...	...	1	335

It will be noticed that in both Tables VI and VII there is one value (in parentheses) far removed from the distribution of the other entries. It seems desirable to determine the correlations with this value included and excluded. Consequently, the correlations will be given both with and without it, since it is highly probable that some error has crept into the determination of the solids for this test.

Table VIII gives the values of the correlation coefficients and the correlation ratios, together with the constants to show the approach to linearity of the regression lines.

TABLE VIII.—*Analytical constants for inter-individual variation of the constituents of cow's milk*

Character correlated.	r	η	Σ_m	η^2-r^2
Weight of milk and percentage of butter fat	-0.0977 ± 0.0156	0.1251 ± 0.0155	0.1213	0.0061 ± 0.0024
Age and percentage of butter fat	$-.0546\pm.0181$	$.1325\pm.0178$	.1917	$.0146\pm.0044$
Weight of milk and percentage of solids-not-fat	$-.0553\pm.0367$	$.1161\pm.0364$	.1857	$.0104\pm.0075$
Age and percentage of solids not fat without doubtful observation	$-.1612\pm.0359$	$.2341\pm.0348$	.3084	$.0288\pm.0124$
Weight of milk and percentage of solids-not-fat	$-.0659\pm.0367$	$.1373\pm.0362$	.2032	$.0145\pm.0089$
Age and percentage of solids-not-fat	$-.2191\pm.0351$	$.2459\pm.0347$	.1883	$.0125\pm.0082$

This table shows that the weight of milk produced in a year is negatively correlated with the percentage of butter fat and of solids not fat contained in this milk. In each case the correlation is low, in neither case being as great as -0.1. For the butter fat percentage the correlation -0.0977 ± 0.0156, although low, is highly significant, since the correlation value is 6.2 times its probable error; or, in other words, could be expected from random sampling only once in slightly more than 100,000 times. The correlation for percentage of solids not fat and milk, -0.0553 ± 0.0367, is only about half that for the percentage of butter fat and milk. Further, this correlation can not be considered significant, as it is only 1.5 times its probable error, or about once out of three trials a correlation as great or greater than this due to random sampling would be expected. It will be noted that even where the abnormal observation is eliminated the correlation does not increase to a value where it becomes significant.

The correlation between the percentage of butter fat and age is slight (-0.0546 ± 0.0181) and in the same direction as that of butter fat and milk production—that is, minus. It may possibly be significant, since it is about 3.1 times its probable error. Even if it were significant, however, it would be scarcely detectable except in a large mass of data where statistical methods were applied.

On the other hand, the correlation between age at test and percentage of solids-not-fat is significant, for, with the doubtful observation, the correlation is 4.4 times its probable error, and without this doubtful observation the correlation is 6.2 times the probable error. Furthermore, the difference between the correlation of percentage of butter fat and percentage of solids-not-fat in cow's milk and the age at which the test is made [1] is probably a significant difference. The difference of the correlation of the percentage solids-not-fat and age and the correlation be-

[1] The probable error of the difference is calculated by the usual formula $\pm0.67449\sqrt{\sigma_1^2+\sigma_2^2}$.

tween the percentage of butter fat and age is slightly over 2.7 times its probable error when the doubtful observation is included in the data. The difference and its probable error for this is 0.1066 ± 0.0400, or 2.7 times its probable error when the doubtful observation is included in the data. The difference and its probable error for this is 0.1066 ± 0.400, or the difference is 2.7 times its probable error. When this doubtful value is not included in the calculations, the difference and its probable error becomes 0.1645 ± 0.0395, or 4.2 times the probable error, a value which certainly represents a greater effect of age on the solids-not-fat content of cow's milk than of age on the butter-fat content of the same milk.

Much the same statement holds for the relation of the percentage of solids-not-fat and weight of milk produced and percentage of the solids-not-fat and age at test. The difference is of the same magnitude as is the difference between percentage of solids-not-fat and percentage of butter fat and age—that is, the difference is only slightly significant if we consider the correlation found in the presence of the doubtful value, and is markedly significant when this value is thrown out of the table.

LINEARITY OF REGRESSION

The analytical constants necessary to test the linearity of regression are given in Table VIII. In every case the correlation ratio is a somewhat larger numerical quantity than the correlation coefficient for the same table. These differences are shown to be of little significance in view of the fact that Σ_m and $\eta^2 - r^2$ are substantially zero. The difference between the correlation ratios and the correlation coefficients are probably not significant. In only one case is the difference $\eta^2 - r^2$ greater than three times the probable error (*3*), and in this case the difference is only 3.3 times the probable error. For this one case the difference is in all probability not significant. For the other correlations the difference is certainly not significant. It may be concluded, therefore, that the regressions are linear and that the correlation coefficient represents the true correlation.

The following conclusion may be drawn from the above analysis concerning the relations between the constituents of cow's milk and the variables, age at beginning of the year test and amount of milk produced during this year test.

1. As the amount of milk given by the cows in this test increases, the percentage composition of the butter fat in this milk decreases. The amount of this decrease is statistically significant. Considered practically, this fall in butter-fat content could not be easily detected in the small samples usually handled.

2. There is a slightly significant fall of the percentage of butter fat contained in the milk as age advances. This slight fall may, however, be accounted for by the rise in milk production which occurs coincident with

this increase in age. For the partial correlation between percentage of butter fat and age, holding the milk production constant, is 0.0105 ± 0.0181, or there is no significant correlation.

3. The quantity of milk produced for the year is entirely independent of the percentage content of the solids-not-fat, or put in another way, the factor or factors causing high or low milk production are separate and distinct from those causing a high percentage of these constituents in the milk.

4. Age is a prominent factor in bringing about the reduction of the percentage of solids-not-fat. This reduction is not due to differences in the amount of milk, as the milk held constant by the partial-correlation method gives practically the same correlation as when the milk production is not considered.

5. Decrease in the amount of milk raises the percentage of butter fat in a greater degree than it affects the percentage of the solids-not-fat.

6. Increased age has a marked effect in reducing the percentage of solids-not-fat. It does not so reduce the percentage of butter fat.

CORRELATION BETWEEN THE BUTTER FAT AND THE SOLIDS-NOT-FAT IN COW'S MILK

This question of the correlation between the butter fat and the solids-not-fat has considerable importance for the problems of the mechanism of milk secretion in the mammary gland. Does this gland secrete a high content of butter fat when it secretes a high content of the other solids—lactose, protein, and ash? Should such an association exist, it becomes evident that the factors leading to a high fat content also lead to a high solids-not-fat content. Taken in connection with either theory to account for the presence of the organic constituents of milk, such a correlation indicates a regulatory mechanism which balances these constituents together in similar proportions in any given individual cow.

Apart from the bearing on the problems of the milk secretion, such a correlation has wider significance. It predicates that the factors in inheritance for this high content of one constituent also transmits the production of high content of the other solids. The problem thus becomes important for the student of inheritance of quantity and quality in cow's milk.

To answer these questions for Holstein-Friesian cows, it is necessary to arrange the data for the yearly records of butter fat in a table of double entry or correlation table. In the arrangement of this table 100-pound intervals were chosen as the basis of division of both the solids-not-fat and butter-fat production of the year tests. In the first two observations of the butter fat it is necessary to group these between 280 and 300 pounds and calculate accordingly. The results are given in Table IX.

Observation easily shows that these two variates, solids-not-fat and butter fat of cow's milk, are highly correlated. The correlation coeffi-

cient in this case is $r=0.8991\pm0.0071$ and the correlation ratio $\eta=0.9023\pm0.0069$. As in the previous tables, the cell which contained the doubtful observation is indicated by parentheses. Where this doubtful observation is left out of consideration, the correlation rises slightly to $r=0.9095\pm0.0063$, and the correlation ratio is $\eta=0.9064\pm0.0066$.

TABLE IX.—*Correlation surface for pounds of solids-not-fat and pounds of butter fat as deduced from individual year records*

Weight of solids-not-fat (pounds).	Weight of butter fat (pounds).								
	200–300.	300–400.	400–500.	500–600.	600–700.	700–800.	800–900.	900–1,000.	Total.
700–800	1	3							4
800–900	1	6	2						9
900–1,000		20	5						25
1,000–1,100		13	28						41
1,100–1,200		2	40	5					47
1,200–1,300			23	24					47
1,300–1,400			5	43	2				50
1,400–1,500			2	33	4				39
1,500–1,600				13	17				30
1,600–1,700				2	16	2			20
1,700–1,800					6	4	1		11
1,800–1,900				(2)		4			6
1,900–2,000						2	1		3
2,000–2,100						1			1
2,100–2,200						1			1
2,200–2,300								1	1
Total	2	44	105	122	45	14	2	1	335

These constituents of cow's milk are shown by these correlations to be highly correlated variates. This correlation can not be accounted for by the regression of solids-not-fat on butter fat, not being a linear regression, as even a glance at the values of the correlation coefficient and the correlation ratio will convince anyone that they are so nearly the same in value as to make it certain that the regressions are linear. Consequently it does not seem necessary to calculate the customary constants for this linearity, as both Σ_m and η^2-r^2 would be negligible quantities. This establishes the conclusion finally that butter fat and solids-not-fat contained in cow's milk are correlated variates. This correlation being positive, a rise in the amount of either constituent also means a rise in the other.

Part of the correlation between the butter fat and solids-not-fat may be due to the rise in the amount of milk of the different individual cows. For the problem of the mechanism of the secretion of these constituents this is of especial importance. Tables X and XI give the correlation surfaces for the variables.

TABLE X.—*Correlation surface for amount of milk and amount of butter fat as deduced from individual year records of Holstein-Friesian cows*

Weight of butter fat (pounds).	Weight of milk (pounds).										
	7,000–9,000.	9,000–11,000.	11,000–13,000.	13,000–15,000.	15,000–17,000.	17,000–19,000.	19,000–21,000.	21,000–23,000.	23,000–25,000.	25,000–27,000.	Total.
280–300	....	2	....	....	....	....	....	....	....	....	2
300–400	2	14	25	2	1	....	....	....	....	....	44
400–500	....	4	36	56	8	1	....	....	....	....	105
500–600	....	....	2	27	(59)	32	2	....	....	....	122
600–700	....	....	....	....	6	25	13	1	....	....	45
700–800	....	....	....	....	....	1	7	4	1	1	14
800–900	....	....	....	....	....	....	1	1	....	....	2
900–1,000	....	....	....	....	....	....	....	....	....	1	1
Total	2	20	63	85	74	59	23	6	1	2	335

TABLE XI.—*Correlation surface for amount of milk and solids-not-fat as deduced from individual year records of Holstein-Friesian cows*

Weight of solids-not-fat (pounds).	Weight of milk (pounds).										
	7,000–9,000.	9,000–11,000.	11,000–13,000.	13,000–15,000.	15,000–17,000.	17,000–19,000.	19,000–21,000.	21,000–25,000.	23,000–25,000.	25,000–27,000.	Total.
700–800	2	2	....	....	....	....	....	....	....	....	4
800–900	....	9	....	....	....	....	....	....	....	....	9
900–1,000	....	9	16	....	....	....	....	....	....	....	25
1,000–1,100	....	....	33	8	....	....	....	....	....	....	41
1,100–1,200	....	....	14	32	1	....	....	....	....	....	47
1,200–1,300	....	....	....	37	10	....	....	....	....	....	47
1,300–1,400	....	....	....	8	38	4	....	....	....	....	50
1,400–1,500	....	....	....	....	22	17	....	....	....	....	39
1,500–1,600	....	....	....	....	2	25	3	....	....	....	30
1,600–1,700	....	....	....	....	....	13	7	....	....	....	20
1,700–1,800	....	....	....	....	....	....	10	1	....	....	11
1,800–1,900	....	....	....	....	(1)	....	3	2	....	....	6
1,900–2,000	....	....	....	....	....	....	....	3	....	....	3
2,000–2,100	....	....	....	....	....	....	....	....	1	....	1
2,100–2,200	....	....	....	....	....	....	....	....	....	1	1
2,200–2,300	....	....	....	....	....	....	....	....	....	1	1
Total	2	20	63	85	74	59	23	6	1	2	335

These tables show that the variables butter fat and solids-not-fat are highly correlated with the amount of milk produced. The correlation coefficient for butter fat and milk is $r=0.8644\pm0.0093$ and the correlation ratio $\eta=0.8638\pm0.0093$. The correlation coefficient for solids-not-fat and milk is $r=0.9497\pm0.0036$ and the correlation ratio is $\eta=0.9484\pm0.0037$. As in the preceding case, these variates are

highly correlated. The regressions are so obviously linear that it does not seem necessary to calculate any of the customary constants for determining this, other than the correlation ratio given above.

In order to obtain the coefficients to measure the relation between the butter fat and solids-not-fat for a constant production of milk, it is necessary to resort to correlation of the first order given by the formula of Pearsons (*21–23*).

$$r_{12.3} = \frac{r_{12} - r_{13}r_{23}}{\sqrt{1 - r_{13}}\sqrt{1 - r_{23}}}$$

When the correlation between the butter fat and solids-not-fat for a constant quantity of milk is thus measured, it is found that the partial correlation coefficient is $r_{12\cdot3} = 0.4964 \pm 0.0278$ for the case where the doubtful observation (shown in parenthesis) is included in the calculations and is $r_{12\cdot3} = 0.5635 \pm 0.0252$ where this doubtful observation is not included. These correlations show that the production by the mammary gland of butter fat and of solids-not-fat are correlated functions. This correlation being plus it means that an increase in the liberation of either constituent of cow's milk means a coincident increase in the other.

This conclusion is important, as it shows that the factors responsible for the increase in the content of butter fat for a given volume of milk are in a high degree responsible for the increase in the solids-not-fat content in this same milk. It means that the physiology of the mammary gland in elaborating the milk solids is such that the release of a certain amount of butter fat to the milk also releases a proportionate amount of solids-not-fat. In order to account for this correlation, it is necessary to explain how the mammary gland sorts out the different elements into the milk to give the proportion of the butter fat and solids-not-fat. This question refers itself back to the fundamental one of how milk is secreted.

DIURNAL VARIATION OF THE CONSTITUENTS IN COW'S MILK

Before discussing the direct bearing of these data on the problem of the milk secretion a few more important data must be presented. It is a well-known fact that the evening milk of a cow is, in general, higher in butter fat than the morning milk. The relation of the evening and morning milk for solids-not-fat is not so well known. Table XII gives this relation for two groups of cows: Those in the first half of their lactation period and those in the last half.

This table shows the variation between morning and evening milk in the percentage composition of the same individual cow. The sample of two consecutive mornings' milk were made, and aliquot parts of each composited for analysis. The same holds true for the evening milk. Each percentage may therefore be said to represent the mean

between two days of lactation for morning and for evening milk. The milkings begin in the morning at 4.45 and in the evening at 3.45. The interval therefore is shorter between the morning-to-evening milking.

TABLE XII.—*Daily variation of the constituents of cow's milk* [a]

Cow No.	First half of lactation.				Last half of lactation.			
	Morning.		Evening.		Morning.		Evening.	
	Fat.	Solids-not-fat.	Fat.	Solids-not-fat.	Fat.	Solids-not-fat.	Fat.	Solids-not-fat.
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	4. 70	8. 35	4. 80	8. 36	4. 20	8. 19	4. 90	8. 76
2	4. 90	8. 49	5. 00	8. 60	4. 40	8. 98	4. 80	9. 18
3	3. 00	8. 48	3. 30	8. 77	4. 40	9. 60	6. 20	9. 55
4	5. 20	8. 55	7. 20	8. 59	3. 20	8. 03	4. 10	7. 95
5	3. 40	8. 76	4. 50	8. 64	6. 80	10. 00	8. 00	9. 96
6	4. 20	8. 79	4. 80	8. 85	4. 90	8. 95	5. 20	9. 12
7	3. 30	8. 34	4. 00	8. 41	3. 50	8. 25	3. 80	8. 47
8	4. 10	9. 05	4. 40	9. 29	3. 60	8. 41	4. 20	8. 11
9	3. 90	9. 00	4. 80	9. 01	4. 50	8. 58	4. 90	9. 00
Average	4. 078	8. 646	4. 756	8. 724	4. 389	8. 777	5. 122	8. 90

[a] The author is indebted to the Chemistry Laboratory of this Station for the careful analysis given in Table XII.

The average composition of morning milk in the first half of the lactation is seen by Table XII to be 4.078 per cent butter fat and 8.646 per cent solids-not-fat. The average composition of evening milk is 4.756 per cent fat and 8.724 per cent solids-not-fat. Thus, the butter-fat constituent increases markedly in the evening milk over that of the morning. The increase of the solids-not-fat is not as marked, although a slight increase does occur. The significance of this increase is supported further by the same kind of relationship exhibited by the morning and evening milk of the cows in late lactation. The numbers are not large, but the consistency of the increase composition of butter fat of the evening milk over that of the morning leads to the conclusion that this relation is certainly significant. For the increase of solids-not-fat the case is not so clear. It is possible that this increase is slightly significant, but this seems doubtful. In no case is this rise of the solids-not-fat as great as that of the butter fat. This, taken in consideration with the fact that the solids-not-fat are more than twice as great in amount as the butter fat, establishes the conclusion that the butter-fat composition of milk is affected to a much greater extent by these different times of milking than are the other solids.

This conclusion is further emphasized by the work of Ingle (*16*) on the same question. In the mixed milk of a herd of 23 animals milk for a period of 18 weeks the average composition of the morning milk

was 2.97 per cent butter fat and 8.87 per cent solids-not-fat, and the evening milk was 4.31 per cent butter fat and 8.86 per cent solids-not-fat.

The significance of these facts as above established on the problem of the mode of liberation of the constituents into cow's milk has been overlooked. Before 1850 the prevailing opinion held that the milk solids were filtered out by the mammary gland from the blood serum. This view was shown to be incorrect by the fact that lactose is not present in the blood and the fat percentage of the serum is not sufficient to account for the fat in a single milking. To replace this old theory, three major hypotheses have been put forth to account for the secretion of the mammary gland:

(1) Cells of the gland break loose bodily and disintegrate in the alveoli to form the milk solids.

(2) The portion of the cells toward the alveoli becomes loaded with solids, breaks loose from the basal portion, and disintegrates to form the milk solids.

(3) The cells of the mammary gland secrete the materials of the milk solids without themselves breaking down.

In opposition to the first theory, it may be said that no such extensive cell multiplication is witnessed in the mammary gland as would be necessary to replace the cell destruction called for on the theory. This disintegration, as pointed out by Heidenhain (*13*) for the milk produced by some cows in one day would require the replacing of all cells in the udder at least five times a day, a replacement of cells unprecedented in our knowledge of cell division.

The second theory, suggested by Langer and ably supported by Heidenhain (*13*), Steinhaus (*28*), and Brouha (*4*), lays its foundation on histological evidence. According to this evidence, the gland cells lengthen out into the lumen of the alveoli. The projecting ends of these cells become loaded with nutrients similar to milk solids. These projecting ends disintegrate to allow the escape of these solids. The basal portions, including a nucleus, are left to rebuild the cell and to enable it to repeat the process. Steinhaus says that, in order to support this rebuilding, mitotic divisions are frequent, and that the daughter nuclei which lie on the outer portion of the cell often degenerate.

The third theory lays its stress on analogy with the other secretory glands without other supporting evidence than the negative evidence of Bertkau (*2*), who says the disintegration appearing in the secretory cells is due to imperfect fixation and that no necrobiosis of any kind appeared.

The above summary of the evidence for the three theories to account for the introduction of the solids into the milk shows how contradictory is the evidence so far presented. This contradiction, however, is not to be wondered at. The examination of the cells of the actively lactating

mammary gland of a Holstein-Friesian cow showed that they were quite small. Considered in the light of this small size, it is likely that observations on the distal end of a cell might be called by one observer the destruction of this portion and by another the cell in its natural shape. This explanation of the confusion in interpretation of observations in these cells is made further probable by the change of shape which cells undergo at different stages of lactation. Thus, in the mammary gland of a bitch when just emptied, Heidenhain says that the cells were high and columnar and in another bitch where milk had not been drawn for 48 hours the cells were flat. The weakness of the histological evidence is obvious. To the final solution of the problem it appears that other evidence beside the histological observations must be presented.

Some evidence from the physiological side has been presented in the foregoing pages of the interaction of the four variables, age, quantity of milk secreted, diurnal variations, and the effect of the content of one solid on the relation of the other variable, butter fat, or solids-not-fat. These variables give criteria to the efficacy of the three contending hypotheses to explain the release of the milk solids.

Consideration of the manner of metabolism and energy requirement for most bodily functions seems to furnish the explanation of the slight negative correlation of butter-fat concentration with the amount of milk produced, where no such correlation exists with amount of milk production and the other solids. Energy has been shown to be required for most bodily functions. There does not seem to be any reason to suppose that the mammary gland is any exception to this rule when milk is produced. Such energy requirement would of necessity be dependent on the amount of production—that is, the high producer will require more energy and to produce this energy will consequently take a slightly greater amount of fat that might have gone into the milk. The other solids would not be required to furnish any of this energy and consequently would show no effect of the amount of milk produced on their concentration. The correlations obtained show the associations that would be expected with this explanation. The conclusion seems justifiable that the energy required in the production of milk causes a slight reduction in the amount of fat present in the milk of high-producing cows.

The maintenance of the fat concentration of the milk throughout life and the decline of the solids-not-fat apparently represent the normal conditions going on throughout the whole body. It is common knowledge that increase in age generally brings with it a relative decrease in the protein upbuild of the body and an increase in the fat. This increased metabolism of fat appears to extend itself to the mammary gland, as well as to other parts of the body, being just great enough to maintain the butter-fat concentration throughout life, whereas the

relative decreased metabolism of the other solids causes a decrease in the concentration of these solids in the milk.

By far the best evidence yet presented for the secretion theory for the liberation of the milk solids (the third hypothesis) is given by the diurnal variation of the constituents of cow's milk shown above. It is very difficult to see why the cell constitution should change in balance between the solids-not-fat and butter fat between morning and evening milking on any other theory. Why should the cells discharged in the evening contain the ratio *a* to *b* of butter fat to solids-not-fat, whereas in the morning the ratio is changed to a markedly lower value of *c* to *b*? On the first and second theories the cell must contain a fixed quantity of solids-not-fat, while the butter fat varies in such a way that, in a longer time between the emptying of the gland, the cell accumulates less fat than in the shorter time; or, taken in another way, the cell accumulates relatively more protein as the interval between milkings is lengthened.

Our knowledge of fat formation by other cells of the body makes it probable that either of these two possible alternatives for the formation of this milk fat on the cell-destruction hypothesis are inconsistent with the facts. In the formation of fat, the cell is first composed chiefly of protein material. In this protein material the fat is accumulated in ever-increasing amounts at the expense and crowding out of the protein constituents. This change of the ratio of the fat to solids-not-fat is just opposite to what must take place in the mammary gland on the cell-destruction hypothesis. The ratio of the fat to the solids-not-fat in the fat cells increases as the cells increase in age; the ratio of the fat to the solids-not-fat in milk, derived from the cell breakdown on the two destruction hypothesis, decreases as the age of the cell increases. It is hard to believe that there is such a difference in fat formation going on in the body. It is much more likely that the mechanism of fat formation in the two cases is the same and that the diurnal variation of the ratio of butter fat to solids-not-fat is only another phase of the changes which take place in known secretions. The large variations current in the amount of milk produced, the variations of the constituents with age, the great and characteristic differences in the composition of the milk of two cows of the same breed all add weight to the view that milk is secreted. By analogy, the mammary-gland mechanism for milk secretion would agree with practically all of the glands which secrete. There is no need to assume that rapid cell division is taking place in order to maintain the necessary number of cells for breakdown, as called for on the cell-destruction hypotheses, where no such amount of mitosis as would be necessary is witnessed microscopically. Further, the secretory theory has supporting evidence of whole granules being secreted into the saliva by the salivary glands in much the same way as is done in the secretion of butter fat. The data supporting the secretory hypothesis

are certainly strong, whereas the proof for the cell-destruction hypotheses seems weak when analyzed in the light of the above facts, and, in truth, in some particulars is contrary to known facts. The conclusion that milk is a true secretion seems justified by what we know of the mechanism behind such glands.

The data above presented give us a criterion to judge the value of any hypothesis for the origin of the milk solids from a common mother substance, such as has been suggested by Thierfelder (*31*) and later by Landwehr (*17*), to account for the derivation of casein and lactose from nucleoproteids or glycoproteids of the gland cells by splitting. The correlation of the solids-not-fat and fat might lead one to suppose such a common origin for some component of such solids and the fat. This can not be the case, however, as the correlation of fat and of solids-not-fat with amount of milk and age precludes that possibility, for if such a common origin occurred, the fat and solids-not-fat would necessarily be correlated to these other variables by comparable amounts. The milk components are not correlated equally with either milk quantity or with age; consequently, the hypothesis of a common origin is not tenable.

The correlations furnish the necessary evidence for the determination of an important genetic relation between the hereditary factors for the concentration of the butter fat and the solids-not-fat. Since the data are taken from the presumably homogenous population of the advanced registry Holstein-Friesian cows, linkage of factors for milk solids can not be used to account for this correlation; for, granting the homogeneity of the population, the factors are as likely to be in opposite chromosomes as in the same chromosomes—that is, calling one "*a*" and the other "*b*," the parental chromosome content would be:

ab	*a*	*b*	
ab	*ab*	*ab*	*ab*
ab	*a*	*b*	
a	*ab*	*a*	*a*
ab	*a*	*b*	
b	*b*	*b*	*b*
ab	*a*	*b*	

or, reducing down, four chromosomes would be present in equal numbers $\frac{ab}{\quad}$, $\frac{a}{\quad}$, $\frac{b}{\quad}$, $\frac{\quad}{\quad}$. These bred together would give the random distribution, considering that crossing over is as likely to occur one way as another. This, however, is not what actually happens in our case. The quantity of fat is correlated to the quantity of solids-not-fat per volume of milk. This must mean that some of the factors responsible for fat concentration are responsible for the concentration of sugar, protein, or ash in cow's milk.

From the practical side of increasing the solids content of cow's milk, the importance of the correlation between the butter fat and the solids-not-fat should be pointed out. This high correlation between these variables allows us to use the determination of the percentage content of one as a means of predicting what the content of the other will be. Thus, butter-fat content is easily determined by almost any one familiar with the Babcock test; but solids-not-fat are not so easily determined nor so frequently recorded. We may, in trying to improve the solid content of milk select as breeders those cows which test well with the Babcock apparatus, and at the same time improve the solids-not-fat content of the milk.

SUMMARY

This paper is the fourth of a series of studies on milk now being conducted in the Biological Laboratory of the Maine Agricultural Experiment Station. The data for this study are taken from the semiofficial year record of the pure-bred Holstein-Friesian cows, compiled and supervised by the Holstein-Friesian Association.

(1) The means, standard deviations, and coefficients of variation are given for these year records. The mean annual production of these animals was 15,417 pounds of milk, 528 pounds of butter fat, 1,303 pounds of solids-not-fat at a mean age of four years. The standard deviations are 3,742 pounds of milk, 134 pounds of butter fat, 260 pounds of solids-not-fat, and two years. The coefficients of variations are, respectively, 24, 25, 20, and 50 per cent.

(2) Comparison is made of Holstein-Freisian milk with the milk of other breeds and other species.

(3) Correlations are presented between the variables butter-fat percentage and amount of milk produced, butter-fat percentage and age at test, solids-not-fat percentage and amount of milk, and solids-not-fat and age at test. These correlations lead to the following conclusions: (a) As the amount of milk given by the cows in this test increases, the percentage composition of butter fat decreases. The amount of this decrease is highly significant, measured statistically. Considered practically, this fall in butter-fat content would not be easily detected in small samples. (b) The correlation between the age at test and butter fat is not significant. (c) The correlation between the amount of milk produced and the percentage of solids-not-fat is not significant; or, put in another way, the quantity of milk produced for one year is independent of the concentration of the solids-not-fat. This, from a genetic viewpoint, means that the hereditary factors for high or low milk production are separate and distinct from those causing a high percentage of solids-not-fat. (d) The correlation of age at test and solids-not-fat is -0.2191 ± 0.0351—that is, as the age of a cow increases, the solids-not-fat percentage of the milk decreases. (e) The constants for the linearity of regression are given. They show the regressions to all be linear. (f)

These conclusions give us two variables which influence the concentration of butter fat and solids-not-fat differently. This difference in action of these variates proves that the butter fat and the solids-not-fat can not have a common mother chemical from which they are derived from splitting.

(4) Correlations are presented between the variables, pounds of milk, butter fat, and solids-not-fat. Each variable is highly correlated, the correlation ranging from $r=0.8644\pm0.0093$ to $r=0.9497\pm0.0036$. In each case the regressions are linear. The partial correlation between butter fat and solids-not-fat for a constant value of the milk is found to be 0.5635 ± 0.0252. This correlation, together with those above, furnishes the data necessary to establish the conclusion that some of the factors responsible for high concentration of butter fat are also responsible for high concentration of some of the solids-not-fat in cow's milk. Another important practical conclusion may be drawn from this correlation; that if it is desired to improve either the butter fat or solids-not-fat concentration in a given herd the determination of the concentration of either solid will also result in an increased concentration of the other solid.

(5) Data on the diurnal variation of cow's milk are presented. These data show that the morning milk is between 0.678 and 0.723 per cent lower in butter fat than in the evening milk throughout the whole lactation. No appreciable difference occurs in the solids-not-fat. These data offer criteria between the theories to account for the secretion of the milk solids. In the cell-disintegration theories the cell must contain a fixed quantity of solids-not-fat, while the butter fat varies so that in the longer interval between milkings the cell accumulates less fat than in the short time; or, taken the other way, the cell contains relatively more protein and sugars than fat as the interval between milkings lengthens. This is contrary to our knowledge of fat formation, for it is commonly accepted that first comes the cells composed largely of protoplasm and that as time goes on this cell is more and more loaded with fat at the expense of the protoplasm. Unless these mammary cells behave very differently in the formation of this fat than other body cells, this variation is enough to discredit seriously the hypothesis of cell disintegration to account for these milk solids; and in fact, to make it an absurdity. Furthermore, so far as our knowledge of the variations of secretory glands goes, the variations of the milk fall in well with the secretory hypothesis to account for these solids.

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NEW BIOLOGIC FORMS OF PUCCINIA GRAMINIS[1]

[PRELIMINARY PAPER]

By E. C. STAKMAN, *Head of Section of Plant Pathology, Department of Plant Pathology, Botany, and Department of Agriculture, University of Minnesota;* M. N. LEVINE, *Field Assistant, Bureau of Plant Industry, United States Department of Agriculture;* and J. G. LEACH, *Shevlin Fellow, University of Minnesota*

COOPERATIVE INVESTIGATIONS BETWEEN THE AGRICULTURAL EXPERIMENT STATION OF THE UNIVERSITY OF MINNESOTA AND THE BUREAU OF PLANT INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE

Several biologic forms of *Puccinia graminis* on wheat (*Triticum* spp.) have been described. Originally *P. graminis tritici* was supposed to be the only form capable of attacking wheat varieties. None of the common wheats (*T. aestivum*) was resistant to this form, although several varieties of durum (*T. durum*), emmer (*T. dicoccum*), and einkorn (*T. monococcum*) were either resistant or almost immune.

The first demonstration that there was more than one form of stemrust on wheat was made in 1916 when *P. graminis tritici-compacti* was described.[2] This form proved to be especially interesting and significant because it could not infect the hard spring wheats normally, but developed well on soft wheats. It was also found that many of the hard winter wheats were resistant to the new form. The range of parasitism of the second form was therefore narrower than that of the ordinary *P. graminis tritici.* But in 1918 Melchers and Parker[3] found that Kanred, Kansas P. 762 (CI 5146),[4] Kansas P. 1066 (CI 2879), and Kansas P. 1068 (CI 5880), three selections from the Crimean group made at the Kansas Experiment Station, were almost immune to *P. graminis tritici.* These selections were also found to be moderately resistant to *P. graminis tritici-compacti,* and they therefore seemed to be resistant to the stemrust of wheat. Later Melchers and Parker[5] found a form which infected Kanred and the two other selections normally. Levine and Stakman[6] and Leach[7] had begun an intensive study of the parasitic capabilities of forms of *P. graminis* on varieties

[1] Published, with the approval of the Director, as Paper 144 of the Journal Series of the Minnesota Agricultural Experiment Station.

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of wheat and other species of Triticum, and also found a form which developed normally on Kanred, P. 1066, and P. 1068. It was clear from these results that the new forms could therefore do something which neither of the two other forms could do.

But none of the known forms could infect White Spring emmer (Minnesota 1165), durum (Mindum, CI 5296), and several other varieties, mostly durums. It was perfectly evident from the work with *P. graminis tritici-compacti* [1] and the two other new forms that the biologic specialization within the genus Triticum could only be determined by testing many species and varieties and that there was a strong probability that forms of rust would be found which were capable of attacking varieties resistant to all known forms of stemrust. This is exactly what has been found. A form was found which infected White Spring emmer and Mindum normally. The work has continued until about a dozen forms have been found up to the present time (Oct. 1, 1918).

About 25 varieties and strains of *Triticum aestivum*, *T. durum*, *T. compactum*, *T. dicoccum*, and *T. monococcum* are being used as differential hosts, and no variety so far tried is resistant to all of the rust forms except Khapli (CI 4103), an emmer originally imported from India. Some of the forms are very virulent on many varieties, while others are weak and can attack only a few varieties successfully. Some forms differ from each other only in their action on one or two varieties; but these differences are definite and consistent. No attempt has yet been made to name the recently discovered forms.

The factors governing the distribution of the forms are not at all clear. Material has been collected from 27 States and most sections of the country are represented. Two distinct forms have often been isolated from the same lot of material, and at least four have been found in Minnesota.

The fact that there are so many biologic forms of stemrust on wheat seems to be of profound significance in at least two ways. It is an additional reason for eradicating the rust-susceptible varieties of barberry (*Berberis* spp.), and it is of the greatest importance in the work of breeding wheats for rust resistance.

Many of the virulent forms seem to occur in the Northern States, where everyone will now concede that the barberry is of tremendous importance in the persistence of stemrust from year to year. Eradicate the common barberry and there is reason to believe that these forms may gradually die out entirely, or at least be reduced to a condition bordering on impotence. The fact also that in the South and on the Pacific coast, where barberry does not rust commonly, the forms of rust seem to be more uniform than in other regions certainly lends some color to the view that the barberry may have some effect on their development. A hypothesis that forms may have originated by hybridiza-

[1] LEACH, J. B. OP. CIT.

tion on the barberry may be worth investigating. Of course mutation and adaptation must be considered also in any attempt to explain how so many forms originated.

The fact that the same variety of wheat may be immune in one locality and susceptible in another is clearly explained. Formerly recourse was taken to the theory that the environmental conditions changed the physiologic processes and materials of wheat varieties so fundamentally that the resistance of the plants broke down. The real explanation of this phenomenon however is the fact that there are many biologic forms of the rust fungus. This has actually been demonstrated in field experiments. There is also preliminary evidence to show that the same thing may be true of *Puccinia triticina* on wheat.

Methods for breeding for rust resistance must be changed fundamentally—if indeed it is worth while to do such work at all until more is known about the specialization of the rust fungus. The breeder must know and work with those forms of rust which occur in the region for which his new variety is intended; and even then breeding must be very largely a regional or even a local problem. For instance, in the breeding plots at the Minnesota Agricultural Experiment Station certain varieties were practically immune to stemrust, but rust forms have been found within 50 miles of the plots which can attack these varieties so heavily as to make them worthless for rust resistance.

The discovery of so many forms of stemrust on wheat complicates the rust problem seriously. Extensive experiments are under way to determine the number, characteristics, and distribution of biologic forms as well as their constancy and probable origin.

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Vol. XVI JANUARY 27, 1919 No. 4

JOURNAL OF AGRICULTURAL RESEARCH

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J. E. GREAVES, E. G. CARTER, and H. C. GOLDTHORPE

(Contribution from Utah Agricultural Experiment Station.)

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON: GOVERNMENT PRINTING OFFICE: 1919

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from State Experiment Stations should be addressed to H. P. Armsby, Institute of Animal Nutrition, State College, Pa.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., JANUARY 27, 1919 NO. 4

INFLUENCE OF SALTS ON THE NITRIC-NITROGEN ACCUMULATION IN THE SOIL

By J. E. GREAVES, *Chemist and Bacteriologist*, E. G. CARTER, *Assistant Bacteriologist*, and H. C. GOLDTHORPE, *Assistant Bacteriologist, Department of Bacteriology, Utah Agricultural Experiment Station*

INTRODUCTION

Salts which may occur in soils and those applied to them in various operations influence the number, species, and activity of the microflora of a soil. These factors are in turn reflected by the yields obtained from the soil. Some substances applied to a soil serve as food for the growing plant; others increase plant growth but not through the direct furnishing of food. This latter effect may be due to a change brought about by the salt on the physical, chemical, or bacterial properties of the soil. The substance may alter the physical properties of the soil to such an extent that the bacterial flora is modified; this in turn may increase or decrease the crop produced upon the soil. Other substances may react chemically with constituents within the soil and in so doing liberate substance which can be directly utilized by the growing plant. Again, they may directly modify the microflora and fauna of the soil both as to numbers and physiological efficiency. Or, in some cases, all three changes may be wrought by one and the same salt. The question therefore arises as to what effect this or that fertilizer or soil amendment is going to have upon the bacterial activities of the soil.

Furthermore, there are millions of acres of land in arid America which contain varying amounts of soluble salts. Some of these soils contain such large quantities of these so-called "alkalis" that no vegetation is found upon them. Other soils contain only a medium amount of soluble salts, and the vegetation is composed chiefly of alkali-resisting plants. Still other soils contain much smaller quantities of soluble salts, and they become injurious only when the soil is improperly handled. The reclaiming of the heavily charged soils and the maintaining of the others in a productive condition can be carried on successfully only when we understand the influence of salts upon the growing plants and their action upon the biological, chemical, and physical properties of the soil.

The purpose of this investigation is therefore to determine the relative toxicity of various substances found in or applied to a soil, as measured in terms of bacterial activities of the soil; also to determine the stimu-

lating influence of various substance upon bacterial activity and the manner in which the stimulation is exerted. The results thus obtained can be directly compared with those obtained for higher plants. Then, if a correlation between the two, the lower and the higher plants, be obtained, it should give a quick method of testing not only alkali soils but also other soils containing various soluble constituents. Furthermore, it makes possible the studying of balanced solutions within the soil by means of bacteriological tests, thus getting in a short time comparative results which with higher plants would be obtained only by an enormous amount of work and time. Knowledge thus obtained can be used in the reclaiming of the alkali lands of the arid West. A careful review of the literature has been made elsewhere and, hence, is not included here (*3*).[1]

EXPERIMENTAL WORK

The soil used in this work, taken from the College farm, is of a sedimentary nature. It was deposited by streams flowing into the valley, laden with débris derived from the near-by mountains, which are composed largely of quartzite and limestone. A physical and chemical analysis of the soil is given in Table I.

TABLE I.—*Physical and chemical composition of soil*

Physical composition.		Chemical composition.	
Soil.	Per cent.	Constituent.	Per cent.
Coarse sand (above 1 mm.)	17.69	Insoluble matter	66.69
Fine sand (1 to 0.03 mm.)	37.39	Potash (K_2O)	.55
Coarse silt (0.03 to 0.01 mm.)	15.19	Soda (Na_2O)	.49
Medium silt (0.01 to 0.003 mm.)	10.36	Lime (CaO)	7.41
Fine silt (0.003 to 0.001 mm.)	10.32	Magnesia (MgO)	4.15
Clay (below 0.001 mm.)		Ferric oxid (Fe_2O_3)	2.93
Moisture and loss	9.05	Alumina (Al_3O_2)	3.49
		Phosphorus pentoxid (P_2O_5)	.25
		Sulphur trioxid (SO_3)	.07
		Carbon dioxid (CO_2)	7.62
		Humus	2.18
		Total nitrogen	.15

The soil used, therefore, was a sandy loam very high in acid-soluble constituents, but the water-soluble constituents were not excessive. The calcium and magnesium contents were very high and mainly in the form of the carbonate. The soil was well supplied with phosphorus and potassium, and there was a fairly large quantity of iron present. In fact, all of the elements of plant food were present in abundance, with the exception of nitrogen, which was low. The soil was very productive, and previous work had shown the ammonifying and nitrifying powers of the soil to be about the average for the soils of the arid regions. The

[1] Reference is made by number (italic) to "Literature cited," pp. 134–135.

nitrogen-fixing powers of the soil were above the average, and previous work had shown it to have an intensely interesting bacterial flora.

Several hundred pounds of the soil were thoroughly mixed, stored in a large box, and kept as near field conditions as possible so that all the work could be done on the same soil. As the soil was needed in the work, portions were brought to the laboratory, air-dried in the dark, then weighed in 100-gm. portions into sterile covered tumblers. To each of these were added 2 gm. of dried blood. The whole was then carefully mixed, and the salt in most cases added from a carefully standardized stock solution. This, together with sufficient sterile distilled water to make the moisture content up to 20 per cent, was thoroughly mixed in the soil. Each series, together with sterile blanks, was incubated at 28° to 30° C. for 21 days, and then the nitric nitrogen determined as follows (*4, p. 200*):

The contents of the beaker, together with 500 cc. of distilled water and 2 gm. of alum, were placed in quart Mason jars and agitated for five minutes in a shaker.

An aliquot part (100 cc.) of the supernatant liquid was pipetted off, and, together with 2 cc. of a saturated solution of sodium hydroxid, was evaporated to about one-fourth of its original volume to free it from ammonia. To this were added 50 cc. of ammonia-free water, 5 gm. of "iron-by-hydrogen" and 30 cc. of sulphuric acid (sp. gr. 1.35). If less than 40 mgm. of nitric nitrogen is to be determined, it is well to take a correspondingly smaller quantity of iron and sulphuric acid. The neck of the reduction flask was fitted with a 2-hole stopper, through which passed a 50-cc. separatory funnel and a bent tube which dipped into a vessel containing water in order to prevent mechanical loss. The acid was slowly added and allowed to stand until the rapid evolution of hydrogen was over. It was then heated to boiling for 10 minutes. The contents of the side vessel were returned to the reduction flask before the reaction was complete, thus insuring the complete reduction of any nitrates which may have been carried over with the first violent evolution of the hydrogen. The contents of the reduction flask were transferred to Kjeldahl flasks, neutralized with sodium hydroxid, and distilled into standard acid. The excess of acid was titrated back with standard alkali, lacomoid being used as an indicator; controls were made on all the reagents, including the alum used as a flocculant.

In every case at least four determinations were made with each concentration of the salt, and, in the absence of agreement between determinations, the series was repeated so that the results as herein reported are in every case the average of four or more closely agreeing determinations. Hence, experimental error has been reduced to as near a minimum as possible in this kind of work.

The solutions of the salts were prepared by weighing gram-molecular quantities of Merck's best grade of the respective salts into 1,000 cc. of

sterile distilled water and then quantitatively determining the amount present. In those cases in which the analysis showed the concentration wrong, it was corrected, so that we have a definite knowledge of the quantity of salt added to the soil, as the varying results reported by different investigators can in many cases be interpreted by the unknown variation in salts added. The solution thus prepared was then added to the soil in such quantities as to make the amount of the anion and of cation the same and directly comparable the one with the other. The comparatively insoluble salts, calcium carbonate, calcium sulphate, etc., were carefully weighed and intimately mixed with the soil. The arranging of the work in this order gives us as nearly absolute results as can be obtained by the present bacteriological methods, and at the same time gives us directly comparable results, which after all is what we have to look for in this work.

The salts tested were the chlorids, nitrates, sulphates, and carbonates of sodium, potassium, calcium, magnesium, manganese, and iron.

INFLUENCE OF SODIUM SALTS

The compounds used in this series were sodium chlorid, sodium sulphate, sodium nitrate, and sodium carbonate. They were in concentrations such that equivalent quantities of sodium in the various forms could be directly compared. The strengths varied from 0 to 1,380 p. p. m. of soil, and represented the actual proportion of sodium in the various forms applied to the soil. The results are reported as percentage, considering the nitric nitrogen produced in the untreated soil in each case as 100 per cent. This method of reporting the results makes them more directly comparable than if stated as milligrams of nitric nitrogen formed in 100 gm. of soil. The average nitrifying power of the untreated soil was 53 mgm. of nitric nitrogen per 100 gm. of soil. The results are given in Table II, and in every case are the average of at least four and sometimes of several times this number of closely agreeing determinations; hence, they should represent very closely the comparative influence of the various sodium salts upon nitrification.

Sodium chlorid is the only one of the sodium salts tested which increases the accumulation of nitric nitrogen in the soil. In this regard nitrification differs widely from ammonification, for in the latter both sodium nitrate and sodium carbonate stimulate. However, sodium chlorid is a much more active stimulant of the nitrifiers than it is of the ammonifiers, and it stimulates in much higher concentration, being the most active when the soil contains 230 p. p. m., and is not toxic until the quantity in the soil exceeds 460 p. p. m. The toxicity rapidly increases above this concentration, and at a concentration of 1,380 p. p. m., the nitric nitrogen present had been reduced to 16.4 per cent of what it was in the original soil.

TABLE II.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of sodium salts*

[The untreated soil is taken as 100 per cent]

Amount of sodium.	Perceutage of nitric nitrogen formed in presence of—			
	Sodium chlorid.	Sodium sulphate.	Sodium nitrate.	Sodium carbonate.
P. p. m.				
None	100. 0	100. 0	100. 0	100. 0
3.6	102. 4	87. 8	92. 7	100. 0
7.2	102. 5	60. 3	88. 3	79. 4
14.4	100. 6	57. 1	94. 4	76. 9
28.8	103. 1	86. 2	101. 0	88. 2
57.5	114. 7	74. 0	75. 9	94. 1
115.0	139. 6	55. 1	71. 6	79. 4
230.0	142. 0	65. 4	69. 4	73. 5
460.0	136. 2	63. 2	48. 0	76. 5
920.0	57. 5	63. 0	17. 1	63. 5
1,380.0	16. 4	50. 8	— 14. 0	58. 8

Although sodium carbonate is toxic in the lowest concentration tested, yet its toxicity does not increase as rapidly as does that of the chlorid, for at the highest concentration it still produced 58.8 per cent of nitric nitrogen. The action of sulphate and carbonate nearly parallel each other throughout the entire series.

Sodium nitrate probably stimulates slightly at 28.8 p. p. m., but above this concentration the nitric nitrogen rapidly decreases and when the concentration of sodium in the form of sodium nitrate reaches 1,380 p. p. m., there is an actual loss of nitric nitrogen from the soil.

It is quite evident from these results that the order of toxicity of these salts are as follows: Sodium sulphate, sodium carbonate, sodium nitrate, and sodium chlorid; but if we consider them at the highest concentrations the order becomes sodium nitrate, sodium chlorid, sodium sulphate, and sodium carbonate.

The results for sodium chlorid confirm the findings of C. B. Lipman (*8*) as opposed to Dehérain (*1*) that sodium chlorid does stimulate the nitrifying bacteria. Although sodium carbonate is toxic in comparatively low concentrations, it is not as toxic in this soil as it was found to be in the soil used by Lipman; moreover, he noted a stimulation with sodium sulphate which does not appear in any of our work. This apparent discrepancy must be due to a difference in the soils used.

At the lower concentrations a given molecular quantity of sodium sulphate is more toxic to the nitrifying organisms than is an equivalent molecular proportion of sodium carbonate, and this is more toxic than is an equivalent molecular proportion of sodium nitrate. Sodium chlorid is less toxic than any of the other salts tested. When 4×10^{-3} mole of sodium chlorid, sodium sulphate, or sodium carbonate are added to 100 gm. of soil, the nitrate accumulation is reduced to about one-half

normal. But when an equivalent quantity of sodium in the form of the nitrate is added, the nitrifying powers are reduced to one-fifth.

The work was so planned that equal molecular proportions of the various salts could be compared as shown in Table III.

TABLE III.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of sodium salts in equal molecular proportions*

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Percentage of nitric nitrogen formed in presence of—			
	Sodium chlorid.	Sodium sulphate.	Sodium nitrate.	Sodium carbonate.
None	100. 0	100. 0	100. 0	100. 0
156×10^{-7}	102. 4	60. 2	92. 7	79. 4
312×10^{-7}	102. 5	57. 1	88. 3	76. 9
625×10^{-7}	100. 6	86. 2	94. 4	88. 2
125×10^{-6}	103. 1	74. 0	101. 0	94. 1
25×10^{-5}	114. 7	55. 1	75. 9	79. 4
5×10^{-4}	139. 6	65. 4	71. 6	73. 5
1×10^{-3}	142. 7	63. 2	69. 4	76. 5
2×10^{-3}	136. 2	63. 0	48. 0	58. 8
4×10^{-3}	57. 5	50. 8	17. 1	58. 8

Sodium chlorid, which has the lowest molecular weight, is the least toxic, whereas sodium nitrate, with the next lowest, comes next in order of toxicity. It is, therefore, quite evident that the toxicity of the sodium salts varies with the electro-negative ion with which sodium is combined and must be due to a physiological influence exerted by it upon the protoplasm of the organisms and not due to a direct osmotic effect.

INFLUENCE OF POTASSIUM SALTS

The compounds used in the potassium series were the chlorid, sulphate, nitrate, and carbonate. The concentrations were the same as those used in the sodium series. The results, as reported in Table IV, are the average of four or more closely agreeing determinations.

Potassium chlorid and potassium nitrate are the only potassium salts which yielded a stimulation with the nitrifying organisms. But after the stimulating concentration is exceeded these compounds rapidly increase in toxicity, so that by the time a concentration of 2,346 p. p. m. of potassium in the form of the chlorid has been added to the soil, the nitric nitrogen present had been reduced to 5.23 per cent. Where a similar quantity of the nitrate was added, there was a marked loss of nitrates.

Potassium sulphate was found to be toxic in the lowest concentration tested, but the toxcity does not increase as rapidly with the increased concentrations of this salt as it does with those salts which are stimulants. For it is only when 1,564 p. p. m. of potassium in the form of potassium sulphate has been added to the soil that we find the nitric-nitrogen accumulation reduced to one-half normal.

TABLE IV.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and different forms of potassium*

[The untreated soil is taken as 100 per cent]

Amount of potassium.	Percentage of nitric nitrogen formed in presence of—			
	Potassium chlorid.	Potassium sulphate.	Potassium nitrate.	Potassium carbonate.
P. p. m.				
None	100. 0	100. 0	100. 0	100. 0
6. 1	93. 5	79. 7	106. 4	99. 1
12. 2	86. 8	95. 7	74. 7	92. 7
24. 4	106. 5	95. 0	98. 6	81. 3
48. 4	102. 3	93. 8	92. 5	80. 3
97. 8	84. 3	73. 4	100. 5	69. 0
195. 5	84. 3	76. 4	71. 1	70. 9
391. 0	84. 8	67. 2	33. 5	63. 7
782. 0	78. 0	62. 3	20. 0	55. 4
1, 564. 0	35. 6	51. 5	2. 5	41. 8
2, 346. 0	5. 2	32. 4	— 41. 2	14. 8

Potassium carbonate in the lower concentrations is less injurious than is the sulphate, but in higher concentrations it becomes much more toxic.

It may be seen that the action of the chlorid and carbonate are very similar, and one must conclude that the anion, in addition to the cation, exerts a very marked effect upon the nitrifying organisms of the soil. The nitrifying powers of the soil are reduced to about half-normal with nearly the same concentration of potassium in the form of the chlorid, sulphate, and carbonate, whereas the nitrate reduces it to half-normal when one-fourth the concentration has been reached.

The influence of equivalent molecular proportions on the various potassium salts is given in Table V.

TABLE V.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of potassium salts in equal molecular proportions*

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Percentage of nitric nitrogen formed in presence of—			
	Potassium chlorid.	Potassium sulphate.	Potassium nitrate.	Potassium carbonate.
None	100. 0	100. 0	100. 0	100. 0
156×10^{-7}	93. 5	95. 7	106. 4	92. 7
312×10^{-7}	86. 8	95. 0	74. 7	81. 3
625×10^{-7}	106. 5	93. 8	98. 6	80. 3
125×10^{-6}	102. 3	73. 4	92. 5	69. 0
25×10^{-5}	84. 3	76. 4	100. 5	71. 0
5×10^{-4}	84. 3	67. 2	71. 1	63. 7
1×10^{-3}	84. 8	62. 3	33. 5	55. 4
2×10^{-3}	78. 0	51. 5	20. 0	41. 8
4×10^{-3}	35. 6	32. 4	2. 5	14. 8

In the lower concentrations a given molecular quantity of potassium carbonate is much more toxic to the nitrifiers than is an equivalent molecular quantity of the sulphate, nitrate, or chlorid, the toxicity of the compounds varying in the order named.

In the highest concentration tested the chlorids reduce the nitrifying powers to 35.6 per cent, the sulphate to 32.4 per cent, the nitrate to 2.5 per cent, the carbonate to 14.8 per cent.

The toxicity of the potassium salts is governed largely by the electro-negative ion combined with the potassium and if the osmotic pressure plays any great part it is masked by other factors.

INFLUENCE OF CALCIUM SALTS

The compounds used in this series were calcium chlorid, calcium nitrate, calcium sulphate, and calcium carbonate. The first two were added to the soil according to the usual method from a standard solution, whereas the sulphate and carbonate were weighed into the soil, carefully mixed, and then treated in the ordinary manner.

A number of determinations were made in each case and compared with sterile blanks, so that the results as reported in Table VI are the average of four or more closely agreeing determinations.

TABLE VI.—*Percentage of nitric-nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of calcium salts*

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Amount of calcium.	Percentage of nitric-nitrogen formed in presence of—			
		Calcium chlorid.	Calcium sulphate.	Calcium nitrate.	Calcium carbonate.
	P. p. m.				
None	None	100. 0	100. 0	100. 0	100. 0
78×10^{-7}	3. 12	87. 9	117. 1	99. 1	99. 4
156×10^{-7}	6. 24	79. 0	115. 9	88. 8	97. 2
312×10^{-7}	12. 35	88. 2	143. 9	92. 5	97. 2
625×10^{-7}	24. 82	84. 0	140. 9	102. 1	85. 3
125×10^{-6}	49. 64	86. 8	125. 0	92. 1	95. 9
25×10^{-5}	99. 28	127. 4	148. 8	100. 8	97. 2
5×10^{-4}	198. 56	160. 3	151. 7	86. 9	79. 0
1×10^{-3}	397. 12	167. 6	180. 6	46. 8	82. 0
2×10^{-3}	794. 24	124. 5	196. 7	— 0. 4	59. 2
3×10^{-3}	1191. 36	99. 2	189. 2	— 20. 2	59. 1

In marked counterdistinction to its action on the ammonifying organisms, calcium carbonate fails to stimulate in any of the concentrations. This, however, is not surprising, as the soil which is being used in this work contains over 12 per cent of calcium carbonate, which is undoubtedly abundant for the maximum activity of these organisms. We do, however, find a gradual increase in toxicity as the quantity of calcium carbonate added to the soil increases, so that by the time 1,191.4 parts of calcium in the form of the carbonate have been added the nitri-

fying powers of the soil have been reduced to 59 per cent of normal, thus indicating that it is possible to add sufficient limestone to a soil to reduce its nitric-nitrogen content. Whether this be due to its direct action upon the bacterial activities or to changing of the calcium : magnesium-carbonate ratio of the soil can not be answered by these results. It is certain it can not be due to the anions alone, for we find the other calcium salts acting as stimulants.

Calcium nitrate stimulates only slightly, and this at the medium concentrations. At low concentrations it is not as toxic as is calcium carbonate, but at concentrations of 397.1 p. p. m. and above of calcium in the form of calcium nitrate it is highly toxic. At the highest concentration tested, 1,191.4 p. p. m., the nitric nitrogen rapidly disappeared from the soil.

Calcium sulphate stimulated in all of the concentrations tested, and in most cases there was a very marked increase in the nitric nitrogen of the soil. This undoubtedly accounts for the increased plant growth noted when gypsum is added to a soil. When 794.2 p. p. m. of calcium in the form of the sulphate had been added to the soil, there was nearly twice as much nitric nitrogen in the treated soil as there was in the untreated soil.

Calcium chlorid is apparently toxic at the lower concentrations, but at the higher concentrations it becomes a marked soil stimulant. Its highest stimulation is noted at a concentration slightly lower than that of calcium sulphate. Furthermore, it becomes toxic again, a condition which has not been observed for the sulphate.

It may be noted that 3×10^{-3} mole of calcium nitrate reduces the nitric-nitrogen content of the soil 120 per cent below normal, while an equivalent quantity of the carbonate reduces it 59.1 per cent below normal. The chlorid at this concentration is without effect, whereas the sulphate is a very strong stimulant.

INFLUENCE OF MAGNESIUM SALTS

The compounds used in the magnesium series were the chlorid, sulphate, nitrate, and carbonate of magnesium. The last-named was applied to the soil in the form of a dry powder, whereas all of the others were added in the usual manner. The results representing the average of four or more closely agreeing determinations are given in Table VII.

Both the nitrate and carbonate of magnesium increase the nitric-nitrogen content of the soil. In the case of the carbonate this is very marked and is markedly different from the results obtained with calcium carbonate.

The nitrate, after reaching its highest stimulating point, rapidly becomes toxic, so that by the time 729.6 p. p. m. of magnesium in the form of the nitrate had been added to the soil, there was a loss of 354.7 per cent of the nitrogen.

TABLE VII.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of magnesium salts*

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Amount of magnesium.	Percentage of nitric nitrogen formed in presence of—			
		Magnesium chlorid.	Magnesium sulphate.	Magnesium nitrate.	Magnesium carbonate.
	P. p. m.				
None	None	100.0	100.0	100.0	100.0
78×10^{-7}	1.9	67.7	95.2	92.9	90.4
156×10^{-7}	3.8	58.0	96.2	85.7	97.7
312×10^{-7}	7.6	68.5	101.2	106.5	108.1
625×10^{-7}	15.2	55.0	87.1	87.0	136.1
125×10^{-6}	30.4	97.1	88.7	95.7	119.8
25×10^{-5}	60.8	123.2	94.6	65.2	140.7
5×10^{-4}	121.6	84.5	90.4	43.5	101.2
1×10^{-3}	243.2	78.6	82.9	.....	116.0
2×10^{-3}	486.4	26.5	63.3	5.0	72.7
3×10^{-3}	729.6	11.8	59.6	−354.7	51.7

Magnesium carbonate reaches its highest stimulation at a concentration of 25×10^{-5} mole, and at a concentration of 3×10^{-3} mole the ammonifying powers had been reduced to one-half normal almost the same as was the case with the calcium carbonate.

It is indeed interesting to note that this soil, which already contains over 8 per cent of magnesium carbonate, has its nitrifying powers increased by the addition of magnesium carbonate. This would lend support to the idea promulgated in the last section that the depressing effect of the calcium carbonate is due to its changing of the lime-magnesia ratio in the soil.

All of the magnesium salts are peculiar in that at the lower concentrations they are toxic, but that at higher concentrations they are stimulants. This peculiarity is especially noticeable in the case of magnesium chlorid.

Both the chlorid and sulphate were toxic in all but one of the concentrations tested. The sulphate and carbonate of magnesium at the highest concentration tested reduced the nitrifying powers to about one-half normal, while the same concentration of the chlorid reduced it to less than one-eighth normal. A similar concentration of the nitrate caused a rapid disappearance of the nitrate from the soil.

It is quite evident from these results that the determining factor in the action of calcium or magnesium salts upon the nitrifying powers of the soil is the electro-negative ion.

INFLUENCE OF MANGANESE SALTS

The compounds used in this series were manganous chlorid, manganous sulphate, manganous nitrate, and manganous carbonate. The results so obtained as the average of a great number of closely agreeing determinations are given in Table VIII.

TABLE VIII.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of manganese salts*

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Amount of manganese.	Percentage of nitric nitrogen formed in presence of—			
		Manganous chlorid.	Manganous sulphate.	Manganous nitrate.	Manganous carbonate.
	P. p. m.				
None	None.	100. 0	100. 0	100. 0	100. 0
78×10^{-7}	4. 3	100. 6	113. 2	121. 3	91. 2
156×10^{-7}	8. 6	112. 9	100. 0	113. 8	72. 2
312×10^{-7}	17. 2	57. 7	106. 6	113. 5	77. 8
625×10^{-6}	34. 4	41. 7	106. 6	125. 4	86. 3
125×10^{-7}	68. 8	31. 9	104. 1	107. 9	108. 4
25×10^{-5}	137. 6	44. 2	97. 6	79. 4	86. 8
5×10^{-4}	275. 2	55. 2	101. 3	49. 2	84. 6
1×10^{-3}	550. 4	55. 2	88. 7	26. 9	84. 6
2×10^{-3}	1, 100. 8	24. 5	80. 2	4. 5	71. 1
3×10^{-3}	1, 651. 2	4. 5	74. 3	— 17. 80	81. 2

All of the manganese compounds tested are strong stimulants to the nitrifying organisms. The extent of the stimulation and the quantity of the salt required to produce the maximum stimulation varies greatly with the salt used. The sulphate produces its greatest stimulation at the lowest concentration tested, whereas the chlorid is most active at 8.6 p. p. m. of manganese, the nitrate at 34.4, and the carbonate at 68.8. Manganous chlorid stimulates the nitrifying organisms greater than it does the ammonifying organisms, whereas with the carbonate the reverse is true. It is quite evident from these results that the stimulation exerted by manganese upon soil organisms is governed by the electronegative ion, which is combined with the manganese and the specific class of organisms on which it is acting.

While manganese is at times added to the soil as a soil amendment, the results reported by different investigators vary, some noting a marked stimulation, while in other experiments it is not so pronounced. The data herein reported, together with those previously reported for ammonification, offer a very plausible explanation of the lack of agreement among various reported experiments.

If we admit that much of the beneficial effect of the manganese on the plant is due to its stimulating influence on the bacterial activity of the soil, thus liberating more available nitrogen, forming organic acids and carbon dioxid, which in turn liberate phosphorus and other elements essential to plant growth, we can readily see that its influence upon a plant growing in a soil well supplied with available nitrogen and phosphorus would not be great. But if the soil contained unavailable plant food, the increased bacterial activity would make more plant food available. This would then be taken up by the growing plant and shown in the increased crop yield.

The manganous carbonate is peculiar in that at the lower concentration it is toxic, but at a concentration of 125×10^{-6} mole it acts as a stimulant. The chlorid is most active at 156×10^{-7} mole, and in the next concentration the nitric nitrogen content of the soil is reduced to one-half-normal, and at a concentration of 3×10^{-3} mole there is only 4.5 per cent of the normal nitric nitrogen present in the soil.

The manganous sulphate stimulates throughout a much wider range of concentrations than does the chlorid, and at the highest concentration tested it had reduced the nitrification to only three-fourths-normal.

Manganous nitrate is a more powerful stimulant than any of the other salts, and stimulates in the same concentrations as does the sulphate, but it is much more toxic in the higher concentrations than are any of the other salts. Although the carbonate is not a very active stimulant at any of the concentrations tested, neither is it very toxic.

INFLUENCE OF IRON SALTS

The compounds used in this series were ferric chlorid, ferric sulphate, ferric nitrate, and ferric carbonate. All except the carbonate were added to the soil in solution. The carbonate was added in the form of a dry powder and carefully mixed with the soil. Considerable difficulty was experienced in getting duplicate determinations to agree when the sulphate was applied to the soil, and the results as reported represent the average of eight sets of determinations. The chlorid, nitrate, and carbonate represent the average of four closely agreeing sets of determinations The results are given in Table IX.

TABLE IX.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of iron salts*

[The untreated soil is taken as 100 per cent]

Amount of iron.	Percentage of nitric-nitrogen formed in presence of—			
	Ferric chlorid.	Ferric sulphate.	Ferric nitrate.	Ferric carbonate.
P. p. m.				
None	100. 0	100. 0	100. 0	100. 0
2. 9	103. 4	102. 0	95. 6	102. 5
5. 8	101. 0	94. 2	95. 4	104. 1
11. 6	115. 5	67. 0	92. 4	102. 9
23. 2	116. 5	82. 8	90. 1	96. 0
46. 5	128. 4	97. 1	89. 7	99. 4
93. 0	103. 4	97. 5	86. 2	100. 7
186. 0	102. 6	98. 9	89. 2	105. 6
372. 3	78. 5	100. 0	53. 4	110. 7
744. 6	32. 6	84. 3	12. 4	117. 4
1, 116. 9	10. 9	87. 9	7. 9	104. 5

From these results it may be seen that all of the iron salts, with the exception of the nitrates, increase the nitric nitrogen of the soil. The maximum stimulation for the chlorid occurs when 46.5 p. p. m. of iron

had been applied to the soil, the sulphate at 2.9 p. p. m., and the carbonate at 744.6 p. p. m. The chlorid is a much more powerful stimulant than are any of the other salts, and in this respect there is a marked similarity between the ammonifying and nitrifying organisms.

The results offer a very likely explanation of why there is an increased yield obtained when iron compounds are applied to the soil, as the stimulation of the soil organisms would greatly increase the available plant food. There would be not only more available nitrogen but the increased bacterial activity would render soluble more potassium and especially more phosphorus; the results reported by Griffiths (5) indicate that the plants growing on soil manured with iron sulphate contain more phosphorus than those growing on unmanured soil. We would have to assume either that the application of iron to the soil stimulates a plant so that it requires more phosphorus or else that the iron compounds increase the availability of the phosphorus, and, hence, the plant takes up more. This latter explanation seems the more reasonable, but here we have to look for an indirect effect, for the iron directly depresses the solubility of phosphorus (2).

The ferric nitrate becomes toxic to the nitrifying organisms at a much lower concentration than any of the other iron salts. Furthermore, its toxicity increases much more rapidly than that of any of the other compounds. The chlorid does not become toxic until 372.3 p. p. m. of the iron has been added to the soil, whereas the sulphate is toxic at 5.8 p. p. m. The carbonate was toxic at none of the concentrations tested.

The highest concentration used 1,116.9 p. p. m. of iron in the form of chlorid, reduces the nitric nitrogen content of the soil to 10.9 per cent, the sulphate to 87.9, the nitrate to 7.9, whereas the carbonate containing soil contains 104.5 per cent of normal.

INFLUENCE OF CHLORIDS

So far in this discussion we have been comparing the action of compounds having the same electro-positive but a varying electro-negative ion. Hence, the results considered have given us an insight into the influence of the anions Cl, SO_4, NO_3, and CO_3 upon the nitrifying efficiency of the soil. It is therefore important that the compounds be compared where the anion is a constant and the cation a variable. This is done in Table X. In this series we have the chlorids of sodium, potassium, magnesium, calcium, manganese, and iron. The experiment was so arranged that equivalent quantities of chlorin in the various forms were applied in 100 gm. of soil. Each reported results is the average of four or more closely agreeing determinations.

TABLE X.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm of dried blood and varying amounts and kinds of chlorids*

[The untreated soil is taken as 100 per cent]

Amount of chlorid.	Percentage of nitric nitrogen formed in presence of—					
	Sodium Chlorid.	Potassium chlorid.	Magnesium chlorid.	Calcium chlorid.	Manganous chlorid.	Ferric chlorid.
P. p. m.						
None	100. 0	100. 0	100. 0	100. 0	100. 0	100. 0
5.54	102. 4	93. 5	67. 7	87. 9	100. 6	103. 4
11.08	102. 4	86. 8	58. 0	79. 0	112. 9	101. 0
22.16	100. 6	106. 5	68. 5	88. 2	57. 7	115. 5
44.32	103. 1	102. 3	55. 0	84. 0	41. 7	116. 5
88.65	114. 7	84. 3	97. 1	86. 8	31. 9	128. 4
117.30	139. 6	84. 3	123. 2	127. 4	44. 2	103. 4
354.60	142. 0	84. 8	84. 5	160. 3	55. 2	102. 6
709.20	136. 2	78. 0	78. 6	167. 6	55. 2	78. 5
1,418.39	57. 5	35. 6	26. 5	124. 5	24. 5	32. 6
2,127.59	16. 4	5. 2	11. 8	99. 2	4. 5	10. 9

All of the chlorids tested increase the accumulation of nitric nitrogen in the soil, and it would appear from the results that the extent of stimulation is governed largely by the cation while the toxicity of the compound is determined by the anion. Measured in terms of the effect upon nitrification, calcium chlorid is the most effective stimulant of the chlorids tested, followed in the order: Sodium chlorid, ferric chlorid, magnesium chlorid, manganous chlorid, and potassium chlorid.

These results undoubtedly account for the varying results noted when sodium chlorid is used as a fertilizer. Some experimenters obtain a good yield from its use; others obtain just as good a yield without it.

Storp (*10*), in an article on sodium chlorid as a manure, attributes the benefit derived from its use as being due to the decomposing of insoluble plant food by the sodium chlorid. If this be the correct theory, we can account for yields such as those obtained by Voelcker (*11*). As an average of five experiments, on land which had been manured with sodium chlorid, the yield of mangels was 36,060 pounds. On the adjoining unmanured ground there were but 26,035 pounds, a difference of a little over 10,000 pounds due to the use of sodium chlorid. Now, if the land was rich in insoluble plant food and the chlorid was able to liberate it, we could expect a large yield. On the other hand, if the land had been poor in unavailable plant food, no good result would have followed its use. Wheeler (*12*) seems to have established the fact that sodium chlorid can not to any great extent take the place of potassium salts. However, he does think that sodium chlorid can liberate phosphorus from insoluble forms, as the following statement will show:

> It may, however, be stated here that sodium salts seem to liberate phosphorus and potassium so that under certain circumstances they may act as an indirect manure.

In a later report (*13, p. 196–202*) he shows that the percentage of phosphorus in a plant is increased by the use of a sodium salt. With radish this was in some cases as much as 0.052 per cent more in the crop from land which had received a full ration of sodium over that which received but a part ration. In the case of turnips there was a difference of 0.121 per cent, the beets 0.035 per cent, the carrots 0.074 per cent, while in the case of the chicory the results are practically the same in the crop from the manured and unmanured land. The report contains many more cases in which the sodium salt increased the phosphorus in the plant. However, the laboratory tests which have been made on phosphates show that sodium chlorid depresses the solubility of a phosphate (*2*).

It is therefore evident from the results obtained that sodium chlorid increases the ammonia and nitric-nitrogen accumulation of the soil, and all indications point to the conclusion that this is due to an increased activity of bacteria, which bring about this transformation. This being the case, there must be an increase of the nitrites produced in the soil by nitrosomonas, and Hopkins and Whiting (7) have demonstrated that these organisms possess the power of rendering soluble the phosphorus of the soil. Therefore we could expect to find an increased yield when sodium chlorid is added to a soil deficient in soluble phosphate but containing considerable insoluble phosphate, this increase being due to the liberation of phosphorus, which we find revealing itself in a greater phosphorus content of the plants, as noted in the above references.

The concentration at which the various compounds are found to exert their greatest stimulating action varies greatly with the compound. The maximum for the different compounds is as follows: Calcium chlorid 709.2 p. p. m., chlorin as sodium chlorid 354.6 p. p. m., chlorin as ferric chlorid 88.7 p. p. m., chlorin as magnesium chlorid 117.3 p. p. m., chlorin as manganous chlorid 11.1 p. p. m., chlorin as potassium chlorid 22.2 p. p. m.

The point at which the specific compound becomes toxic varies greatly with the cation. With the exception of calcium chlorid there is nearly the same quantity of nitric nitrogen in all soils at the highest concentration, 2,127.6 p. p. m. of chlorin in the various combinations.

The difference in toxicity of the compounds becomes greater when we compare equal molecular portions of the different compounds, as may be seen from Table XI.

TABLE XI.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and to which were added varying amounts and kinds of chlorids in equal molecular proportions*

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Percentage of nitric-nitrogen formed in the presence of—				
	Sodium chlorid.	Potassium chlorid.	Magnesium chlorid.	Calcium chlorid.	Manganous chlorid.
None	100. 0	100. 0	100. 0	100. 0	100. 0
156×10^{-7}	102. 4	93. 5	58. 0	79. 0	112. 9
312×10^{-7}	102. 5	86. 8	68. 5	88. 2	57. 7
625×10^{-7}	100. 6	106. 5	55. 0	84. 0	41. 7
125×10^{-6}	103. 1	102. 3	97. 1	86. 8	31. 9
25×10^{-5}	114. 7	84. 3	123. 2	127. 4	44. 2
5×10^{-4}	139. 6	84. 3	84. 5	160. 3	55. 2
1×10^{-3}	142. 0	84. 8	88. 6	167. 6	55. 2
2×10^{-3}	136. 2	78. 0	26. 5	124. 5	24. 5
4×10^{-3}	57. 5	36. 6	11. 8	99. 2	4. 5

This would be taken to indicate that the toxicity of the salt is due to physiological action upon the bacteria and not the osmotic pressure exerted by the several compounds.

INFLUENCE OF SULPHATES

The compounds used in this series were the sulphates of potassium, sodium, calcium, magnesium, manganese, and iron. The quantity of the salt used in each case was such that equivalent quantities of sulphate in the various forms were added to 100 gm. of soil. It was also of such a concentration, with the exception of the iron sulphate, that equal molecular proportions of the various salts were added to 100 gm. of soil The results, as averages of a number of closely agreeing determinations, are given in Table XII.

TABLE XII.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of sulphates*

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Amount of sulphate.	Percentage of nitric nitrogen formed in presence of—					
		Sodium sulphate.	Potassium sulphate.	Calcium sulphate.	Magnesium sulphate.	Manganous sulphate.	Ferric sulphate.
	P. p. m.						
None	None.	100. 0	100. 0	100. 0	100. 0	100. 0	100. 0
78×10^{-7}	7. 5	87. 8	79. 7	117. 1	95. 2	113. 2	102. 0
156×10^{-7}	15. 0	60. 2	95. 7	115. 9	96. 2	100. 0	94. 2
312×10^{-7}	30. 0	57. 1	95. 0	143. 0	101. 2	106. 6	67. 0
628×10^{-7}	60. 0	86. 2	93. 8	140. 9	87. 1	106. 6	82. 8
125×10^{-6}	120. 1	74. 0	73. 4	125. 0	88. 7	104. 1	97. 1
25×10^{-5}	240. 2	55. 1	76. 4	148. 8	94. 6	97. 6	97. 5
5×10^{-4}	480. 3	65	67. 2	151. 7	90. 4	101. 3	98. 9
1×10^{-3}	960. 6	63. 2	62. 3	180. 6	82. 9	88. 7	100. 0
2×10^{-3}	1, 921. 2	63. 0	51. 5	196. 7	63. 3	80. 2	84. 3
3×10^{-3}	2, 881. 8	50. 8	32. 4	189. 2	59. 6	74. 3	87. 9

Sodium sulphate and potassium sulphate are the only sulphates which fail to increase the nitric-nitrogen content of the soil, and in this respect they are similar to the ammonifiers (*3*). The degree and concentration at which the various salts stimulate vary with the compound, being greatest with calcium sulphate and least with magnesium sulphate.

Calcium sulphate is the most powerful soil stimulant known. This is due not to a direct nutritive value but to the liberation of plant food which may in a measure be due to the direct interchange between calcium and potassium. However, these results clearly indicate that its main influence is upon the bacterial activities of the soil, especially the ammonifying and nitrifying organisms of the soil. In this manner the available nitrogen of the soil is increased. Furthermore, in the metabolic processes of these bacteria there are formed acids and other compounds which act as solvents for the potassium and phosphorus of the soil. It is well known that the addition of gypsum increases the potassium of the crop, and some other experiments show that it increases the phosphorus, for example, Boussingault (*9, v. 1, p. 327*) found a greater amount of phosphorus in land manured with gypsum. The phosphorus in the clover from the manured land was 10.57 kilos; that from the unmanured 4.80 kilos. The following year, although no more manure was applied, the phosphorus from the hay grown on the manured land was 6.93 kilos more than from the unmanured.

Although magnesium, manganous, and iron sulphate all increase at some concentration the nitric nitrogen of the soil, they are not nearly as active as is calcium sulphate.

The toxicity of sodium and potassium sulphate is very marked even at the lowest concentrations tested, and increase with increasing quantities of the salt. Although the toxicity of potassium sulphate does not increase as rapidly at the lower concentrations as does that of sodium sulphate, yet at the highest concentration tested, 3×10^{-3} mole, the sodium-sulphate-treated soil contains 50.8 per cent normal of nitric nitrogen, whereas that of the potassium sulphate treated soil contains 32.4 per cent.

Calcium sulphate is not toxic at any of the concentrations tested, whereas magnesium sulphate probably first becomes toxic at a concentration of 628×10^{-7} mole, and at 3×10^{-3} mole the nitric nitrogen content of the soil had been reduced to about one-half normal.

Manganous sulphate and iron sulphate are similar in that they are only slightly toxic even at the higher concentrations. It is evident from these results that the stimulating action is due mainly to the electropositive ion, whereas the electro-negative ion determines the toxicity of the compound.

INFLUENCE OF NITRATES

The compounds used in this series were sodium nitrate, potassium nitrate, calcium nitrate, magnesium nitrate, manganous nitrate, and ferric nitrate. The quantity added to the soil was such that in each case equivalent quantities of nitrate in the various forms were added to the soil. Hence, the varying factor is the electro-positive ion, the electro-negative remaining the same in each case. The average results for a number of closely agreeing determinations are given in Table XIII as percentages of nitric nitrogen found in 100 gm. of soil, the untreated soil being taken as 100 per cent.

TABLE XIII.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of nitrate salts*

[The untreated soil is considered as 100 per cent]

Amount of nitrate.	Percentage of nitric nitrogen formed in presence of—					
	Sodium nitrate.	Potassium nitrate.	Calcium nitrate.	Magnesium nitrate.	Manganous nitrate.	Ferric, nitrate.
P. p. m.						
None	100. 0	100. 0	100. 0	100. 0	100. 0	100. 0
9. 7	92. 7	106. 4	99. 1	92. 9	121. 3	95. 6
19. 4	88. 3	74. 7	88. 4	85. 7	113. 8	95. 4
38. 8	94. 4	98. 6	92. 5	106. 5	113. 5	92. 4
77. 6	101.	92. 5	102. 1	87.	125. 4	90. 1
155. 2	75. 9	100. 5	92. 1	95. 7	107. 9	89. 7
310. 4	71. 6	71. 1	100. 8	65. 2	79. 4	86. 2
624. 8	69. 4	33. 5	86. 9	43. 5	49. 2	89. 2
1, 241. 6	48. 0	20. 0	46. 8	.....	26. 9	53. 4
2, 483. 2	17. 1	2. 5	— 0. 4	— 5. 0	4. 5	12. 4
3, 724. 8	— 14. 0	— 41. 2	— 20. 2	—354. 7	— 17. 8	7. 9

Sodium nitrate does not stimulate the nitrifying organisms in any of the concentrations, whereas potassium in the lowest concentration does. Otherwise these two compounds are very similar in action. Both in low concentrations depress the accumulation of nitric nitrogen in the soil, but at a higher concentration this toxicity disappears. When the quantity of nitrates added to the soil exceeds 310.4 p. p. m., the nitric nitrogen rapidly disappears from the soil. At the highest concentrations tested the added nitric nitrogen was rapidly disappearing.

Calcium and magnesium nitrate in the lower concentrations are quite similar in action, but at 38.8 p. p. m. of nitric nitrogen in the form of magnesium nitrate is more active as a soil stimulant than the calcium in any of the concentrations tested. At the higher concentrations the nitric nitrogen disappeared from the soil more rapidly where the magnesium salt was added than where the calcium salt was added.

Manganous nitrate is the strongest nitrate stimulant tested, at one concentration increasing the nitric-nitrogen content of the soil one-

fourth. But even in the presence of large quantities of this salt there is a notable disappearance of nitric nitrogen from the soil.

Ferric nitrate is peculiar, in that it fails to stimulate in any of the concentrations tested, and there is a gradual increase in toxicity from concentration to concentration. However, even where the largest quantity of iron nitrate was added, there was no loss of nitric nitrogen from the soil.

The occurrence of the negative results where the sodium, potassium, calcium, magnesium, and manganous nitrate are added to the soil raises the question, Has denitrification taken place or does it stimulate other bacterial activities so that the nitric nitrogen is rapidly transformed into protein nitrogen?

In order to answer this question, a set was prepared in which the various salts were added to the soil and incubated for 21 days, and the total nitrogen determined. The results are given in Table XIV. Each is the average of four determinations.

TABLE XIV.—*Quantity of nitrogen obtained from 100 gm. of soil receiving various treatments*

Treatment.	Nitrogen in 100 gm. of soil.	Excess in nitrate-treated soil.	Gain or loss in nitrogen over soil receiving no nitrate.
	Mgm.	*Mgm.*	*Mgm.*
Dried blood, no nitrate	333.9	.....	.....
Dried blood, 84.06 mgm. of nitric nitrogen as sodium nitrate	419.3	85.4	1.34
Dried blood, 84.06 mgm. of nitric nitrogen as calcium nitrate	460.5	126.6	42.54
Dried blood, 84.06 mgm. of nitric nitrogen as ferric nitrate	455.0	121.1	37.04
Dried blood, 84.06 mgm. of nitric nitrogen as magnesium nitrate	466.5	132.6	48.54
Dried blood, 84.06 mgm. of nitric nitrogen as manganous nitrate	443.1	109.2	25.14
Dried blood, 84.06 mgm. of nitric nitrogen as potassium nitrate	412.0	78.1	— 5.96

It is evident from these results that the loss of nitric nitrogen from this soil is not due to denitrification, for we find in every case, with the exception of where the potassium nitrate was added to the soil, that there was a gain of combined nitrogen. This is remarkable, for we have here a soil which was low in nitrogen but to which had been added 2 per cent of dried blood greatly stimulated in its nitrogen-fixing powers. The quantity of nitrogen fixed by this soil on the addition of the various nitrates in some cases is from four to eight times that normally fixed by the soil. Whether the azofiers would continue to fix nitrogen at this speed is doubtful, but experiments are under way in this laboratory to decide this point. If they will, it opens up an interesting and practical field for investigation.

INFLUENCE OF CARBONATES

The compounds used in this series were the carbonates of sodium, potassium, calcium, magnesium, manganese, and iron. The results as percentages of nitric nitrogen, the untreated soil being considered as 100 per cent, are given in Table XV.

TABLE XV.—*Percentage of nitric nitrogen formed in 100 gm. of soil containing 2 gm. of dried blood and varying amounts and forms of carbonates*

[The untreated soil is taken as 100 per cent]

Fraction of molecular weight in 100 gm. of soil.	Amount of carbonate.	Percentage of nitric nitrogen formed in presence of—					
		Sodium carbonate.	Potassium carbonate.	Calcium carbonate.	Magnesium carbonate.	Manganous carbonate.	Ferric carbonate.
	P. p. m.						
None	None.	100.0	100.0	100.0	100.0	100.0	100.0
78×10^{-7}	4.7	100.0	99.1	99.4	70.4	91.2	102.5
156×10^{-7}	9.4	79.4	92.7	97.4	97.7	72.2	104.1
312×10^{-7}	18.7	76.9	81.3	97.2	108.1	77.8	102.9
625×10^{-7}	37.5	88.2	80.3	85.3	136.1	86.3	96.0
125×10^{-6}	75.0	94.1	69.0	95.9	119.8	108.4	99.4
25×10^{-5}	150.0	79.4	71.0	97.2	140.7	86.8	100.7
5×10^{-4}	300.0	73.5	63.7	79.0	101.2	84.6	105.6
1×10^{-3}	600.0	76.5	55.4	82.0	116.0	84.6	110.7
2×10^{-3}	1,200.0	63.5	41.8	59.2	72.7	71.1	117.4
3×10^{-3}	1,800.0	58.8	14.8	69.1	51.7	81.2	104.5

Neither sodium nor potassium carbonate stimulate in any of the concentrations tested, and in this respect they differ sharply from their action on the ammonifiers. Both compounds become toxic at a concentration of 156×10^{-7} mole, but the toxicity of the potassium salt increases much more rapidly than the toxicity of the sodium salt. The latter, at a concentration of 3×10^{-3} mole, reduces the nitric-nitrogen content of the soil to 58.8, while the former, at the same concentration, reduces it to 14.8 per cent.

Calcium carbonate gradually increases in toxicity from the lower to the higher concentrations, whereas the magnesium carbonate, up to a concentration of 1×10^{-3} mole, is a strong stimulant. Although the calcium is toxic at the lowest concentration, and although magnesium stimulates, yet at the highest concentration tests, 3×10^{-3} mole, there is considerable more nitric nitrogen in the calcium-carbonate-treated soil than in the magnesium-carbonate-treated soil.

Manganese stimulates slightly when 125×10^{-6} mole are added to the soil, above which it becomes slightly toxic, next to iron carbonate; manganese carbonate is the least toxic of the carbonates tested. Iron carbonate is the most powerful of the stimulants tested and becomes toxic only in two concentrations, 625×10^{-7} mole and 125×10^{-6} mole. Above and below these concentrations it is a stimulant.

RELATION BETWEEN BACTERIAL ACTIVITY AND HIGHER PLANTS

The results herein reported, together with those published by Dr. Harris (*6*), make it possible to compare the influence of some of the salts upon the nitrifying powers of a soil with their influence upon the higher plants. This comparison is quite justifiable, for the same soil was used in the experiments with seedlings as has been used in the work on bacterial activities. The comparison is made in Table XVI. The results, as reported, are the quantities of the respective salts which are necessary to reduce nitrification and the production of dry matter in wheat seedlings to about half-normal.

TABLE XVI.—*Percentage of various salts in loam soil necessary to reduce nitrification, germination, and dry matter produced in wheat to about half-normal*

Salt.	Reduction of nitrification to about half-normal.	Reduction of wheat seedling to about half-normal.	Excess required by bacteria.
Magnesium chlorid	0.006	0.40	—0.394
Magnesium nitrate	.074	.45	— .376
Potassium nitrate	.101	.40	— .299
Potassium carbonate	.138	.70	— .562
Sodium nitrate	.170	.30	— .130
Sodium carbonate	.212	.30	— .088
Sodium chlorid	.234	.20	.034
Potassium chlorid	.298	.25	.048
Potassium sulphate	.349	.60	— .251
Magnesium sulphate	.361	.70	— .439
Sodium sulphate	.568	.55	.018

Four of the salts tested, sodium chlorid, potassium chlorid, sodium sulphate, and calcium chlorid, are less toxic to the nitrifying organisms than they are to wheat seedlings. All of the other compounds are much more injurious to nitrifying bacteria than they are to wheat seedlings. In many cases the wheat seedlings will withstand many times as much of the salt as will the plant. This is especially noticeable in the case of magnesium nitrate and magnesium chlorid. The plant being able to withstand 70 times as much of this latter compound as will bacteria.

It is certain from these results that a test of the ammonifying power of an alkali soil gives a better index of its crop-producing power than does a determination of its nitrifying power, for a close correlation was found to exist between the toxicity of many of these salts for ammonifying organisms and wheat seedlings (*3*).

RELATIVE STIMULATION OF THE VARIOUS SALTS

It has been noted repeatedly throughout this work that many of the salts tested increase the accumulation of nitric nitrogen in the soil. The extent of this stimulation and the concentration of the specific salts re-

quired to produce the maximum effect vary greatly with the salt. These facts are summarized in figures 1 and 2.

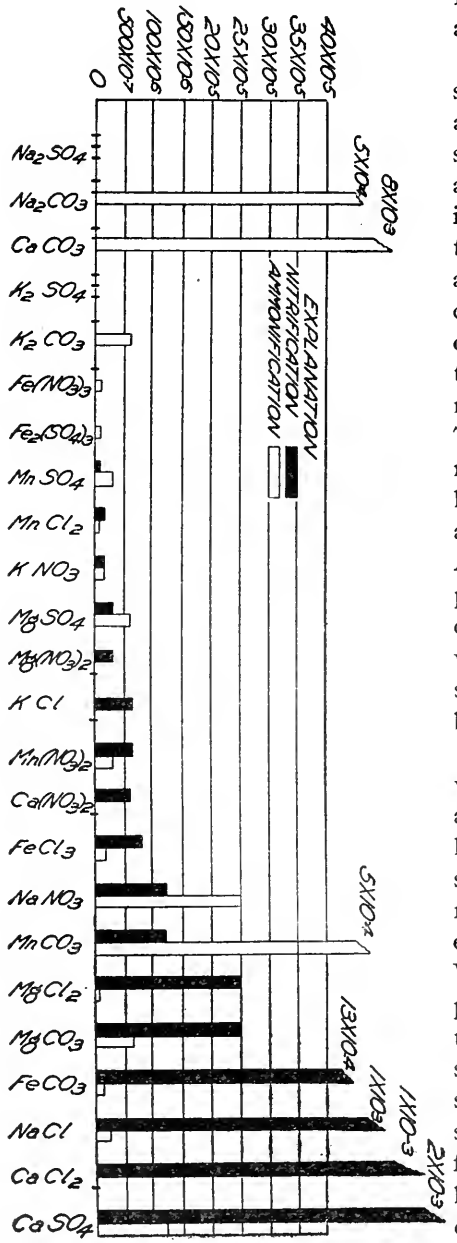

FIG. 1.—Graphs showing molecular concentrations at which the highest stimulation is noted

Only 6 of the compounds tested, sodium sulphate, sodium carbonate, potassium sulphate, potassium carbonate, calcium carbonate, and ferric nitrate, failed to increase the nitric-nitrogen content of the soil. The 18 others all increased the nitric-nitrogen content of the soil. There is no correlation between the stimulation of the ammonifying and nitrifying processes of the soil. This is remarkable when we remember that the speed of the latter is undoubtedly controlled and dependent upon the other. And the results herein reported probably indicate that there are other side reactions taking place which are influenced by these salts but which are not measured by these methods.

Averaging the molecular weights for the 12 compounds acting as the strongest stimulants, we find them to be considerably lower than the average molecular weight of those which exert little stimulating influence. We really find some of the compounds with the lowest molecular weight—for instance, sodium chlorid—the greatest stimulants. Hence, it would seem that Grutzer's generalization for animal stimulants does not hold for either the ammonifying or nitrifying organisms. Some of the strongest stimulants for plants, sodium chlorid and calcium sulphate, increase to the greatest extent the nitric-nitrogen content of the soil. Therefore it is certain that the increased plant growth is due to a

great extent to the increased available plant food yielded by the accelerated bacterial activity of the soil.

The quantity of the salt necessary to produce maximum stimulation varies greatly with the salt. It is usually the case that those compounds which are the greatest soil stimulants must be added in larger quantities to produce maximum stimulation than those which are not as active stimulants and which produce their greatest effect at lower concentrations.

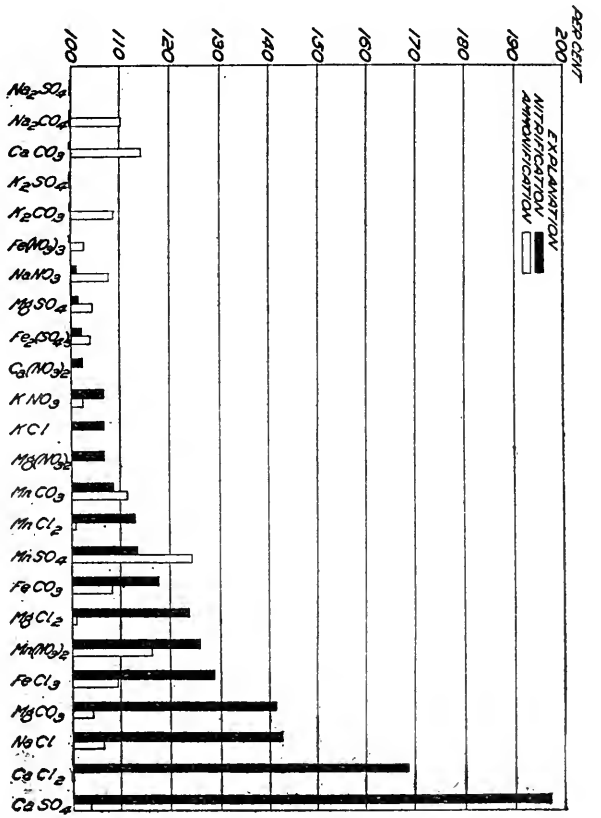

FIG. 2.—Graphs showing the percentage of stimulation at the above noted molecular concentrations (see fig. 1), the untreated soil being counted as producing 100 per cent of nitric nitrogen.

RELATIVE TOXICITY OF THE VARIOUS SALTS

The salts used in this work may be compared as to toxicity from three viewpoints: First, the lowest concentration of the salt at which a toxic effect is noted toward the nitrifying organisms; second, the molecular concentration at which nitric-nitrogen accumulation is reduced to three-fourths normal; and third, the percentage of nitric-nitrogen produced in the presence of the largest quantity of the various salts, which is 2×10^{-3} mole of the salt in 100 gm. of soil. These results are reported in figures 3,

4, and 5. Not one of the compounds tested was toxic at the lowest concentration tested, 78×10^{-7} mole. All of the others became toxic at some

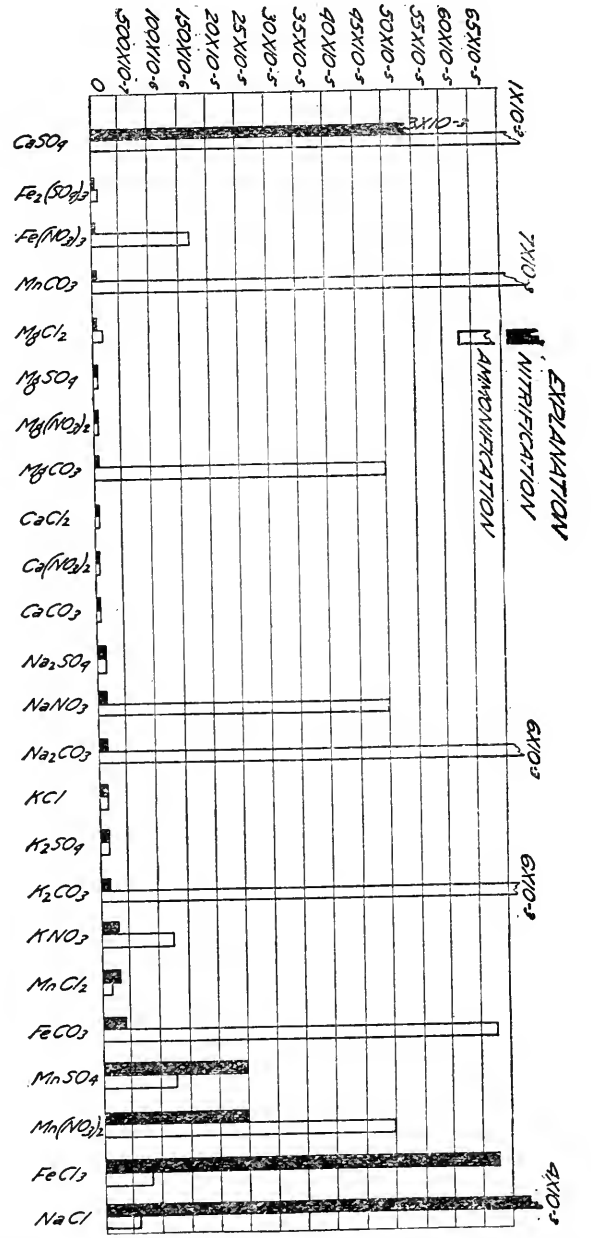

FIG. 3.—Graphs showing the molecular concentrations at which the various salts are toxic to nitrification

of the concentrations tested. In 11 out of the 20 cases tested the point of toxicity for the ammonifiers and nitrifiers were the same, whereas

in the remaining cases the quantity required to become toxic to the ammonifiers was much greater than it was for the nitrifiers. In only

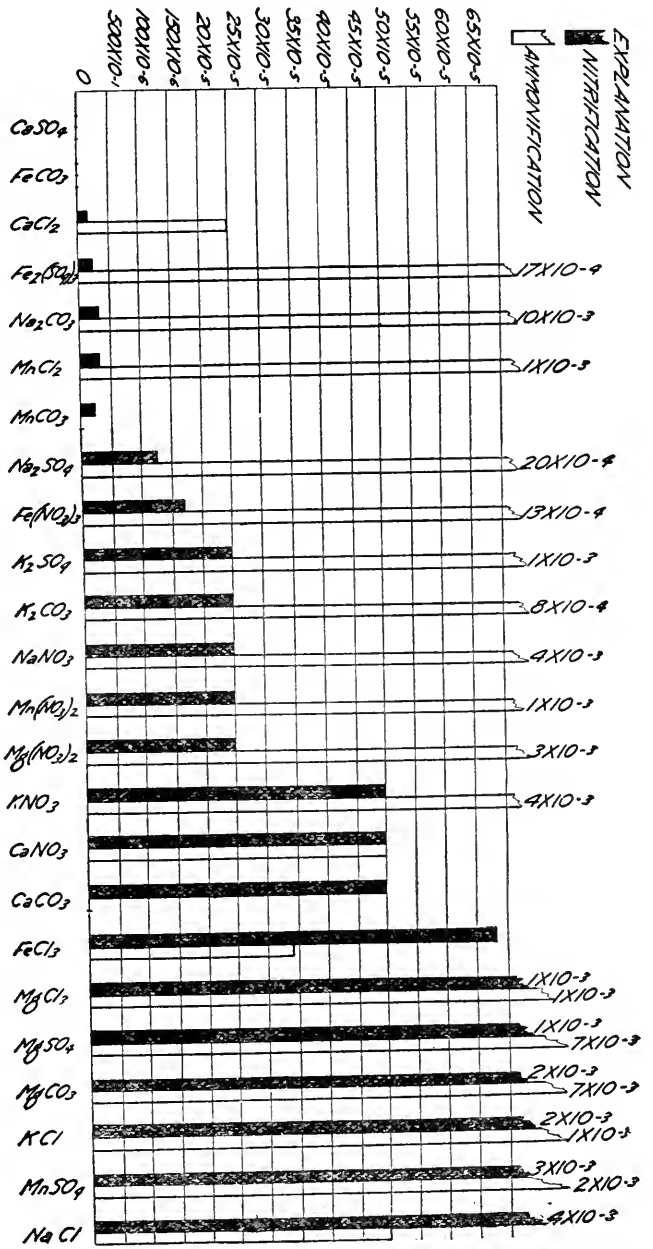

FIG. 4.—Graphs showing the molecular concentrations which reduce the nitrification to three-fourths normal

three instances were the salts more toxic to ammonifiers than to nitrifiers.

It is evident from these results that while the increased osmotic pressure exerted by the salts added to a soil plays an important part in the retarding of the bacterial activity, it is not the only factor nor probably the main one. The principal factor is probably a physiological one caused by the action of the substance upon the living protoplasm of the cell, changing its chemical and physical properties so that it can not function normally. However, we do not find a relationship between the toxicity of the compound and its power to precipitate colloids. It appears, therefore, that while the precipitation of the colloidal cellular

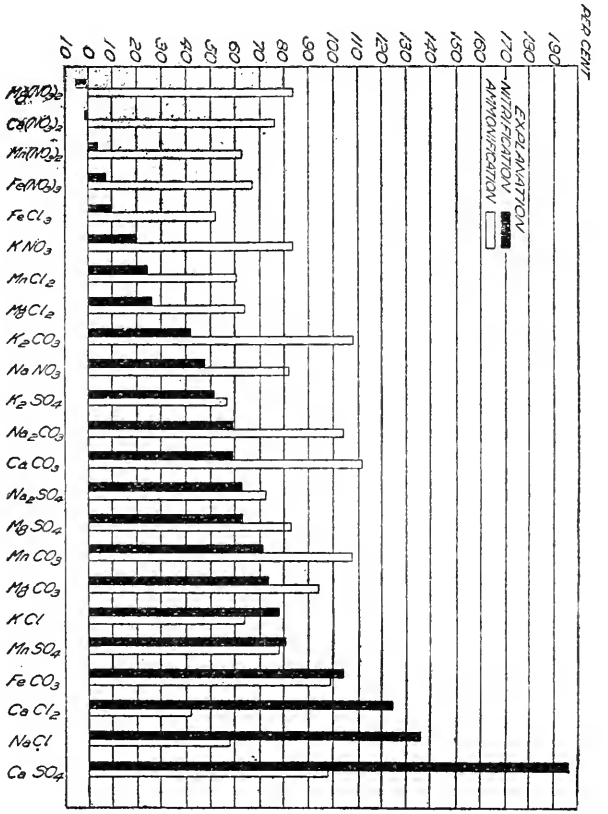

FIG. 5.—Graphs showing the percentages of nitric nitrogen produced in 100 gm. of soil to which had been added 2×10^{-3} mole of the various salts, the untreated soil being counted as producing 100 per cent.

material often causes death of the organisms, it is not necessarily the determining factor in the toxic action of these salts.

As can be seen from figure 4, it is not necessarily those compounds which become toxic at the lowest concentration which have the greatest far-reaching effect upon the bacterial activities of the soil. This condition holds for both the ammonifying and nitrifying organisms. It requires in almost every case more of the specific salt to reduce ammonifi-

cation to three-fourths normal than is required to produce the same effect upon the nitrifiers. It is evident from these results that the common soil alkalis, calcium chlorid, sodium carbonate, sodium sulphate, and sodium nitrate, are very toxic to nitrifying organisms, and if present to any great extent, will greatly reduce the nitric-nitrogen content of the soil.

The toxicity of the compound to ammonification was found to be controlled largely by the cation, but no such a relationship is found to exist in the case of the nitrifiers, as can be seen from figure 5.

The toxicity of the compounds to ammonification was found to be controlled largely by the cation, but no such relationship is found to exist in the case of the nitrifiers (fig. 5).

SUMMARY

The toxicity of the chlorids, nitrates, sulphates, and carbonates of sodium, potassium, calcium, magnesium, manganese, and iron as determined by nitrification is determined by the specific salt and not by the electro-negative ion, as was the case with the ammonifiers. With the exceptions of the manganous chlorid and sulphate and the chlorids of iron and sodium, the salts tested all became toxic at a lower concentration to the nitrifiers than to the ammonifiers.

The quantity of a salt which can be applied to a soil without decreasing the nitric-nitrogen accumulation in the soil varies with the salt, and for the soil under investigation it is in the order of decreasing toxicity of the salts as follows: Sodium sulphate, sodium carbonate, calcium carbonate, potassium sulphate, potassium carbonate, ferric nitrate, sodium nitrate, magnesium sulphate, ferric sulphate, calcium nitrate, potassium nitrate, potassium chlorid, magnesium nitrate, manganous carbonate, manganous chlorid, manganous sulphate, ferric carbonate, magnesium chlorid, manganous nitrate, ferric chlorid, magnesium carbonate, sodium chlorid, calcium chlorid, and calcium sulphate.

It is not necessarily those compounds which become toxic in the lowest concentrations which are most toxic in higher concentrations, as the toxicity of some salts increase more rapidly than the toxicity of others.

It is quite evident from the results reported that the increased osmotic pressure exerted by the salt added to the soil plays a minor part in the retarding of the bacterial activity. The main factor is probably a physiological one due to the action of the substance upon the living protoplasm of the cell, changing its chemical and physical properties so that it can not function normally.

The common soil "alkalis," calcium chlorid, sodium sulphate, sodium carbonate, and the less common one, calcium nitrate, are very toxic to the nitrifying organisms, and if present in soil to any great extent will greatly reduce the nitric-nitrogen accumulation in such a soil.

Sodium sulphate, sodium carbonate, calcium carbonate, potassium sulphate, potassium carbonate, and iron nitrate failed to increase the nitric-nitrogen accumulation in a soil. All of the others, however, in some of the concentrations tested acted as stimulants. The extent of the stimulation and quantity of salt necessary for maximum stimulation varied with the specific compound. Naming them in the order of increasing efficiency, they are: Sodium nitrate, magnesium sulphate, ferric sulphate, calcium nitrate, potassium nitrate, potassium chlorid, magnesium nitrate, manganous carbonate, manganous chlorid, manganous sulphate, ferric carbonate, magnesium chlorid, manganous nitrate, ferric chlorid, magnesium carbonate, sodium chlorid, calcium chlorid, and calcium sulphate. The last two increased the nitric-nitrogen accumulation of the soil 67 and 97 per cent, respectively.

Those compounds which are the strongest plant stimulants are also the most active in increasing the nitric-nitrogen accumulation of the soil and it is very likely that the effect upon the plant is due mainly to the action of the compound upon the bacteria which in turn render available more plant food.

Many of the nitrates caused large losses of nitric nitrogen from the soil; this is due to the stimulation of other species which transform the nitric nitrogen into protein nitrogen and not to denitrification.

Magnesium nitrate, ferric nitrate, calcium nitrate, and manganous nitrate are very active stimulants of the nitrogen-fixing organisms. In some cases these compounds increased nitrogen fixation many times over that in the normal soil.

The ammonifying powers of a soil containing alkalis are a better index of its crop-producing powers than are the nitrifying powers.

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Vol. XVI FEBRUARY 3, 1919 No. 5

JOURNAL OF AGRICULTURAL RESEARCH

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(Contribution from Bureau of Plant Industry)

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., FEBRUARY 3, 1919 NO. 5

PHYSODERMA DISEASE OF CORN

By W. H. TISDALE

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INTRODUCTION

In recent years the Physoderma disease of corn (*Zea mays*) has been reported as doing considerable damage in the southern part of the United States. The uncertainty as to the distribution of the disease and its economic importance, together with the lack of a knowledge of the life cycle and parasitism of the causal organism and the possibility of its becoming a serious pest in the Corn Belt, led the Office of Cereal Investigations to undertake an exhaustive investigational study of the problem. This work was undertaken by the writer in December, 1916. Since that time certain phases of the problem have been more or less completely developed, while others are in need of further study.

HISTORY OF THE DISEASE

Shaw (*8*)[1] in 1912 reported the occurrence of the disease in India as early as 1910, and gave a short description of the causal organism. At the annual meeting of the American Phytopathological Society at Cleveland, Ohio, 1912, Barrett[2] reported the occurrence of the disease in Illinois in 1911. In a personal interview Barrett stated that he received specimens of diseased corn from Ohio and North Carolina. Barre (*1, p. 23*) states that the disease was known to be present in South Carolina as early as 1911, and since that time has been doing considerable damage. Reports of the occurrence of the disease in Georgia in 1910 have come to the writer indirectly, but he has never been able to confirm them. There is no reason, however, to doubt its cocurrence in Georgia at that time, since it is now known to be so widespread throughout the country. Prof. J. M. Beal, of the Mississippi Agricultural College, noted the disease in Mississippi as early as 1914. Mr. A. P. Spencer, of the Florida Agricultural College, claimed that considerable damage was caused by it in Lake County, Florida, in 1915. In the summer of 1915 Melchers (*6*) collected

[1] Reference is made by number (italic) to "Literature cited," p. 154.

[2] BARRETT, J. T. PHYSODERMA ZEAE-MAYDIS SHAW IN ILLINOIS. Not published. Reference to title only in Phytopathology, v. 3, no. 1, p. 74. 1913.

specimens of the disease at Manhattan, Kansas, but, not being sure of its identity, kept the specimens until 1917 before making his report. A number of farmers throughout the South have told the writer that the disease has been present on their farms for many years. It was no doubt present in this country a long time before being reported by pathologists.

DISTRIBUTION AND PREVALENCE

In the third issue of the Plant Disease Survey Bulletin (*9, p. 52*), September 15, 1917, the writer published a map showing the known distribution of *Physoderma zeae-madyis* and the localities in which there was noticeable damage. The disease at that time seemed to be rather thoroughly

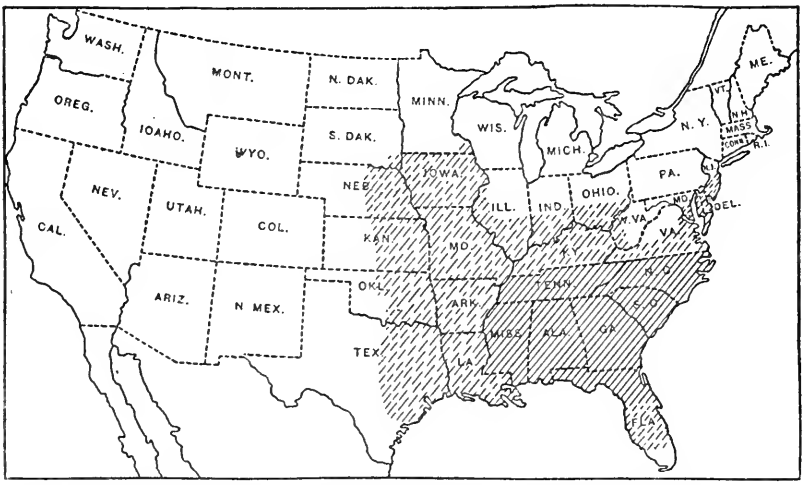

FIG. 1.—Map showing the distribution of *Physoderma zeae-maydis* in the United States. Broken lines, *P. zeae-maydis* present: solid line, *P. zeae-maydis* causing damage.

distributed throughout the Southern States as far north as North Carolina and Tennessee and west to the Mississippi River. Only single instances of its occurrence were known in Illinois and Ohio. Melchers (*6*) since that time has reported its occurrence in Kansas as early as 1915. In September and October, 1917, in cooperation with the Office of Plant Disease Survey, a detailed study was made of the prevalence and importance of the disease in representative localities of the infested area of the Southeast, not including Florida. The survey was also extended to determine the distribution of the disease throughout the United States. The disease was found to be prevalent practically throughout all localities where the detailed survey was made, very few fields being entirely free from it. The writer found diseased corn plants on the Blue Ridge Mountains of North Carolina at an elevation of about 3,000 feet. However, it was much less abundant than on the lowlands of the State. In the more extensive survey the disease was found to occur in Arkansas,

Delaware, Indiana, Kentucky, Maryland, Minnesota, Missouri, Nebraska, New Jersey, Oklahoma, South Dakota, Texas, Virginia, and West Virginia. It was also found in other sections of Kansas, southern Illinois, and Ohio. The disease, however, has not yet been found beyond the eastern part of Texas and Oklahoma, northward to southeastern South Dakota and Minnesota. Likewise the northern border of the infested zone extends from southern South Dakota and Minnesota through southern Illinois, Indiana, Ohio, West Virginia, and Virginia, and along the coast regions of Maryland, Delaware, and New Jersey (fig. 1). The disease is apparently much less prevalent in the area west of Mississippi, and north of North Carolina and Tennessee. The nature of the survey, being less intensive, may be responsible to a certain extent for this conclusion. It is possible that the disease has spread almost, if not quite, to its northern and western limits as permitted by certain weather factors.

ECONOMIC IMPORTANCE

The nature of the fungus causing the disease is such that its ability to produce serious injury to corn is limited by weather conditions, and in any ordinary season only local damage need be expected. However, in certain humid sections of the South, where the time for corn planting may extend over a period of three or four months in each year, weather conditions will more than likely be such as to favor a serious development of the disease on some of the plantings. This holds true especially for the South Atlantic and Gulf coasts and lower Mississippi Valley.

Barre (*2, p. 25*) says:

> The corn disease caused by *Physoderma* sp. as mentioned in the report last year has caused serious loss again this year. This disease was collected during the past season at a number of widely separated points in the state and seems to be more wide spread than ever before. This disease certainly deserves some attention and it is hoped that an investigation of its life history and habits can be undertaken in the near future.

The disease was known to be causing loss to the corn crop in Florida and Mississippi in 1915.

During the survey of 1917 the most pronounced losses were found along the Atlantic and Gulf coasts and in the Mississippi Valley. In the lowlands of North and South Carolina, and in the Gulf and Delta sections of Mississippi, frequent reports of as much as 5 per cent loss were given by the survey men. In some cases the damage was estimated at 6 to 10 per cent of the crop. The writer visited a few fields in the eastern part of South Carolina where the damage was perhaps as much as 10 per cent. Fields of this kind, however, were seldom found. These estimates were based entirely on grain loss, whereas the foliage, which is not considered of great importance except where the plants are used for silage, etc., was often badly injured. Smaller areas sustaining con-

siderable damage were reported throughout the Southeast and as far north as the Mississippi Valley sections of southern Illinois and Missouri. These areas were usually very limited and were confined for the most part to low, wet lands. Considering the infested area as a whole, however, the percentage of damage, at least in the year 1917, was not very great.

In 1918, up to July 15, the disease had not developed to any great extent in the South. This fact was due, no doubt, to the very dry season in that section, which will be discussed later. However, mid-July is not too late for considerable injury to develop should conditions so change as to favor the disease.

FACTORS INFLUENCING THE DEVELOPMENT OF THE DISEASE

Seemingly the more important factors in the development of the disease are moisture and temperature. The fungus requires considerable moisture, with a fairly high temperature, for a high percentage of germination and infection. If these conditions are realized before the corn plants are more than half-grown, the disease probably will become severe if there is abundant spore material present. The following information regarding these factors has been noted:

(1) Serious injury has been confined largely to the South, where the summer temperature is continuously high, and to localities in which there has been considerable rainfall during the early growth of the corn crop. Plants may become infected in the later stages of growth, but the damage is not likely to be great in cases of this kind. The warm summer showers which may occur daily for a week or more furnish ideal conditions for the development of severe attacks by *P. zeae-maydis*. For instance, in the coast district of the Carolinas, where the disease was most severe in 1917, there was considerable rainfall in early summer. This would also hold true for the Delta in Mississippi and for southwestern Tennessee, where there was considerable rainfall in early June.

(2) Where the seasons were dry the disease was more pronounced on corn growing near water, or on low, wet land where the atmosphere was moist. Plants growing under these conditions are more likely to retain the sheath and bud water until the spores can germinate and produce infection. Conditions of this kind were noticeable at the South Carolina Station in 1918, where corn on the low bottom lands had considerably more infection than highland corn. In the lowlands the foliage of plants is less subject to drying by winds.

(3) Where the early corn season was dry and the late season wet the disease was more severe on late corn, and the reverse. Striking examples of the former were noted at the Mississippi Station in 1917, where there was less than 10 per cent infection on early corn and as high as 40 per cent infection on late corn, and at the Kansas Station, where early corn was almost free from the disease, while late corn showed considerable

infection. The latter, however, might have been influenced by temperatures. Early corn at the Pee Dee Station at Florence, S. C., was dwarfed by dry weather in 1918 and was not attacked to any great extent by the fungus. At Clemson College, S. C., very early and very late corn sustained considerable injury in 1917, while corn of intermediate ages suffered much less damage. The midsummer was very dry at this station, while the early and late seasons were rather wet.

(4) Apparently the more vigorous plants in certain cases sustain the severest attacks. These plants, however, will not continue to look vigorous after the disease has had time to develop. The fact that these vigorous plants are capable of shielding the free water which is held behind the sheath and around the growing point, or bud, from the drying effects of the wind and sun offers more favorable conditions for spore germination and no doubt accounts for the greater percentage of infection on plants of this type. In low, wet fields small plants are injured the same as large ones. This greater injury to large, vigorous plants was more noticeable in dry territory.

(5) Where seasons were wet—for instance, in the sections where greater damage was done in 1917—there was little noticeable difference in the amount of infection on corn growing on high and on low lands. At the South Carolina Station the most severe injury was caused to very early corn grown on comparatively high land. As previously mentioned, the early season was fairly wet at this point.

(6) The disease was found on the Blue Ridge Mountains of North Carolina at an elevation of about 3,000 feet, where it is claimed that the summer nights are always cool. In 1917, corn foliage was killed by frost on September 11 at this point. Very little of the disease was found at this elevation, however, even on wet lands. The disease is probably held in check to a certain extent by low temperatures which prevail at that elevation. A similar explanation was offered by the writer, in a summary of the survey work which was given by Lyman (*4*, *5*), for the absence of serious injury by the disease in the Northern States. It was also thought probable at that time that the disease had reached its northern limits. This supposition was drawn from the results of temperature studies of the germination of sporangia in the laboratory. Since that time, however, further investigation has shown that the sporangia of the fungus will germinate at a considerably lower temperature than was then supposed. However, the minimum temperature at which they are known to germinate is rather high (23° C.) as will be explained later, and it is probable that this temperature does not occur commonly during and immediately after the cold rains of early summer in the north. So far as is known at present, it would require a temperature not lower than 23° C. continuously for three days, with sufficient surface water for germination, in order for severe attacks to develop provided the sporangia are present on the plants. There is a question

as to whether these conditions are realized to any great extent in the Northern States, and it is hoped that the disease will not become a serious one in the Corn Belt.

(7) The rare occurrence or absence of the disease farther west is no doubt due to the semiarid conditions which exist there. The moisture requirements suitable for the development of an epidemic of the disease perhaps are seldom, if ever, realized in this section. However, further investigations are needed to determine in detail what the weather conditions are for the given sections and to study the possibilities for further development and spread of the disease in the Corn Belt.

HOSTS

So far as is known, all varieties of corn, including pop corn and sweet corn, are susceptible to the disease. Of the numerous varieties observed in the South there seems to be little, if any, difference in the degree of susceptibility shown by them. *P. zeae-maydis* also occurs on teosinte (*Euchlaena mexicana*), a near relative of the corn plant. It is possible that the disease was introduced from Mexico or Central America with this plant. The fact that it has been found in considerable quantities on corn in comparatively isolated fields where corn was never grown before and where no corn products were applied to the land suggests the possibility that there are other hosts for the fungus among wild plants.

SIGNS OF THE DISEASE

The disease occurs on the blade (Pl. A), sheath, and culm (Pl. B), and in rare cases it has been seen on the outer husks of the ears. Infection is usually more abundant on the lower half of the plant. Its first appearance on the thin parts of the blades resembles the early stages of the corn rust caused by *Puccinia sorghi*. It is first evidenced by slightly bleached or yellowish spots, which become darker within a few days when sporangia are formed. This darkening continues until the spots are brown to reddish brown, with a somewhat lighter margin. These spots are very small, seldom becoming more than 1 mm. in diameter, except where two or more of them coalesce. The spots may in some instances be so numerous as to give the entire blade a rusty appearance. For this reason the disease is often considered a true rust by persons who are not familiar with its nature. This rusty appearance is not uncommonly seen in bands across the blades, owing to the nature of infection, which takes place through zoospores in the bud water. On the midrib of the blade and on the sheath the spots become considerably larger. Often a single spot will measure 0.5 cm. across. They are irregular in shape and sometimes may be almost square in outline. This is due to the fact that they are definitely limited by the cell walls. In the very early stages these spots are evidenced by a color which is a somewhat darker green than the normal tissue surrounding them. This seems to indicate a

stimulating effect caused by the presence of the invading fungus. A few days later these spots are dark brown in the center, owing to the formation of the dark brown sporangia of the fungus. This change in color spreads until the entire spot is a dark or chocolate brown. These infections are often so abundant as to coalesce, and sometimes the entire sheath may become brown (Pl. 10). Where the infections are as numerous as this, the entire leaf often is killed before the plant is mature. However, the disease is usually more abundant on the parts of the sheath which are beneath the overlapping parts where the moisture is held. The disease often is accompanied by a reddening of the sheath and midrib, and especially the latter, which may almost entirely mask the brown spots. After the plants begin to mature, the epidermis becomes loose over these areas and they appear as brown blisters. This dry epidermis breaks easily and the spores are liberated as a brown spore dust. The entire parenchyma tissues of the invaded parts are destroyed by the disease, leaving the vascular system as so many free threads after the spores have been liberated (Pl. 11, C, E). On the culms the spots are very much like those on the sheath and midrib. They are usually more abundant at the nodes and just below the nodes, where spores are more likely to lodge and where free water is held by the sheath. The culms often are completely girdled at the nodes and are very easily broken by the winds after the tissues have been invaded. The disease is responsible for considerable lodging of corn in the South in the early stages of maturity. Only the lower nodes as a usual thing become so thoroughly invaded by the fungus as to be easily broken (Pl. 11, A, B). Considerable damage may result from severe attacks of this kind. After the plants have fallen, the pith at the infected nodes will be found to be filled with a brown mass of spore material (Pl. 11, D).

The signs of the disease on teosinte (*Euchlaena mexicana*) are very similar to those on corn, and therefore a separate description will not be necessary.

The pronounced signs of the disease have led farmers to apply various significant terms to it in the way of common names. The writer has heard the following names applied to it: "Rust," "corn measles," "corn pox," "dropsy," "frenching," and "spot disease." None of these terms, however, is in general use, and some of them—for instance, "rust" and "frenching"—would be incorrect, as corn is known to be affected by other distinct diseases called by these names. The name "falserust" has been suggested as a desirable common name for the disease. There would be a strong tendency, however, on the part of the layman to drop the word "false," thus causing a confusion with the true rust. Furthermore, the disease on the sheath and the culm bears very little resemblance to a rust. Since no satisfactory term suggests itself at present and since the scientific term "Physoderma," seems to be gaining favor as a common name, the author suggests that this term be retained.

CAUSAL ORGANISM

There still remains some doubt as to the correctness of the classification of the causal organism. According to the description of Cladochytrium and Physoderma as given by the leading mycologists, the organism evidently belongs in one of these genera. The essential difference between the two genera lies in their method of reproduction. The genus Cladochytrium may have both thick-walled sporangia, or so-called resting spores, and thin-walled sporangia, or presporangia, while the genus Physoderma is characterized by having only thick-walled sporangia (resting spores). As the species on corn is not known to produce the thin-walled sporangia, its thick-walled sporangia definitely place it in the genus Physoderma. Shaw's (*8*) description of the species from India was based almost entirely on the resting spores (sporangia). Barrett [1] found the disease in the State of Illinois in 1911 and in 1912 declared the organism identical with the species described by Shaw. Measurements of the sporangia of the fungus in America are practically identical with those given by Shaw (*8*). Measurements given by Shaw are 18 to 24 by 20 to 27 μ, while the writer finds the sporangia of the organism in this country to measure 18 to 24 by 20 to 30 μ. Therefore, so far as size of sporangia are concerned, the fungus in America is apparently identical with that described by Shaw from India as *Physoderma zeae-maydis*.

DESCRIPTION OF THE ORGANISM

The sporangia of the fungus are smooth, brown, thick-walled, 18 to 24 by 20 to 30 μ, slightly flattened on one side where the outline of a definite cap or lid can be seen by careful observation. On germination this trapdoor lid opens, or is carried up by the top of the thin-walled endosporangium, which finally ruptures at the apex and liberates a number of uniciliate zoospores. These zoospores are 3 to 4 by 5 to 7 μ, with a cilium three to four times the length of the spore itself. The zoospores have a comparatively large central oil droplet or nucleus. After their active stage these motile spores come to rest, in most cases lose their cilia, spread slightly in an ameboid fashion, and germinate by putting out fine fibrous hyphae. The mycelium is composed of very fine fibers, about 1 μ thick, which connect the large vegetative cells which Clinton (*3*) and Von Minden (*7, p. 397–410*) term "*Sammelzellen.*" These enlarged cells, which may occur singly or in groups of two or more, produce sporangia directly or send out short fibers which produce terminal sporangia. The fungus is apparently an obligate parasite, and the mycelial stage is seen only within the tissues of the host plant. After the sporangia are mature no traces of the mycelium can be seen (Pl. 17, *B*).

[1] BARRETT, J. T. OP. CIT.

GERMINATION OF THE SPORANGIA

The more essential factors influencing sporangium germination and zoospore formation are moisture, temperature, and fresh air. After the sporangia have become thoroughly dried, it is very difficult to obtain germination. Sporangia which have just matured germinate readily when taken directly from the green corn plant. Germination was best obtained by placing the sporangia in a small amount of water in a watch glass or other shallow vessel, which was in turn placed in a large moist chamber. The sporangia seemed to germinate equally well in either distilled or tap water. The moist chamber was kept in an incubator or placed in the open room where the temperature was high enough for the germination of the sporangia. An incubator, in order to give good results, should be large and well regulated so as to keep the air as fresh as possible. The moist chamber should be kept thoroughly damp and should be rather large. The temperature should be kept constantly between 23° and 30° C., and preferably at 28° to 29°, as this seems to be the optimum range of temperature for germination. Sporangia placed as nearly as possible under these conditions often fail to germinate for some unknown reason. At other times sporangia from the same source germinate readily.

With the proper conditions of temperature, moisture, etc., the endosporangium absorbs water and begins a process of swelling, which causes the lid or cap of the resting spores to open in doorlike fashion or to be carried at the apex of the protruding endosporangium (Pl. 13, *b–d*). The lids begin opening in from 30 to 48 hours after the sporangia have been placed in the incubator. The granular content of this endosporangium begins rounding up into small nuclei, or oil droplets, which finally become the central bodies of the zoospores. Within a few hours after the sporangia start opening, zoospores are formed with the small oil droplets as centers. A small projection, or papilla, develops at the apex of the thin-walled endosporangium, and after the zoospore formation is complete a movement of the contents of the endosporangium can be seen to take place toward the projection, which breaks open, whereupon the zoospores are liberated in rapid succession (Pl. 13, *e*). These zoospores, which are usually 20 to 50 in number, swim away at first with a very jerky motion, which gradually becomes more uniform until their motile stage has ended. This occupies from one to two hours, depending somewhat on the temperature to which they are subjected. With cooler temperatures their active period is shorter. After their motile stage is over they settle down and in most cases lose their cilia and spread slightly in an ameboid fashion before germinating. The various stages of germination and zoospore formation and activity were studied with the aid of the high power lens of the microscope which was immersed in the zoospore suspension contained in a watch glass. The water in this case serves very nicely as an

immersion fluid. After the majority of the zoospores have escaped from the zoosporangium, it is very often the case that the collapsing wall of the endosporangium catches one or more of them within, where their motile stage can be easily studied (Pl. 13, *f*). A single zoospore has plenty of room to swim around within the empty sporangium.

ZOOSPORE GERMINATION AND HOST PENETRATION

Within one to two days after the zoospores have come to rest, they begin to germinate by sending out very fine, fibrous hyphæ (Pl. 13, *h*), which cease to grow after they have reached a few microns in length if they fail to come in contact with the host plant. Few of them ever reach this stage if they are kept in ordinary tap water, for they serve as a prey to bacteria and numerous protozoa. They break down completely and apparently disappear under adverse conditions. The small hyphæ which were produced by the germinating spores were made visible by applying to the spore solution a few drops of .5 per cent potassium iodid with enough iodin dissolved in it to give the solution a dark-brown color. This gradually killed the zoospores and stained them slightly at the same time. This method was used for staining both the cilia and hyphæ of the zoospores, which are so small that it requires a high power lens to make them visible. In this case the immersion lens was used in the manner previously described.

If the zoospores are in contact with the host epidermis, the fibers continue to grow after germination and penetrate the epidermal cell walls, thereby producing infection (Pl. 14). Very delicate technic was required in order to determine this point. The bud of a young corn plant was unfolded until the very thin white tissue, which was free from cholorphyl, was obtained. Sections somewhat smaller than a cover slip were cut from this thin leaf tissue and placed on slides. Drops of a zoospore suspension containing numerous spores were placed on these thin sections. The slides were then placed in the moist chamber and incubated at the same temperature required for resting spore germination. After two days a drop of the iodin solution was placed in the drop of spore suspension, a cover slip placed over the section, and an examination made. It was necessary to use the immersion lens to see what was taking place. Oil could not be used on a loose cover placed in this manner because it was so viscous as to hold the cover slip while the slide was being moved, and thereby disturbed the sections. A drop of water was used instead of oil and was found to be fairly satisfactory. At the end of two days the fine, fibrous hyphæ had, in cases, penetrated the epidermis, and some had produced the large swollen cells within the epidermal cells of the host (Pl. 14, *c*, *e*, *f*). The germinating zoospores were more commonly found attached to the host near the dividing wall of two epidermal cells. In some instances more than one hypha was seen passing through the host cell wall from a single zoospore (Pl. 14, *a*, *b*, *d*).

DEVELOPMENT OF THE FUNGUS WITHIN THE HOST TISSUE

So far as is known, the fungus is an obligate parasite. After zoospore germination and host penetration the fine mycelial fibers invade a number of the surrounding parenchyma cells, forming numerous enlarged cells, or *Sammelzellen* (Pl. 15). These enlarged cells are always intracellular, and are often in groups of two or more. A number of the small fibers are usually found extending in various directions from these cells. These fibers penetrate the host cell walls at any point (Pl. 16, *a–e*), passing directly into the adjoining cells which are likewise invaded through the same process of enlarged cell and fiber formation. Commonly where the host cell walls are penetrated the cell wall and the hypha of the fungus seem to be unmodified. In some cases, however, there may be a slight enlargement of the mycelium or a slight thickening or modification of the host cell wall, or perhaps both (Pl. 16, *a–e*). The hyphæ of the fungus are so small that no opening can be seen where they pass through the cell walls. The enlarged cells of the fungus may apparently develop directly into sporangia or send out short fibers which produce a single terminal sporangium. In cases where the enlarged cell develops directly into sporangia there is a rounding up of the content of the enlarged cells around a denser part of the protoplasm, which is to all appearances a nucleus. Where fibers grow out to produce terminal sporangia, they may arise directly from the enlarged cells without any noticeable disturbance to the nuclear structure. In some instances a double nucleate condition is seen, and a thread or fiber develops from this structure to produce a terminal sporangium (Pl. 16, *g*). A very common form of the enlarged body is an elongated structure containing from two to four cells (Pl. 16, *f*, *g*), but more commonly two cells. These structures, which have very thin walls and less dense protoplasm than some of the more compact structures, are often found to produce sporangia on the thin fibers. Where there are only two cells present one may give up its contents and collapse while the other may develop directly into a sporangium or produce a sporangium at the end of a hypha. In some cases both cells reproduce in one way or the other. The same is true for the three-celled and four-celled bodies, one or two cells of which apparently may collapse while the others produce sporangia. The double nucleate condition was more noticeable in structures of this kind.

After the formation of sporangia is complete, the invaded host cells are usually filled with them (Pl. 17, B). No traces of the mycelium or vegetative cells can be seen at this stage. These parts are entirely absorbed or broken down in the process of reproduction. The host cells appear to be slightly enlarged, owing to invasion by the fungus. They die as soon as the sporangia are formed, as their protoplasm is almost completely destroyed by this time. In the early stages of invasion there is a stimulation of the invaded cells which is brought about by the presence of the parasite. This no doubt accounts for the noticeably slight enlargement of cells.

FIXING AND STAINING METHODS

Material was obtained from the different parts of infected plants at various stages of the development of the disease and killed, preferably in Flemming's medium fluid. The material was put through the regular process of washing until it had been passed through 70 per cent alcohol. It was then placed in a 10 per cent solution of hydrofluoric acid and allowed to remain for three or four days to remove any silicates which might be present. Sections not treated in this manner injured the knife and could not be sectioned satisfactorily. The material was then returned to 70 per cent alcohol and the regular process followed until it was embedded in paraffin.

Three different stains were used for differential staining of host and fungus tissue—namely, Delafield's hematoxylin, and Flemming's triple and Pianeze stains. Both the triple and Pianeze stains proved to be desirable for this purpose, while the hematoxylin was not satisfactory. The orange G in the triple stain was taken up very readily by the fungus and was very desirable for staining the small hyphæ. The Pianeze stain gave the fungus tissue a pinkish color and served to bring out more of the details of structure, especially in the reproductive bodies. Sections from both corn and teosinte were stained, and the more successful sections were obtained from the thicker parts of the sheath tissue of teosinte.

ARTIFICIAL INOCULATIONS

Artificial inoculations were first tried in the greenhouse at Madison, Wis., in the winter of 1916–17. A special section of the house was obtained for this work, one in which a fine spray of water was kept going to keep the room damp. The temperature was kept at as near 30° C. as possible, which was perhaps a little too high for the germination of the sporangia. The plants were inoculated by spraying a suspension of sporangia behind the sheaths and in the bud. None of these plants were infected, for some reason. Inoculations were made in a similar manner on plants in a small isolated plot at West Raleigh, N. C., on July 23, 1917. At the same time these inoculations were made there was a daily occurrence of summer showers which continued three days after the date of inoculation. These plants were kept under observation by Dr. F. A. Wolf, Pathologist of the Station, who observed the first signs of the disease 10 days after the inoculation had been made. Two weeks after the inoculations had been made, he reported the presence of the dark-brown spots caused by the presence of abundant sporangia. The writer received specimens from these plants for his collections. In May of this year (1918) inoculations were made on corn growing in the greenhouse at the Arlington Experimental Farm at Arlington, Va. The weather was very warm, and it was necessary to spray these plants twice daily in order to keep the bud and sheath water from evaporating. On the writer's return

from a field trip four weeks later, June 14, he found that the disease had developed to a more or less extent on most of the plants which were inoculated (Pl. 12).

In cases where infections were obtained, the upper leaves, which were in the bud at the time of inoculation, showed the disease near their tips, while leaves which were mature were diseased at the base. This explains in part the fact that in the field the disease may be present only on the tips of blades or confined to the basal parts. The occurrence of the disease in bands of alternating heavy and light infection across the blades, which is often very noticeable, is no doubt due to the effect on sporangia germination of alternating periods of favorable and unfavorable temperature and moisture conditions while the leaves are emerging from the bud. The part of the blade which happened to be in contact with the bud water while the zoospores were being liberated became infected.

OVERWINTERING OF THE SPORANGIA

The sporangia of *Physoderma zeae-maydis* pass the winter in the old infected plants and in the soil, and germinate the following summer to produce new infections. Through the kindness of Dr. H. E. Stevens, of the Florida Agricultural College, fresh sporangial material was obtained from Florida at four-week intervals from January to April, 1917. These sporangia were found to be viable each time. Sporangia collected at the Alabama and Mississippi stations in May and June of the same year germinated readily. Sporangia buried about 3 inches deep at Clemson College, S. C., September 7, 1917, germinated in small percentages as late as July 20, 1918. These sporangia were taken from the soil on July 10. Sporangia left aboveground at the same point germinated also. Sporangia taken from the field on January 5 at Agricultural College, Miss., and buried 3 to 5 inches deep germinated about 50 per cent on June 20. Sporangia which remained aboveground in an open box gave a lower percentage of germination. This may be due to the fact that the sporangia aboveground were much drier when the tests were made. The winter was severe at this point, the temperature at one time being as low as zero Fahrenheit. This temperature apparently does not injure the sporangia, as the author had allowed sporangia to freeze in a cake of ice at the Wisconsin Station when the temperature was $-8°$ F. After several days the ice was melted from the sporangia, and some of them germinated. Sporangia collected in Alabama, Florida, and Mississippi in June, 1918, showed a high percentage of viability. Sporangia collected in South Carolina as late as July were found to be viable. Later tests than these have not been made, and it may be possible that these sporangia live in the old infected plants and in the soil for a number of years. Material has been prepared for further tests.

DISSEMINATION

This fungus, as is the case with many others, is no doubt disseminated in numerous ways. After the infected plants are mature the sporangia are liberated in large quantities and are free to be carried by such agencies as wind, running water, insects, and various animals, including man.

Wind is certainly responsible for considerable dissemination of sporangia. This was demonstrated, as will be shown by Tables I and II. Table I shows the results of sporangium catches on common microscopic slides at Agricultural College, Miss., in January, 1918. These slides were coated on one side with ordinary vaseline (a method used by Mr. H. D. Barker, of this Office, for catching wind-blown spores) and were placed on stakes 1 inch square at heights of 1, 2, and 3 feet. A slide was placed on each of the four sides of the stake at the different heights and held in place by rubber bands passing around each end of the four slides and the stake. The stakes were placed in the field so that the slides were facing the four points of the compass. A larger number of spores were caught on the slides facing the prevailing winds at the time the experiment was conducted. The slides were brought into the laboratory and marked off crosswise in narrow strips by running a sharp pencil through the vaseline. These lines served as guides while counting the spores with a microscope.

TABLE I.—*Catches of wind-blown sporangia of Physoderma zeae-maydis at Agricultural College, Miss., in January, 1918*

Stake No.	Date.	Direction of wind.	Relation of stake to cornfield.	Direction slide exposed.	Number of sporangia on slides.		
					1 ft.	2 ft.	3 ft.
1	Jan. 10–13....	Storm from northeast, changeable afterwards.	In infested field near diseased plants.	North...	2	20	145
1	.....do.....	.....do.....	.....do.....	East....	40	9	17
1	.....do.....	.....do.....	.....do.....	South...	2	11	7
1	.....do.....	.....do.....	.....do.....	West...	12	10	4
2	.....do.....	.....do.....	At northern edge of infested field.	North...	0	2	2
2	.....do.....	.....do.....	.....do.....	East....	46	12	17
2	.....do.....	.....do.....	.....do.....	South...	57	8	71
2	.....do.....	.....do.....	.....do.....	West...	4	9	30
3[a]	Jan. 19–21....	Northeast to south.	30 yards from west side of infested field.	North...	1	0	0
3	.....do.....	.....do.....	.....do.....	East....	2	0	0
3	.....do.....	.....do.....	.....do.....	South...	2	0	1
3	.....do.....	.....do.....	.....do.....	West...	0	0	0
4	.....do.....	.....do.....	30 yards from east side of infested field.	North...	7	4	1
4	.....do.....	.....do.....	.....do.....	East....	3	3	0
4	.....do.....	.....do.....	.....do.....	South...	3	2	2
4	.....do.....	.....do.....	.....do.....	West...	34	0	0

[a] Stake 3 was blown down during the experiment.

The results given in Table I show that the sporangia of *P. zeae-maydis* may be carried by the wind in considerable numbers. With the abundance of sporangial material which is present in the fields after the corn plants have matured it is quite possible that many of the sporangia are carried by even the very slight breezes.

Further experiments were conducted at Clemson College, S. C., in April, 1918, to obtain additional information on the dissemination of sporangia of the fungus by wind. Slides prepared as mentioned above were placed on stakes without regard to direction in a field where the corn plants had been badly injured by *P. zeae-maydis* in 1917. The plants were cut in the fall of 1917 for shredding, but not until the sporangia had been liberated in large numbers. At the time the slides were placed the field was being planted to cabbage and most of the remaining parts of the corn plants had been plowed under. There was considerable wind and rain during the time the slides were kept in the field. Table II gives the results of these sporangium catches.

TABLE II.—*Results of sporangium catches of Physoderma zeae-maydis on slides placed in fields at Clemson College, S. C., in April, 1918*

Date of experiment.	Stake No.	Number of sporangia per slide at different heights.				
		½ ft.	1 ft.	1½ ft.	2 ft.	2½ ft.
Apr. 4–8	1	8	4	4	21	Lost.
Do	2	8	3	5	6	Lost.
Do	3	18	0	3	3	1
Do	4	Lost.	6	10	Lost.	1

The results given in Table II show that a considerable number of sporangia are carried by wind even after the plants have been removed and the soil has been plowed. These sporangia were doubtless blown from the soil surface as well as from the small parts of diseased plants which remained uncovered. Some of them, however, might have reached the lower slides through spattering rain drops.

Overflowing streams or surface water flowing through infested corn fields after heavy rains may carry large numbers of sporangia to be deposited along their courses. The disease has been found to be more abundant on overflow lands and near streams. Within the infested area the sporangia doubtless reach their host plants in some cases through spattering rain water.

Man is perhaps one of the most important agents by which the fungus is disseminated. In removing diseased plants for stover, fodder, silage, etc., large quantities of sporangia are carried to the barns, and sometimes they may be shipped considerable distances with this material. After these products have been used as feed for animals, the barnyard

manure is utilized as a fertilizer, and no doubt carries with it a large number of viable sporangia which serve as a source of infection to corn plants grown on the land in the future. There is considerable doubt as to the possibility of the sporangia's remaining viable after passing through the silo or the body of the animal, as no experiments have been conducted to determine this point. However, sufficient sporangial material to serve as a means of dissemination escapes the action of the silo and the digestive processes of the stomach of the animal. This would be especially true with stover and fodder, where the tough, thick parts are not eaten by the animals. Hay, unhusked corn, or any other plant products removed from infested fields and shipped to various parts of the country would serve as an ideal means of disseminating *P. zeae-maydis*. This would hold true especially in late fall, when the sporangia are being liberated in so great an abundance.

There is a remote possibility of the dissemination of the fungus to a certain extent by being carried on the seed. However, very little of the disease has been seen, even on the outer husks of the ears. When the ears are husked, the only chance for sporangia to be present on the seed is for them to be brought in by some foreign agent, as they are not produced inside the husks. If the husking is done in the field it is quite probable that some of the loose sporangia will lodge on the husked ears. If, however, a few of them are present on the surface of the kernels of corn when planted the chances are that they will not be able to infect the resulting crop because they are buried with the seed in the drill row, where no cultivation is given and there is practically no chance for them to be disturbed.

If they were to germinate in the soil, the zoospores would have no chance to reach the surface to infect the corn plants. There is a possibility, however, that the sporangia might live over in the soil until the following year, or even longer, and finally come in contact with corn plants, though the possibilities of dissemination in this manner seem to be comparatively small.

POSSIBLE CONTROL MEASURES

No definite means of control has yet been discovered for the disease. However, certain measures may be recommended for reducing the amount of sporangial material present which would have a tendency to reduce the severity of the attacks by the fungus. These measures may be outlined as follows:

(A) The quantity of sporangia present could be greatly reduced by burning the old infected plants after the corn has been harvested, but this would be a destructive practice, especially in the South, where the disease is important and where organic matter is so badly needed in the soil. If the plants could be cut into very fine pieces and plowed under deep enough so that they would not be disturbed by ordinary cultivation the

quantity of infectious material for the following year would be greatly reduced. Where the corn is used for silage or stover, the plants should be cut as near the ground as possible and as early as feasible, in order that a large part of the sporangial material may be removed. Barnyard manure containing these and other corn products should not be used to fertilize corn and should not be put on land where the disease has not been known to occur previously.

(B) Crop rotation may have a tendency to reduce the amount of injury caused by the disease. The most severe cases known have been on land where corn has been grown continuously for a number of years. In a system of rotation the corn plot should be removed as far as possible from the previous plot, as the sporangia of the fungus are wind-borne. Hence, a change of only a short distance in the location of the corn plot probably would have but little effect as a means of reducing the amount of disease present. The longer the duration of the rotation, the better the results are likely to be.

(C) Where the disease continues to be severe, there is a possibility of selecting disease-free plants and from these obtaining a strain that will be resistant to attacks by the fungus. In order to keep them pure, these plants would have to be selected from pure varieties and grown under conditions where crossing could not take place. It seems hardly possible that a variety will be found which naturally resists the disease, as no indications of such a variety have as yet been seen in the various varietal experiments in the South.

(D) Control through seed treatment, no doubt, is worthy of little consideration because there is slight chance for the sporangia to be seed-borne. However, when seed corn is transported from infested territory to territory free from *P. zeae-maydis*, seed treatment would be a desirable precaution, provided an effective method and means of treatment can be found. Sporangia immersed for 10 minutes in a 4 per cent solution of copper sulphate remained viable. This treatment was used to kill the bacteria on the surface of the sporangia.

SUMMARY

(1) The Physoderma disease of corn was discovered in India by Shaw in 1910, and in the State of Illinois by Barrett in 1911.

(2) The disease occurs throughout this country as far westward as central Texas and Nebraska and northward to southern Minnesota and New Jersey. It has perhaps almost reached its northern limits, owing to low temperatures, and its western limits, owing to semiarid conditions.

(3) Considerable damage is caused to corn in the Atlantic and Gulf Coast States and in the Mississippi Valley. The amount of damage varies with weather conditions, moist and warm weather being more favorable for its development.

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(4) The disease is caused by a species of Physoderma, one of the Phycomycetes, which is probably identical with Shaw's Indian species, *P. zeae-maydis.*

(5) The sporangia of the fungus live over the winter on the old, diseased corn plants and in the soil, and germinate the following summer by producing numerous zoospores which infect the corn plants and cause the disease.

(6) The sporangia require free water and a high temperature, 23° to 30° C., for germination and host penetration.

(7) The fungus is disseminated by wind and probably by other agencies—for example, flowing water, insects, and various animals, including man.

(8) There is a possibility of controlling the disease to a certain extent through sanitation, crop rotation, and resistant varieties, although this has not been positively proved.

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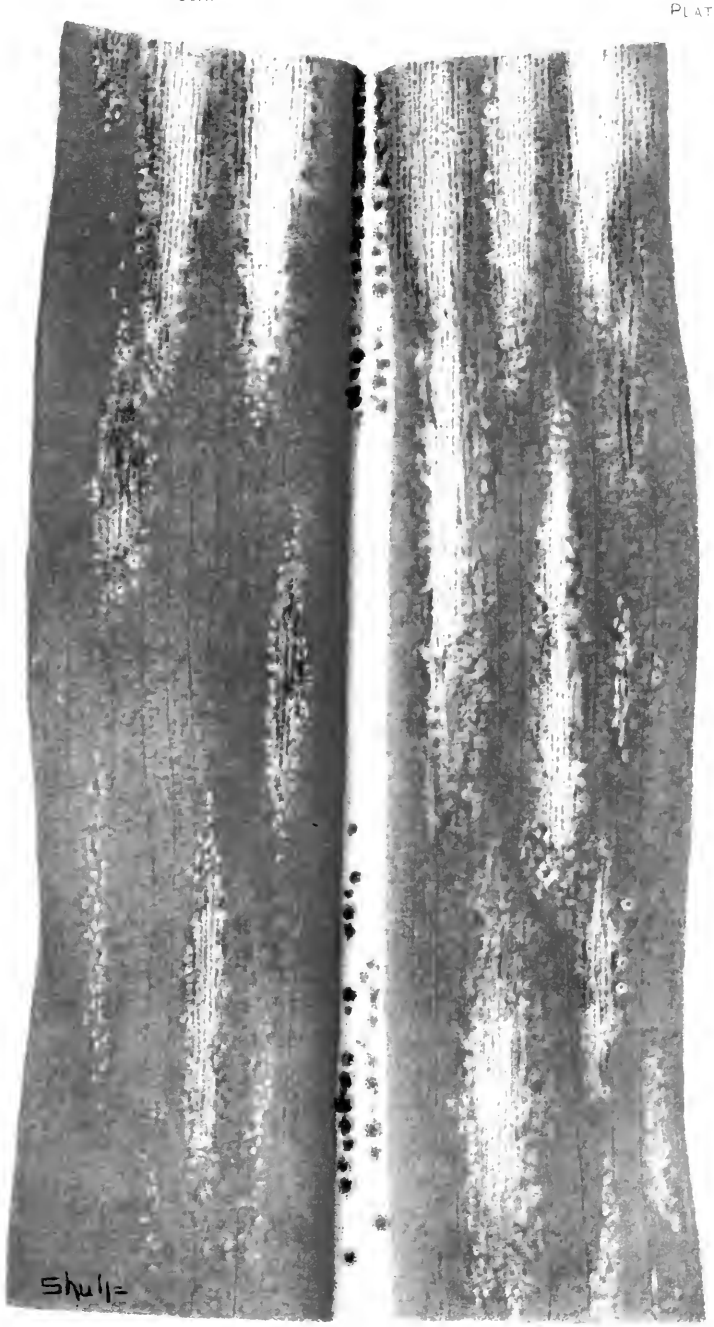
PLATE A

Corn leaf showing the effects of an attack by *Physoderma zeae-maydis*. Notice the light-brown, rustlike appearance on the thin parts of the blade and the larger, dark spots on the midrib. This difference in color is due to the fact that a greater number of the dark-brown sporangia are produced in the fleshy tissues of the midrib.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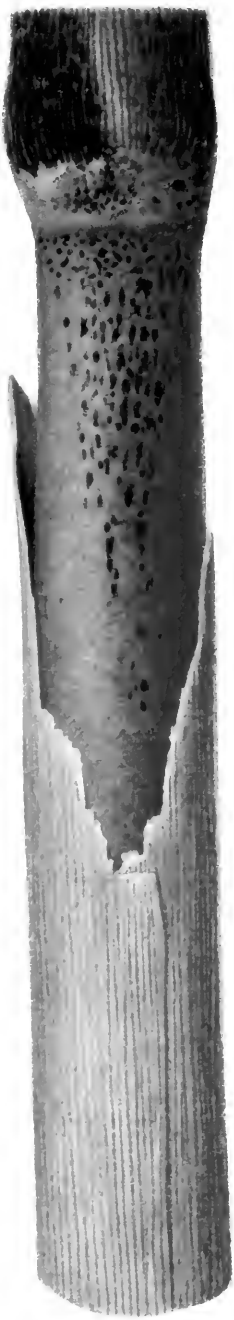


PLATE B

Sheath and culm of corn plant showing the effects of *Physoderma zeae-mazydis*. In these parts there is a large production of sporangia, which causes the dark spots, as is true in case of the midrib of the blade as seen in Plate A. In some cases the entire sheath is darkened and the nodes completely girdled by the fungus.

PLATE 10

Blade and sheath of corn plant showing the effects of a severe attack by *Physoderma zeae-maydis*.

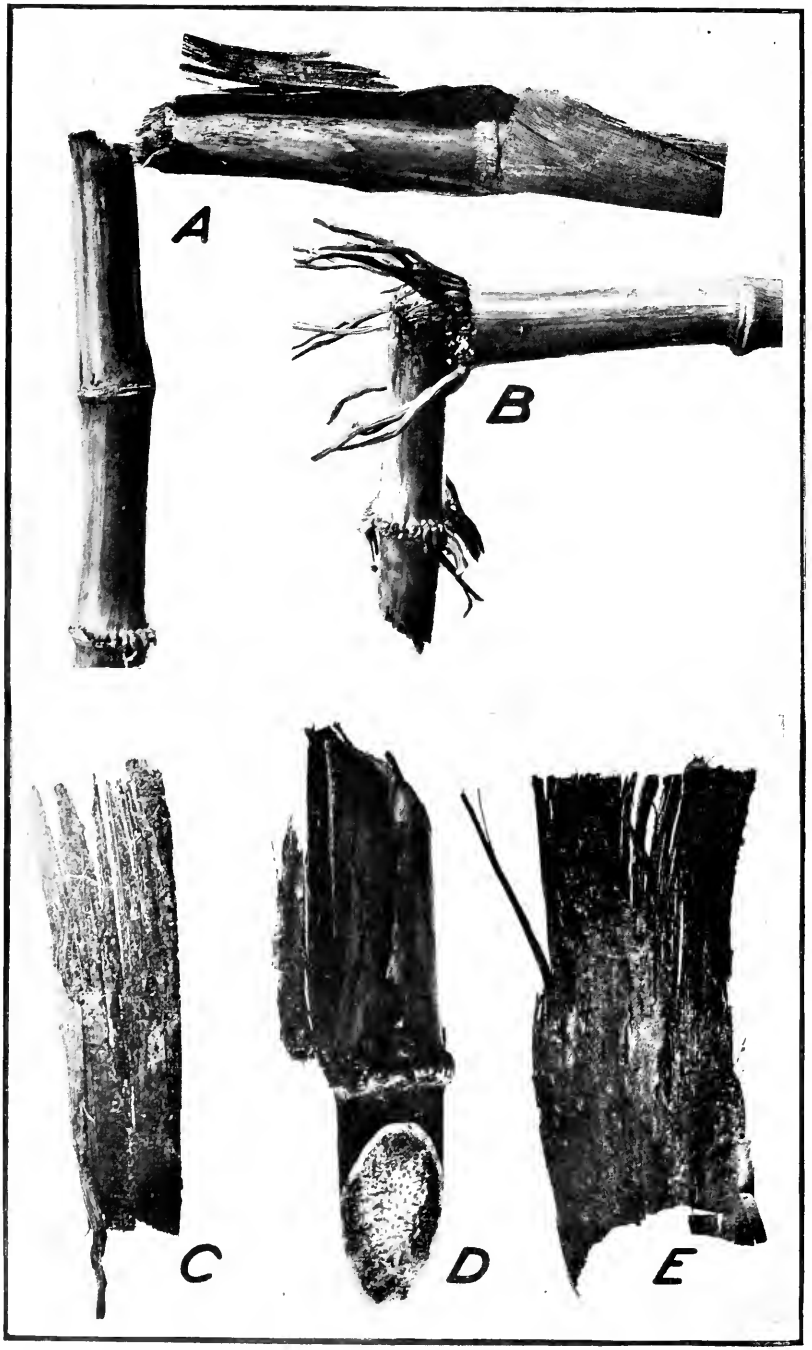

A
B
C
D
E

PLATE 11

Old sheaths and culms of corn showing effects of severe attacks by *Physoderma zeae-maydis:*

A, B.—Badly diseased stalks broken over at weakened, infected lower nodes. These are the effects of the disease in regions where it is most destructive.

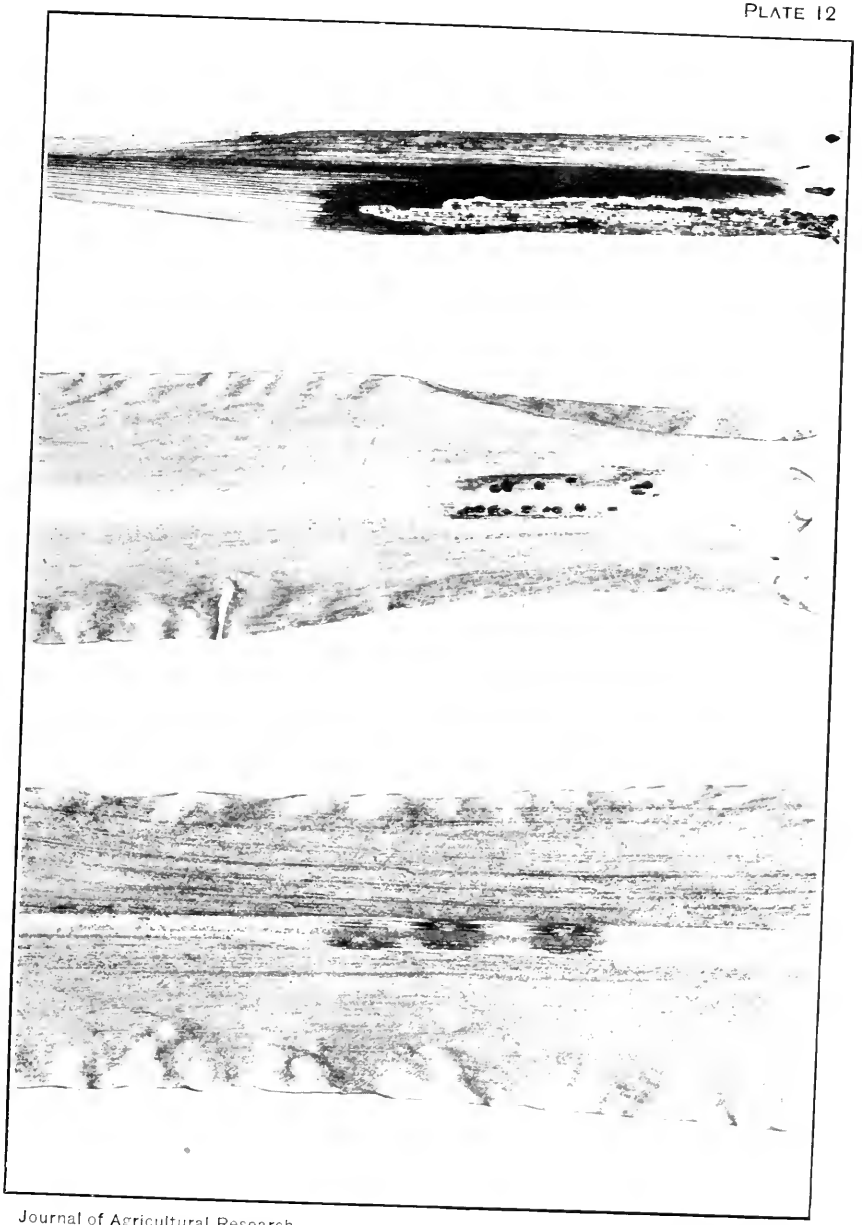
C, E.—Portions of old attacked sheaths showing characteristic shredding. These old infected sheaths contain countless numbers of resting spores which become freed and are then blown and washed about when sheaths become weathered and shredded.

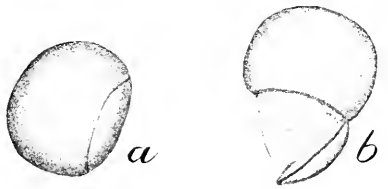
D.—Portion of an old infected stalk showing discoloration both on outside and in pith due to the attacks of the fungus. Numerous spores are found in all attacked portions.

PLATE 12

Blades and sheath of corn showing the Physoderma disease produced by inoculating plants in the greenhouse with a suspension of the sporangia of *P. zeae-maydis* in tap water. The suspension was poured behind the sheaths and in the bud of the plants and the plants were sprayed twice daily to keep them damp. Notice the dark area around the diseased tissue in the bottom figure. This is a dark-red color which is often seen on diseased plants in the field.

PLATE 12

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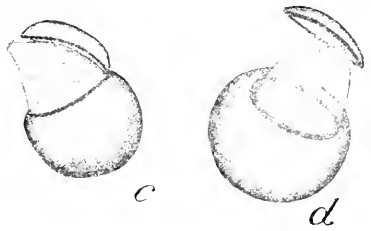
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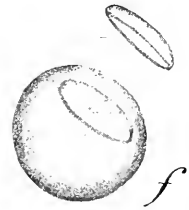

f


g
h

PLATE 13

Physoderma zeae-maydis: Various stages in the germination of sporangia, formation of zoospore, and germination of zoospore.

a.—Sporangium.

b, c, d.—Opening sporangia showing the early stages of zoospore formation.

e.—Mature zoospores escaping through the ruptured apex of the endosporangium.

f.—The collapsing endosporongium after the zoospores have escaped. A single zoospore remained within the sporangium. The active stage of zoospores remaining within the sporangium was easily studied.

g.—Zoospores.

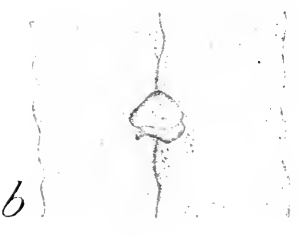
h.—Germinating zoospores.

PLATE 14

Physoderma zeae-maydis:

a–f,—Zoospores germinating by fine threadlike hyphæ which have penetrated the epidermal cell walls of a tender leaf of Indian corn. In *c*, *e*, and *f* the enlarged cells have begun to form in the epidermal cells of the host.

a



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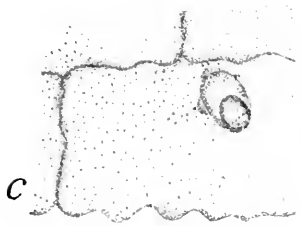

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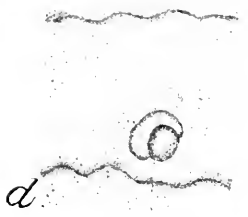

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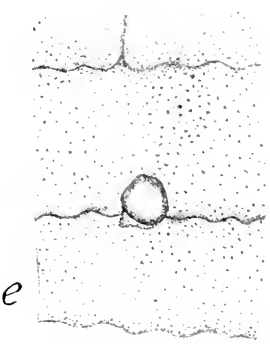


e

f


a
b
c
c
d
f
g

PLATE 15

Physoderma zeae-maydis: Mycelial stages within the host cells.

a–d.—Drawings from ordinary high-power magnifications showing the fibers and enlarged cells of the mycelium.

e–g.—Drawings magnified with oil-immersion lens.

b, d, g.—Notice the young sporangia at the ends of the short hyphæ.

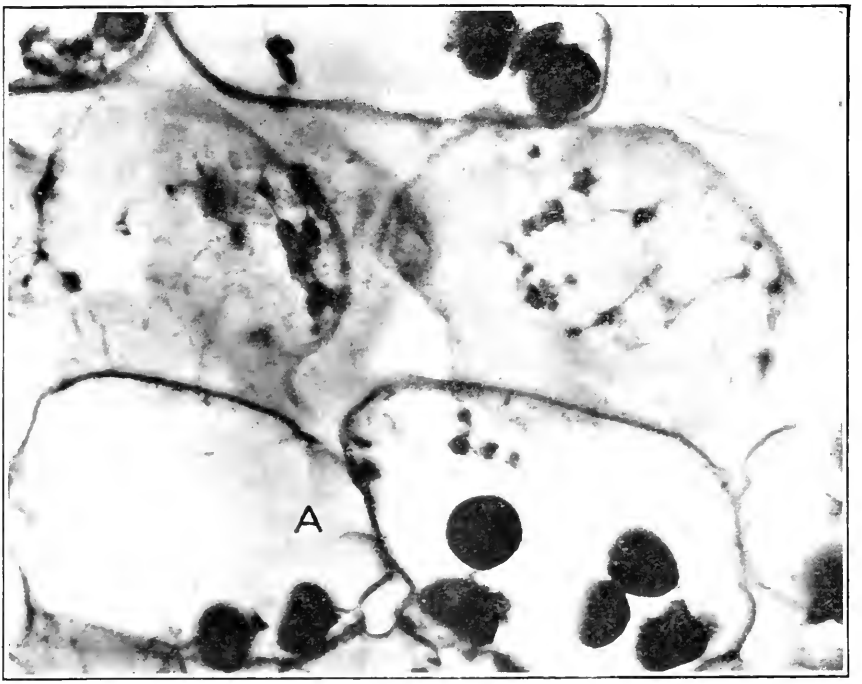
PLATE 16

Physoderma zeae-maydis:

a–e, Mycelial fibers penetrating the cell walls of the host tissue.

f, *g*, Different types of reproductive bodies. Notice the double nucleate condition in figure *g*.


a
b
c
d
e
f
g


A

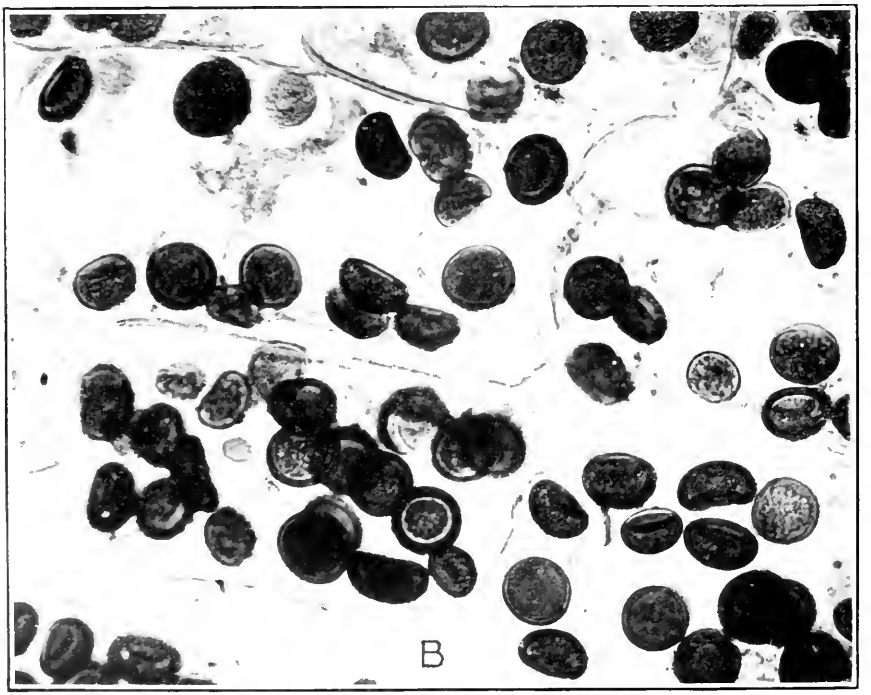

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PLATE 17

Physoderma zeae-maydis: Photomicrographs showing the different stages of the development in the host tissue (teosinte).

A.—Notice the reproductive bodies connected by the very fine threadlike hyhæ in the central cells of the figure.

B.—Host cells filled with mature sporangia.

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Vol. XVI FEBRUARY 10, 1919 No. 6

JOURNAL OF AGRICULTURAL RESEARCH

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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., FEBRUARY 10, 1919 No. 6

INJURY TO CASUARINA TREES IN SOUTHERN FLORIDA BY THE MANGROVE BORER

By THOMAS E. SNYDER

Specialist in Forest Entomology, Forest Insect Investigations, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

In southern Florida many thousand casuarina, or "Australian pine," trees (*Casuarina equisetifolia* Forster) have been and are being planted for shade and ornament along roads and avenues, on reclaimed swamp land, on golf courses, along the seashore, and as windbreaks for fruit trees (Pl. 18, A). The tree makes a rapid growth, is not affected by salt spray from the ocean, and is utilized for the same purposes as eucalyptus trees in California. It is indigenous to tropical Asia and Australasia and, in addition to southern peninsular Florida and the Florida Keys, it has been introduced throughout the West Indies and other tropical regions of North and South America.

Reports of serious injury to casuarina trees in Florida by a bark- and wood-boring insect (*Chrysobothris tranquebarica Gmelin*) [1] led to special investigations by the writer which resulted in the discovery that this buprestid beetle was a common and destructive enemy of the red mangrove (*Rhizophora mangle* Linnaeus), and that, therefore, the mangrove was the source of the trouble affecting the casuarina trees.

The fact that this beetle has so changed its normal habits as to attack and breed in a plant so different botanically from its common host, together with the economic importance of this changed habit to property owners who have made extensive plantings of the casuarina, has rendered the subject of special scientific interest and practical importance.

The first reports of insect injury to the casuarina came from Hobe Sound and Miami Beach in April, 1916. These and other localities in southern Florida were visited by the writer in May, 1916, March and April, 1917, and April and May, 1918, in order that a thorough investigation of the insect, the conditions relating to its attack, and the methods of combating it might be made.

[1] Determination by Mr. W. S. Fisher, Bureau of Entomology.

CHARACTER AND EXTENT OF THE INJURY

It was found that the mangrove borer attacks only living red mangrove and casuarina. The casuarina trees attacked range from 2 to 6 inches in diameter; those over 5 years old usually are not attacked, except high in the tops or branches. Small casuarina trees are attacked near the base as a rule. In case of small trees the trunk may be girdled before the larvæ attain their growth, and in most cases the damage is done before the presence of the insect is noticed. Many casuarina trees were killed at Miami Beach in 1915 (Pl. 18, B) and more in 1916. The infestation in 1917 at Miami Beach was apparently less than in 1916, it having been estimated that among trees planted during the winter of 1916–17, within half a mile of the mangrove swamp, not more than 1 tree out of 20 was lost.

In the mangrove swamp along Biscayne Bay many red mangrove trees were found in 1916 to have been killed by the borer. In 1917 a great accumulation of dead and stag-headed mangrove trees which had been gradually killed by the borer was noted, and many newly infested trees. In 1918 many additional mangrove trees were found infested and it was noted that the infestation extended for many miles north of Miami. The dead trees and the stag-headed, partially killed trees, many of which are of large size, are strikingly evident against the sky line.

At Hobe Sound, Jupiter Island, Fla., which is farther north than Miami Beach, quite a few casuarina trees were killed in 1915; the trees are nearly 5 years old and, hence, not so liable to attack. At this locality the red mangrove is low and scrubby, being apparently too far north for favorable growth. In the swamps near by the borer was found in the red mangrove, but the infestation was not heavy.

On the ocean keys or reefs south of Miami the red mangrove apparently is not infested by *C. tranquebarica*. At Adam Key, about 27 miles south of Miami, neither the red mangroves nor the casuarinas which have been planted there are infested, and no damage to mangrove by the borer has been noticed. On Key Biscayne, just south of Miami, there was formerly a heavy infestation in the casuarinas, but the trees have now reached an age at which they are out of danger of further attack. Infested red mangroves apparently do not occur in swamps continuously from Miami Beach to Hobe Sound; therefore there are broken centers of infestation. No infested trees have been found south of Key Biscayne.

STAGES, HABITS, AND SEASONAL HISTORY OF THE BEETLE

Although *C. tranquebarica* was collected by Mr. H. K. Morrison at Key West in 1886 and by Mr. E. A. Schwarz on cordwood of red mangrove at the same locality in 1887 and although the beetle has been known to science since 1787, it appears that nothing has been recorded regarding its various stages, seasonal history, habits, etc.

Because of its thorough establishment in the red mangrove it is evident that this beetle was not introduced into Florida with the casuarina; in fact, specimens had been collected at Key West before the casuarina was planted in Florida.

The beetle's habitat is the West Indies, where the red mangrove tree is also native.

In India the casuarina is a common tree, but the red mangrove does not occur. *C. tranquebarica*, despite its specific name,[1] does not occur in India. Tranquebar is on the east coast of Madras.

THE ADULT

The adult of the mangrove borer (Pl. 20, D; 21) is metallic greenish bronze and has two lighter-colored and one smaller basal impressions on each elytron. There are also impressions on the thorax. Adults can be told from those of any other species of Chrysobothris found in the United States by the fact that the eyes are nearly contiguous on top of the head. The female is larger than the male, and the front of the head is green. The length ranges from 13.5 to 17 mm. The smaller, more active male ranges in length from 12.5 to 14 mm.; the front of the head is bright red. There are other sex differences in the last ventral segment of the abdomen (Pl. 19, A) and, of course, in the genitalia.

Adults of both sexes are fond of bright sunlight and are commonly found flying from 10 a. m. to 3 p. m. (central time) in open places in the swamps and on the casuarina trees. Oviposition takes place in either morning or afternoon.

Both male and female beetles feed on the tender, succulent bark of the trees which they infest. They may be found resting on the trunks of trees in the bright sunlight chewing through the outer bark to the cambium.

The beetles, owing to their rapidity of movement, strong powers of flight, and shyness, are probably able to survive enemies and live for two or three weeks, or possibly a month or so. They are difficult of detection when resting on the bark of red mangrove, but when flying in the sunlight they are conspicuous on account of the bright-green color of the body. The beetles are never active unless the day is warm, sunny, and not windy.

As the beetles are strong fliers and are fond of flitting from one sunny tree trunk to another, and as they lay many eggs each, it is probable that one female may be responsible for the death of many trees.

On April 13, 1918, in a mangrove swamp along Biscayne Bay, opposite Miami, Fla., females were found ovipositing at 1.10 p. m. (central time), and the operation observed. After a short exploration of the bark, made with extended ovipositor (Pl. 19, B), a proper crevice was

[1] FISHER, W. S. CHRYSOBOTHRIS TRANQUEBARICA GMEL. VERSUS IMPRESSA FABR. *In* Proc. Ent. Soc. Wash., v. 20, no. 8, pp. 173-177 November, 1918 (1919).

found under loose bark and the beetle remained with its ovipositor in the crevice for one and one-half minutes. During this time there was a perceptible pumping motion near the basal end of the ovipositor, and 4 eggs were laid in an irregular row. The tree is attacked anywhere from the large aerial roots to high up on the trunk, but usually in the middle trunk.

THE EGG

The egg (Pl. 19, C) may be compared in shape to a scallop shell, and one end, which is broader than the other and flattened, is irregularly ribbed. It is white and ranges from 1 to 1.5 mm. in length; the average width is approximately 0.75 mm.

The red mangrove has the bark separated into plates; in the process of growth loose bark occurs at the dividing lines (Pl. 19, C). The eggs are inserted under this thin outer layer of loose bark in an irregular longitudinal row. Four eggs are the largest number that have been found together. Eggs occur singly and in twos and threes. One female may lay eggs in several trees. Twenty-three full-sized eggs were dissected from one female, many eggs being in the distended oviduct, and many immature ovules were present.

The period of incubation was not determined but probably one week is required. Young larvæ 5.5 mm. in length were found on April 23, 1918, in a red mangrove tree near Miami Beach.

THE LARVA

The larva[1] is white and a typical "flatheaded" borer (Pl. 20, B; fig. 1). It is of the common Chrysobothris type, moderately compressed, and sparsely covered with coarse, light-colored bristles. The first thoracic segment is large and oval; the second wider and shorter than the third; the third wider than the first abdominal, which is narrower than the second abdominal; the third to eighth abdominal are of about equal width, the ninth and tenth successively narrower; the lateral folds of the second to ninth abdominal segments are well developed; the dorsal plate of the first thoracic segment is marked with a well developed, inverted V of grooves and pointlike rugosities; the ventral plate has a well developed groove extending back three-fourths of the distance from the anterior margin, and rugosities which tend to form ridges. The length is 30 mm. and the width of the first thoracic segment 7 to 8 mm.

The young larvæ upon hatching from the eggs bore through the cambium to the surface of the wood and as they feed on the cambium and grow they extend the burrows horizontally, spirally, or longitudinally (Pl. 20, A). The entire length of the burrow is packed with boring dust. The length of the larval period is nearly one year. When

[1] Description by Mr. H. E. Burke, Bureau of Entomology.

full grown or mature, the larva ranges from 29 to 35 mm. in length. At this stage it bores into the wood to a considerable depth and exca-

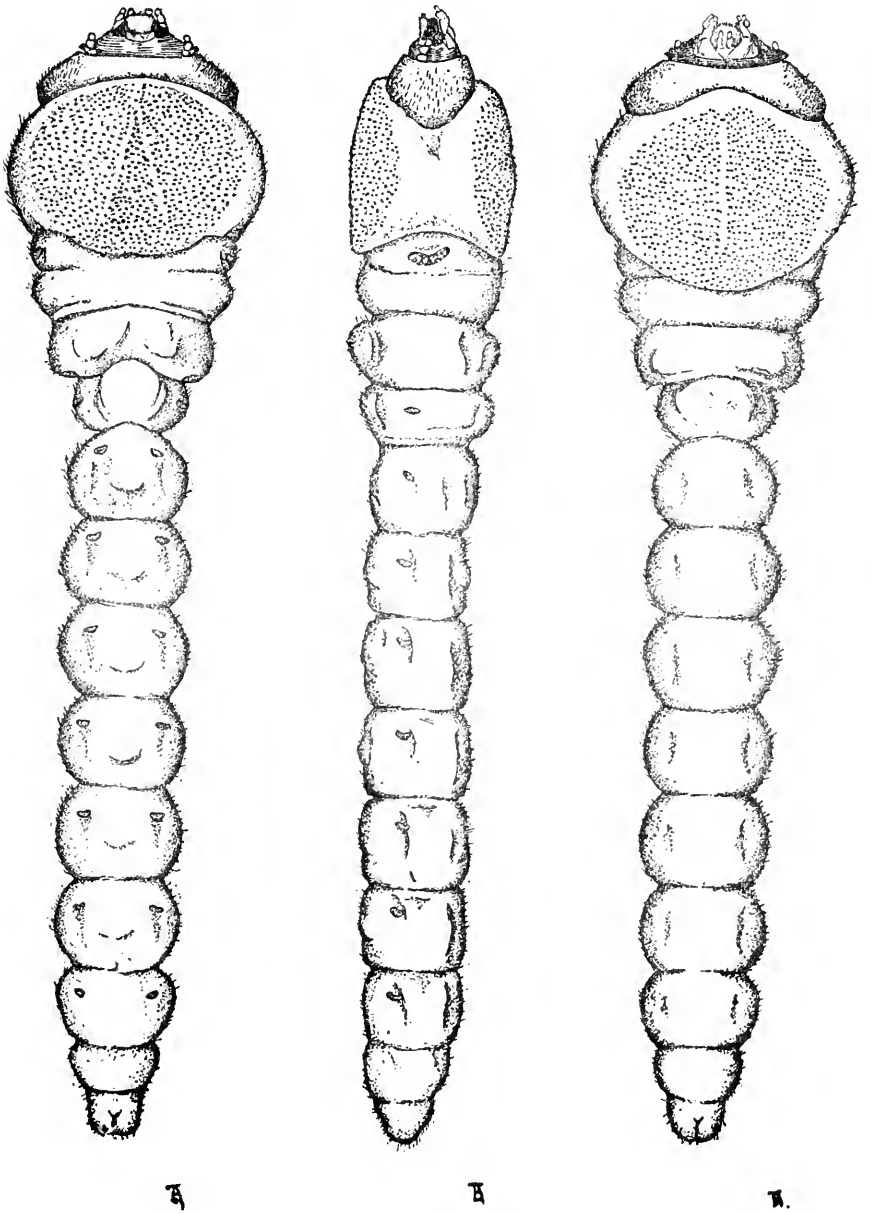


FIG. 1.—*Chrysobothris tranquebarica:* Larva, dorsal, lateral, and ventral views. × 5.

vates its pupal cell. A hole for the exit of the beetle is also excavated by the larva from the pupal cell to or near to the surface and is there

finally packed with coarse boring chips. In some large, heavily infested red mangrove trees as many as three pupal cells per linear 2 inches were found.

THE PUPA

The pupa is white and of the shape characteristic of buprestid pupæ (Pl. 20, C; fig. 2). It is of the common Chrysobothris type, with the head resting on the breast and the legs and wings folded on the ventral surface. The developing insect gradually acquires characters of the adult beetle. The size varies with the individual and there is also a sex difference; the length ranges from 15 to 20 mm.

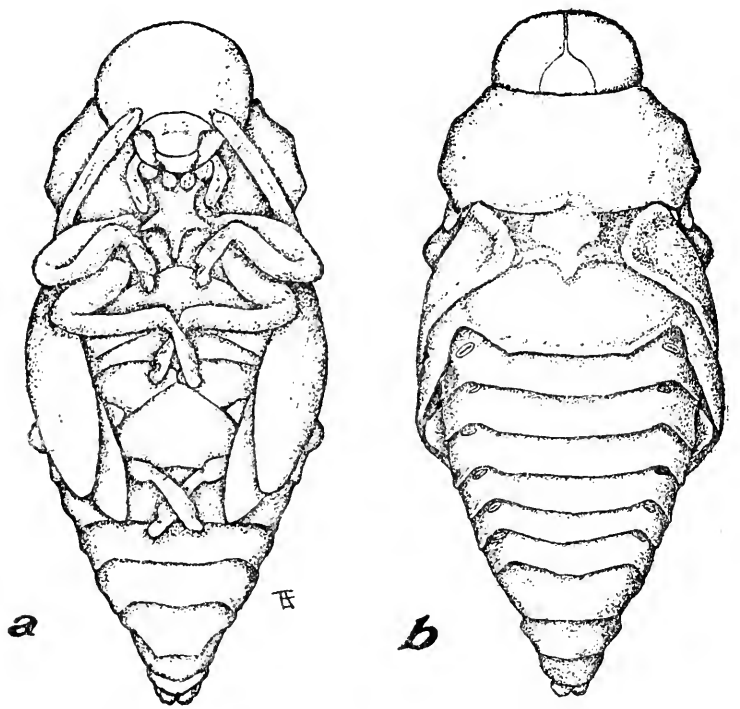


FIG. 2.—*Chrysobothris tranquebarica: a*, Female pupa, ventral view; *b*, same, dorsal view.

The average duration of the pupal period is about two weeks. When the adult becomes mature it chews its way out through the plug of wood fiber, cuts an oval hole through the bark, and escapes. This hole is often mistaken by property owners for the point of entrance of the borer.

SEASONAL HISTORY

One year is required for the development of the mangrove borer from egg to adult. Adult beetles first begin to emerge about the 1st of April. The period of maximum activity of the beetles on the wing is from the

middle of April to the 1st of June, but a few stragglers are found as late as August. Most of the eggs are probably laid from the middle of April to June. The larvæ seem to be full grown by August, and the majority form the pupal cells before winter. The species probably passes through a dormant period, or one of comparative inactivity, during the months of December, January, and February, as mature larvæ under the bark or in the pupal cells. On March 19, 1917, such mature larvæ, together with pupæ and immature adults, were found in infested trees at Miami Beach. On April 4, 1917, many pupæ were changing color, indicating that they would soon transform to the adult stage. In 1918, on April 8, mature larvæ, pupæ, and adults were in pupal cells in infested trees at Miami Beach. The first eggs were found on April 13, 1918, and the first young larvæ on April 23, at Miami Beach, in infested red mangrove trees.

PREDATORY ENEMIES AND PARASITES

The flicker (*Colaptes auratus*) and the red-headed woodpecker (*Melanerpes erythrocephalus*) pick out larvæ and pupæ from infested trees, and often obtain a high percentage of the insects infesting a few trees. Predacious beetle larvæ account for other borers. On April 3, 1917, larvæ of a predacious trogositid beetle (*Tenebroides* sp.)[1] were found under the bark of a red mangrove tree infested by *C. tranquebarica*, in a swamp near Miami Beach.

On April 9, 1918, in the same general locality, larvæ of an elaterid beetle (*Adelocera* sp.)[2] were found under the bark of a red mangrove tree infested with the beetle. Presumably they were predacious enemies of the mangrove borer.

Two species of hymenopterous parasites have been found. One species, *Atanycolus rugosiventris* Ashmead,[3] was found to be fairly common at Miami Beach in 1917 and 1918. Its cocoons occur in a mass at the end of the larval burrow of the beetle. Adults were found emerging from the cocoons on March 19 and April 10, 1917, and on April 9, 1918. The other species, *A. labena* n. sp.,[3] constructs a single cocoon in the pupal cell of *C. tranquebarica*, in infested casuarina trees.

Notwithstanding the numerous natural enemies of *Chrysobothris tranquebarica* it is evident that reliance can not be placed upon them to control this borer without help from man.

CONTROL OF THE BORER

In view of the large number of casuarina trees which have been and are being planted in southern Florida and the varied uses to which they are adapted, it will be seen that the problem of controlling this injurious borer is important. Since 1916 owners of these large plantations have

[1] Determination by Dr. Adam G. Böving, Bureau of Entomology.
[2] Determination by Mr. J. A. Hyslop, Bureau of Entomology.
[3] Determination by Mr. S. A. Rohwer, Bureau of Entomology.

been acting upon the advice of the Bureau of Entomology in efforts to prevent injury, but the problem is greatly complicated at Miami Beach by large areas of heavily infested red mangrove trees in near-by swamps.

In 1916 and 1917, at Miami Beach, badly infested young casuarina trees were removed or topped, and borers were killed in the pupal cells by cutting them out. Some trees were sprayed with poisoned kerosene emulsion. Supporting stakes of red mangrove were removed. In 1917 the infestation appeared to have been reduced, but in 1918 it was again severe. In the red mangrove swamps there appeared to be a steady yearly increase of infestation.

The infestation at Hobe Sound, the farthest north that *C. tranquebarica* has yet been found, has not been so severe. The casuarina trees are now (May, 1918) about 5 years old and of large size. In May, 1916, when these trees were younger and the injury more severe, the trunks were thoroughly and repeatedly sprayed with the poisoned kerosene emulsion. About 900 casuarina trees growing in avenues were sprayed at a cost of approximately 10 cents per tree. As the old formula, used at this time, contains a larger proportion of sodium arsenate than is necessary, the cost per tree can be lowered. The outfit consisted of three men and a team of mules to haul the standard orange-tree spray pump. Almost any good spraying outfit, however, would answer the purpose of spraying the trunks of small trees.

In addition to spraying, the rough bark at the bases of trees at Hobe Sound was scraped and the borers killed by cutting them out of the pupal cells. The infestation of 1916 was less and there was a still further decrease in that of 1917, after the use of the same control methods. A few borers were still found in the tops of the casuarina trees in 1918 but these have been cut out. The infestation in the low scrubby red mangrove tree here is not and has not been heavy.

METHODS RECOMMENDED FOR COMBATING THE INSECT

Investigations have shown that many trees can be saved by carrying out the following methods of control: All badly damaged casuarina trees should be cut and burned between September and March to kill the insects before they emerge. The trees may be entirely removed, cut off near the ground, or merely topped so that they will sprout from the stump and make new growth. Since the borer usually attacks the young trees near the base, where there are rougher bark and more suitable places for egg laying, care should be exercised that no infested stumps remain. Trees only slightly damaged and showing evidence, in the rapidly healing wounds, of recovery should not be cut. The wounds will soon heal, and as the trees grow will disappear.

Casuarina trees between 1½ and 6 inches in diameter, growing in proximity to mangrove swamps or near other infested casuarina trees, should be examined carefully in September and March and the young larvæ

killed by spraying the affected part of the trunks with poisoned kerosene emulsion [1] made in accordance with the following formula, recently revised by Mr. F. C. Craighead:

Standard miscible oil	pint	1
Water	gallons	5
Sodium arsenate	pound	¼

Dissolve the arsenate in water, stir, then add 1 pint of miscible oil.

From April to June, when large numbers of the adult beetles are flying and feeding on the bark, they should be killed by spraying the tree trunks with the poisoned kerosene emulsion.

No pruning of casuarina trees should be attempted between April and August, since the consequent flow of sap will attract the flying beetles to the trees.

Mangrove stakes should not be used to support young, recently set-out trees, as they will attract the borers.

According to the host-selection principle [2] as advocated by Dr. A. D. Hopkins, the beetles that breed for one or two generations or more in the casuarina will be much more likely to reinfest this host than they are to go back to the original host; and, since the beetle became established in the mangrove before the casuarina was introduced, it is to be expected that only occasional individuals, among the thousands of beetles that breed in the mangrove, will deposit eggs on the casuarinas. It is of primary importance, therefore, to keep as many of the beetles as possible from reaching maturity in the casuarinas.

[1] CRAIGHEAD, F. C. A NEW MIXTURE FOR CONTROLLING WOOD-BORING INSECTS—SODIUM ARSENATE EMULSION. *In* Jour. Econ. Ent., v. 8, no. 6, p. 513. 1915.

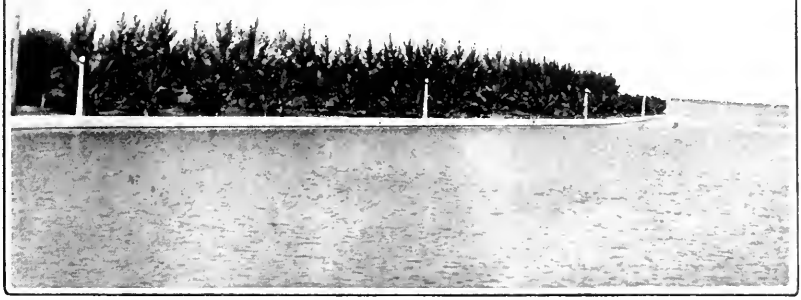
[2] U. S. DEPARTMENT OF AGRICULTURE. PROGRAM OF WORK [1916]/1917, p. 353. Washington, 1916.

PLATE 18

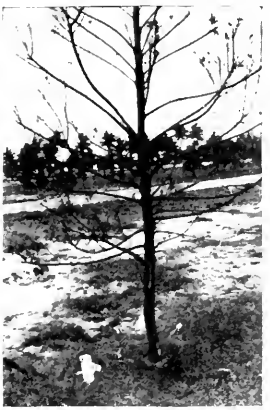
A.—Casuarina trees planted along the water front, Belle Isle, Miami Beach, Fla., June, 1918. Photographed by W. E. Brown.

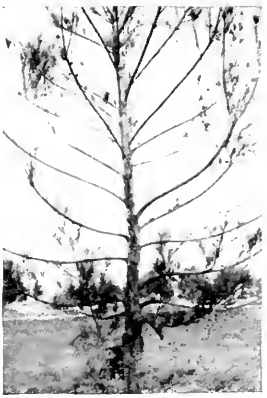
B.—Casuarina trees disfigured and killed by the mangrove borer (*Chrysobothris tranquebarica*) at Miami Beach, Fla.

A.



B.





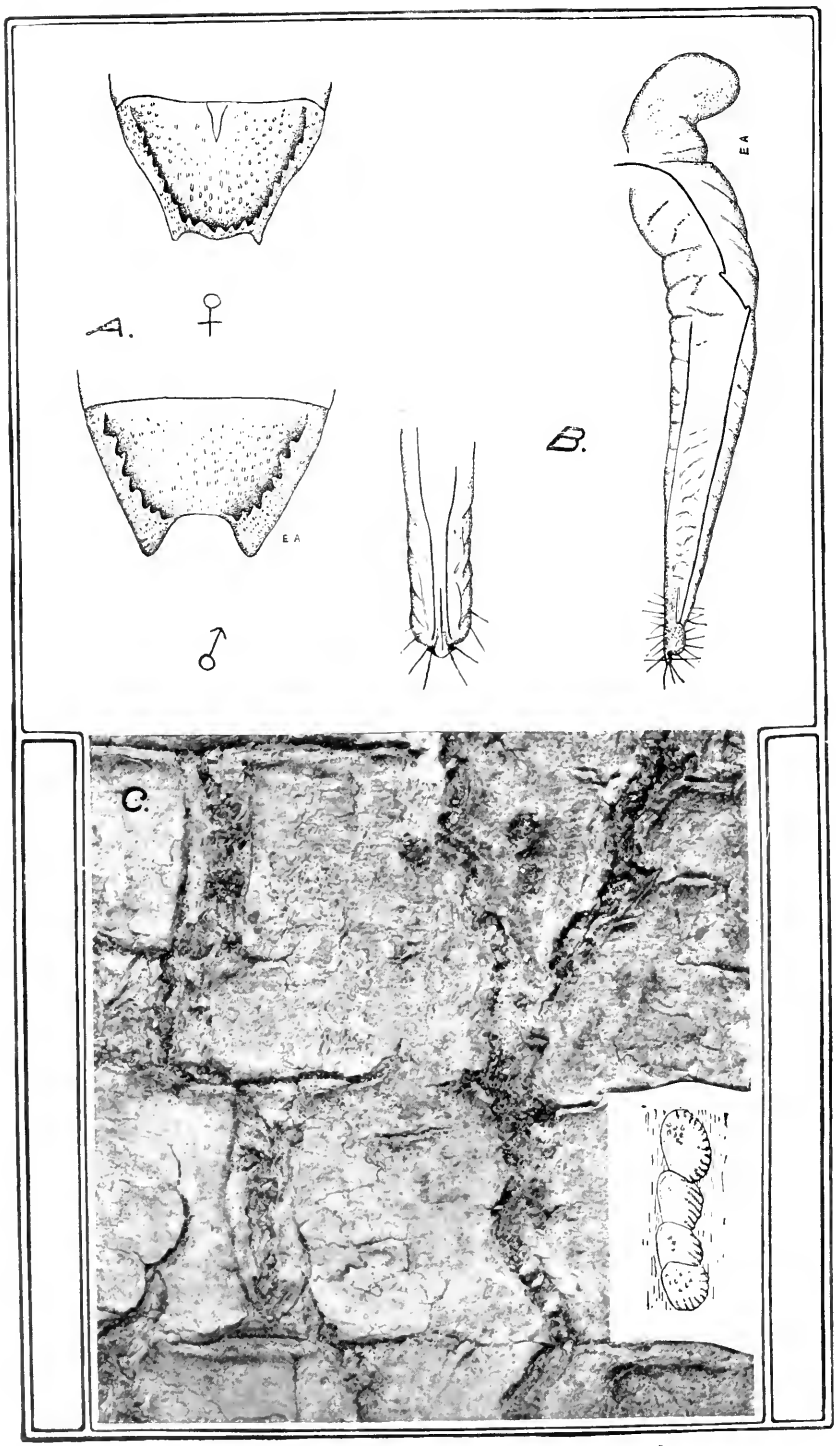

A.
♀
E A
♂
B.
E A
C.

PLATE 19

Chrysobothris tranquebarica:

A.—Sex differences in the last abdominal segment. × 9.
Drawn by E. Armstrong.
B.—Lateral and dorsal view of ovipositor. × 9.
Drawn by E. Armstrong.
C.—Bark of red mangrove (*Rhizophora mangle*) showing how it is divided into plates. Natural size. The eggs are superficially inserted under the thin outer layer, where the bark is loose, at a crack. Eggs × 4.

PLATE 20

Chrysobothris tranquebarica:

A.—Larval burrow in cambium of Australian pine (*Casuarina equisetifolia*), Miami Beach, Fla. Note how the burrow is packed with frass, the exit hole and the cambium growing over the wound. Natural size.

B.—Larvae, ventral and dorsal views. × 3.

C.—Pupa, dorsal and ventral views. × 2½.

D.—Female and male adult beetles. × 2½. Photographed by Mr. William Middleton.

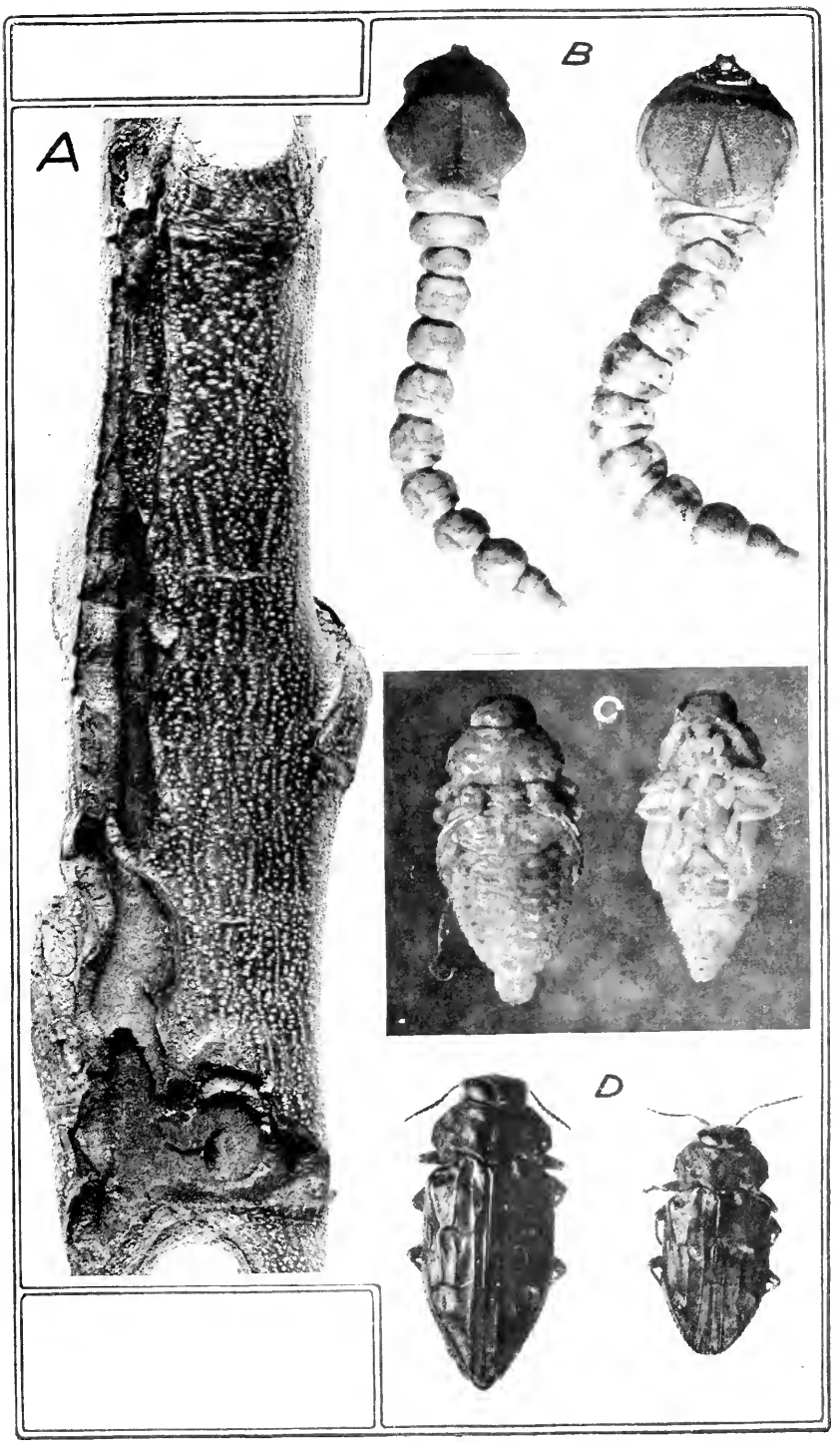

A
B
C
D

PLATE 21

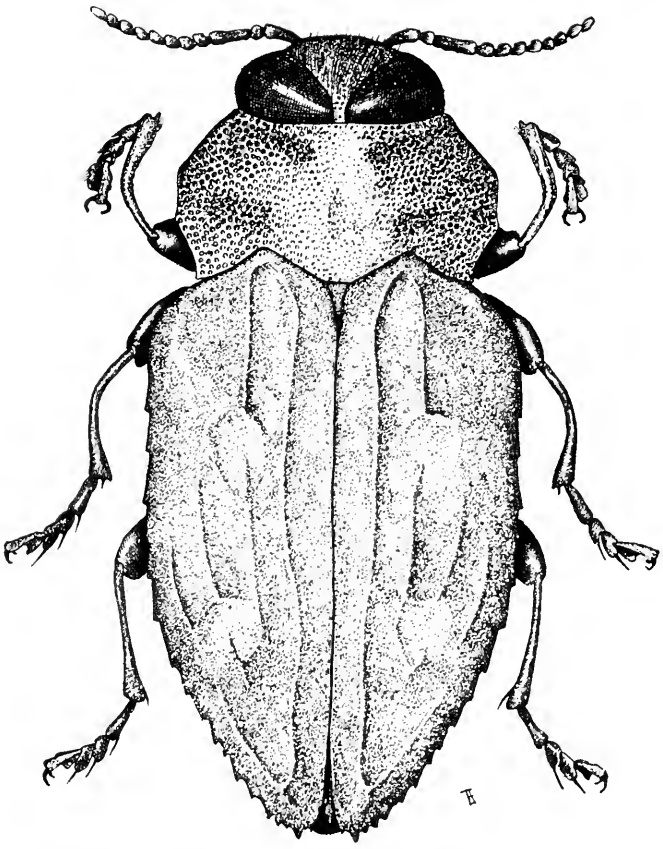


PLATE 21

Chrysobothris tranquebarica:

Adult male, dorsal view. × 7.

LIFE-HISTORY OBSERVATIONS ON FOUR RECENTLY DESCRIBED PARASITES OF BRUCHOPHAGUS FUNEBRIS

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INTRODUCTION

The parasitic Hymenoptera referred to in this paper were observed, together with others, while the writer was making a detailed study of the chalcis-fly *Bruchophagus funebris* infesting the seeds of alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*).

Observations and notes were made concerning the life habits of these new parasites, as the opportunity presented itself, to determine any economic value which one or more of these species may have in the control of *Bruchophagus funebris*.

LIFE-HISTORY SUMMARY OF THE HOST

The host insect, *Bruchophagus funebris* Howard, completes its development from the egg to the adult stage within the seed of alfalfa, red clover, or wild species of Medicago. Upon reaching maturity the adult gnaws an opening through the seed shell and makes its escape. *B. funebris* hibernates in its larva stage within the infested seeds remaining upon the field. It passes through several generations in a single season.

METHOD OF STUDYING THE PARASITES

In order that the development of these parasites could be observed, it became necessary to dissect several thousand alfalfa seeds under the microscope, locate parasite larvæ, and remove them together with their host for study. Each host, with its parasite, was then transferred to a single little cage consisting of an 8-mm. cork with a small cavity in one end and covered by a medical capsule. Here each parasite could be observed from day to day until it had completely destroyed its host, developed, passed through the pupa stage, and transformed to the adult stage.

LIODONTOMERUS PERPLEXUS GAHAN

The two species of Liodontomerus discussed in this paper belong to the hymenopterous superfamily Chalcidoidea, family Callimomidae, and subfamily Monodontomerinae. The genus Liodontomerus was erected by Mr. A. B. Gahan,[1] of the Bureau of Entomology, for specimens of *Liodon-*

[1] GAHAN, A. B. DESCRIPTIONS OF NEW GENERA AND SPECIES WITH NOTES OF PARASITIC HYMENOPTERA. *In* Proc. U. S. Nat. Mus., v. 48, p. 155-168, Dec. 16, 1914. p. 159: Liodontomerus, new genus.

Journal of Agricultural Research,
Washington, D. C.
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Vol. XVI, No. 6
Feb. 10, 1919
Key No. K-75

tomerus perplexus Gahan reared by the writer from *Bruchophagus funebris* infesting alfalfa seeds at Yuma, Arizona.

Liodontomerus perplexus was first reared by the writer from alfalfa seeds collected at Yuma, Ariz., during August, 1912. It was again reared September 20, 1912, from El Centro, California, and from Chino, Cal., on November 4, 1912. Infested alfalfa seeds dissected and subjected to a microscopic examination soon showed this species as being parasitic upon *Bruchophagus funebris*. In the year 1913 it was first reared on July 19 from Corcoran, Cal.; on July 25, from Glendale, Cal.; and in 1914 the first rearing dates from new localities were July 24, Brawley, Cal., and September 8, Red Bluff, Cal. On August 4 it was reared from *B. funebris* infesting bur clover (*Medicago hispida nigra*) at Tulare, Cal.

Examinations of various chalcids reared by different members of the Bureau of Entomology from alfalfa seeds infested by *Bruchophagus funebris* showed that *Liodontomerus perplexus* was reared by C. N. Ainslie at Newell, South Dakota, November 15, 1913, and at Mitchell, S. Dak., in 1914. A single specimen was labeled "Red Oak, Iowa."

Liodontomerus perplexus was described by Mr. Gahan as a new species [1] from the type specimens reared by the writer from *Bruchophagus funebris* infesting alfalfa seeds at Yuma, Arizona, in August, 1912.

STAGES OF HOST SHOWING PARASITISM

Liodontomerus perplexus is primarily parasitic upon the larva stages of *Bruchophagus funebris*. It feeds externally upon its host and frequently destroys the entire host larva with the exception of the head. In exceptional cases this parasite has been found to be parasitic upon the pupa stage of *B. funebris*. Of 97 larvæ of *L. perplexus* under observation 86 were parasitic upon the larva stage and 9 upon the pupa stage of their host. A single specimen of this species was found to be a secondary parasite and feeding upon the larva of a different species after the latter had destroyed the host larva.

HIBERNATION

The larvæ, which become fully developed late in the summer, or in the fall, mostly hibernate until the following spring. This takes place within the alfalfa seeds in which the host was attacked. While hibernation is normal in the larva stage, occasional individuals have been observed to hibernate in the pupa stage under the mild climatic conditions of the Southwest.

APPEARANCE IN THE FIELD

This species does not seem to appear in the field in large numbers as early in the season as might be expected. In southern California and western Arizona it becomes active in April and slowly increases in numbers throughout May. In August the abundance of adults is probably greatest, while a few individuals may be found as late as November (Table I).

[1] GAHAN, A. B., OP. CIT., p. 159.

TABLE I.—*Dates of emergence of adults of Liodontomerus perplexus which developed from larvæ spending the winter in hibernation*

Date.	Number of male adults.	Number of female adults.
March 1–15	.....	.....
March 16–31	.....	.....
April 1–15	1	1
April 16–30	2	1
May 1–15	2	.....
May 16–31	3	.....
June 1–15	8	13
June 16–30	1	5
July 1–15	5	2
July 16–31	3	6
August 1–15	2	1
August 16–31	1	4
September 1–15	.....	.....
Percentage	45.89	54.09

OVIPOSITION

The adult female locates the green and tender seed pods of alfalfa in which seeds are infested by larvæ of *Bruchophagus funebris*, inserts the ovipositor through the seed pod, and deposits an egg upon or near the host larva within the green seed.

LARVA

DEVELOPMENT.—The different larval instars were not studied by the writer through lack of time for this particular subject. Field and laboratory observations, however, showed that the larvæ develop very rapidly upon their host and under favorable conditions require from 8 to 12 days to make their growth. They do not always transform to the pupa stage as soon as they become full grown.

DORMANT PERIOD.—After the larva of *Liodontomerus perplexus* has become fully developed upon its host within the alfalfa seed it may at once enter the pupa stage, but if the seed is exposed to dry climatic conditions a dormant period in the larva stage frequently follows. This dormant period may begin at almost any time throughout the summer and continue right on into hibernation. Transformation to the pupa stage is then delayed until the following spring. In the laboratory a few larvæ that became dormant in the summer continued so throughout the following winter and through the next summer, and hibernated again the second winter before transforming to the pupa. A hibernating larva was taken from the field on December 18, 1913. It remained in the larva stage until March, 1915, then transformed to a pupa, and emerged as an adult on April 19, 1915. This particular habit is undoubtedly of great value to the species and enables it to be carried over the long continued dry seasons of the desert sections of the Southwest.

DESCRIPTION.—The larva of *Liodontomerus perplexus* (fig. 1) varies in color from white to smoky gray. The length averages 1.5 mm. and the thickness averages 0.7 mm. The general appearance is grublike, while a side view shows the general shape, suggesting an interrogation mark. The head of the larva shows the eye lobes and on each a small tubercle. The front of the head contains about eight fine setæ. Mandibles, slightly chitinous, are usually inconspicuous, but sometimes distinctly visible. Segmentation of the 13 body segments is very marked. The body is covered with bristle-like setæ which are from 0.04 mm. to 0.1 mm. in length. Two rows, and a broken third row, are present in the first segment. The second and third segments each bear one row with a broken second row. The other segments each bear one row encircling the segment. Setæ on the dorsal portion of the body are much coarser than those of the ventral side. The last segment is dorso-ventrally bilobed and bears setæ on each of the lobes.

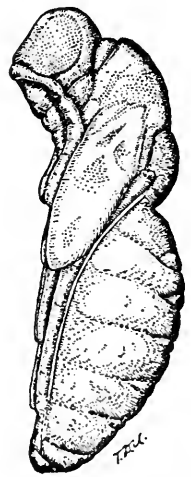


FIG. 1.—*Liodontomerus perplexus:* Larva.

PUPA

PUPATION.—After the pupa has completed its development within the larval skin the latter breaks open along the antero-dorsal margin and is slowly worked back to beyond the tip of the abdomen by a slight movement of the newly formed pupa.

DESCRIPTION.—The pupa (fig. 2) is white when newly formed. It is about 1.5 mm. long and 0.5 mm. thick. The eyes are at first white, but after a few days turn to pale brown. The head and thorax bend slightly forward. The antennæ, legs, and wing pads are folded close to the body and the ovipositor sheath is bent back across the end of the abdomen. In the last few days of the pupa stage the pupa turns almost black, with dark-brown eyes and pale-brown antennæ, legs, and ovipositor.

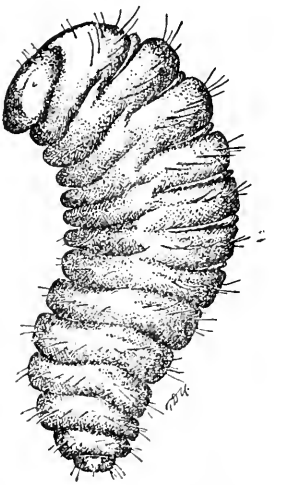


FIG. 2.—*Liodontomerus perplexus:* Pupa.

LENGTH OF PUPAL PERIOD.—The length of the pupal period varies greatly according to the season during which pupation occurs. Hibernating larvæ under observation began entering the pupa stage as early as March; others did not pupate until July and August, and a few remained in the larva stage until the following year before pupating. Twenty-six pupæ, which proved to be males, averaged 23.7 days in the pupa stage; and 31 pupæ, which proved to be females,

averaged 27.9 days in that stage. The longest pupal period observed was 45 days and the shortest was 8 days. These observations were made in the laboratory under natural temperatures. It is very probable that under the most favorable field conditions the pupal period may require even less time than the minimum period recorded.

ADULT

EMERGENCE.—The adult (Pl. 22, A), upon emerging from the pupal skin, finds itself surrounded by the thin seed wall and within the alfalfa seed pod. It proceeds at once to gnaw a small irregular opening through the seed in which the host has been destroyed, then through the seed pod, and thereupon escapes.

RELATIVE PROPORTION OF SEXES.—Both sexes of this species seem to be well represented in all of the localities from which specimens were reared. A count made of 859 adults showed 121 to be males and 738 females, or a ratio of 1 to 6.92.

ADULT VARIATION.—Some adults of this species vary from the true type in that they show a stigmal cloud in the forewing. In a few individuals this clouded area was very conspicuous.

SEASONAL HISTORY

Observations show that about 30 days, under very favorable conditions, are required for the complete development of a single generation and that in alfalfa seed fields of Arizona and southern California there may be as many as three generations in a single season. Other individuals subjected to different local conditions may require an entire season for their development.

PARASITIC IMPORTANCE

This species appears to be a parasite of considerable economic importance in helping to reduce the ravages of *Bruchophagus funebris* in alfalfa seed throughout the western Arizona seed-growing districts. It is apparently not present in sufficient numbers throughout the California, Idaho, and Utah seed-growing sections to be of value in reducing the destructive work of the seed chalcis-fly.

LIODONTOMERUS SECUNDUS GAHAN

Liodontomerus secundus was first collected by the writer on September 5, 1914, at Albany, Oregon, where it was found ovipositing in the green ovaries of florets on red-clover heads. On September 16 the writer reared specimens from red-clover seeds infested by *Bruchophagus funebris* at Caldwell, Idaho; and on September 23 it was reared from infested red-clover seeds taken at Albany, Oreg. Microscopic examination of the seeds showed that this species was parasitic upon the larvæ of *B. funebris*. It was also present among chalcids reared from red clover in 1915 at Elk Point, South Dakota, by C. N. Ainslie.

Liodontomerus secundus was described as new by Mr. A. B. Gahan[1] from specimens reared by the writer from infested red-clover seeds taken at Caldwell, Idaho.

HIBERNATION

Examination of red-clover seeds infested by *Bruchophagus funebris* revealed larvæ of *Liodontomerus secundus* hibernating in the larva stage within the seeds which had been destroyed by their respective host larvæ. The hibernating larvæ pupated in the months of April, May, and June, spending from 24 to 40 days in the pupa stage under laboratory conditions before emerging as adults.

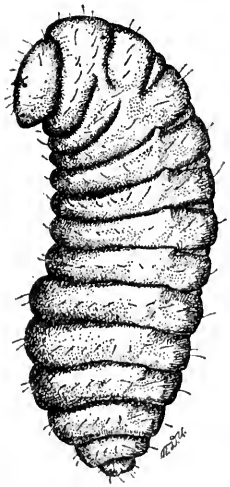


FIG. 3.—*Liodontomerus secundus:* Larva.

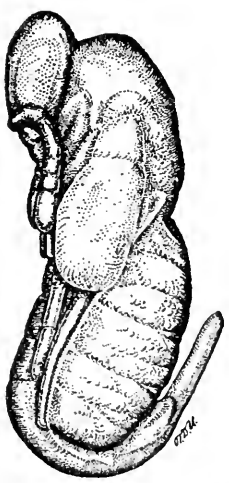


FIG. 4.—*Liodontomerus secundus:* Pupa.

LARVA

The larva (fig. 3) is smoky white in color and averages 1.72 mm. long and 0.8 mm. in thickness. The general shape is grublike. The head, of medium size, shows the eye lobes with a tubercle apparently more distinct in this species than in *L. perplexus*. Pointed mandibles show a slight tinge of brown. The body is covered with pubescence about 0.055 long, the pubescence longest on dorsal portion of first two body segments. Pubescence finer than on larvæ of *L. perplexus*.

PUPA

The pupa (fig. 4) is white when newly formed, but later it turns smoky white and finally brownish black. The eyes turn brown. It averages 1.6 mm. long. The sheath of the ovipositor is folded around the end of the abdomen and back half way along the dorsal side.

ADULT

The adults (Pl. 23, B) emerge from the infested red-clover seeds in spring. Some continue to emerge from the old seeds as late as July. They apparently have two or more generations in a single season and are active in the fields until late in fall.

Observations show that both sexes are well represented in localities where the species was taken.

[1] GAHAN, A. B. DESCRIPTIONS OF SOME NEW PARASITIC HYMENOPTERA. *In* Proc. U. S. Nat. Mus., v. 53, p. 195–217. May 26, 1917. p. 208: *Liodontomerus secundus*, new species.

EUTELUS BRUCHOPHAGI GAHAN

Eutelus bruchophagi belongs to the superfamily Chalcidoidea, family Pteromalidae, and subfamily Pteromalinae.

This insect was first reared by the writer from alfalfa seeds infested by *Bruchophagus funebris*, collected at Blackfoot, Aberdeen, and Caldwell, Idaho, and from Nephi, Gunnison, Manti, and Salt Lake City, Utah, in September, 1914, and from Susanville, California, on September 12, 1917.

Mr. T. R. Chamberlin, of the Bureau of Entomology, reared males of this species from alfalfa seeds collected at Salt Lake City in June, 1914.

Upon careful examination of the infested seeds this species was soon found to be parasitic upon *B. funebris*.

Specimens reared by the writer at Nephi, Utah, were described by Mr. A. B. Gahan, of the Bureau of Entomology, as a new species.[1]

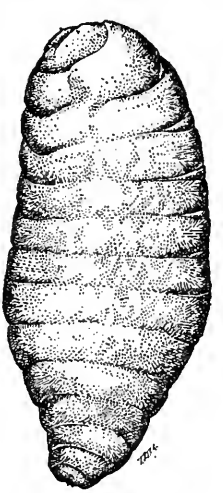


FIG. 5.—*Eutelus bruchophagi:* Larva.

FIG. 6.—*Eutelus bruchophagi:* Pupa.

HIBERNATION

Eutelus bruchophagi hibernates in the larva stage within the infested alfalfa seeds and seed pods remaining on the fields. The warm spring days hasten pupation, and a few weeks later the newly formed adult gnaws an opening through the seed wall and makes its escape.

LARVA

The larva (fig. 5) is grublike in appearance and averages 1.5 mm. in length. Its body is white with a glossy surface free from pubescence and having a clouded appearance under the epidermis. The head is of medium size and the eye lobes rather shallow. The setæ on the eyes are distinctly visible. The anal segment is bilobed and shows three very fine setæ.

PUPA

The pupa (fig. 6) is white when newly formed and after a few days shows the eyes turning to salmon brown. It averages 1.5 mm. in length, and turns black before changing to the adult stage.

ADULT

The adults (Pl. 22, B) live for one or two months under favorable conditions and locate, for oviposition, on the newly-forming seed pods of alfalfa

[1] GAHAM, A. B. OP. CIT., 1917, p. 212.

plants which have become infested by *Bruchophagus funebris*. Apparently there are at least two generations in a single season.

The specimens reared showed a much larger percentage of males than of females.

TIMEROMICRUS MACULATUS GAHAN

Trimeromicrus maculatus (Pl. 23, A) belongs in the hymenopterous superfamily Chalcidoidea, family Pteromalidae, and subfamily Pteromalinae. Specimens reared by the writer were determined by Mr. A. B. Gahan of the Bureau of Entomology as belonging to a new genus. The genus Trimeromicrus was therefore erected by Mr. Gahan [1] for this species.

This species was first reared by the writer from alfalfa seeds infested by *Bruchophagus funebris* and taken at Yuma, Arizona, in September, 1912. Larvæ of this species were later dissected from alfalfa seeds where they were found to be parasitic upon the larvæ of *B. funebris*. It was later reared from the following localities:

El Centro, Cal., September, 1912.
Glendale, Cal., September, 1912.
Chino, Cal., November, 1912.
Corcoran, Cal., July, 1913.
Tulare, Cal., June, 1914.
Red Bluff, Cal., September, 1914.
San Diego, Cal., August, 1915.
Susanville, Cal., September, 1917.

Examination of the undetermined collections and the field notes made by different members of the Bureau of Entomology showed that this species was also reared from infested alfalfa seeds as follows:

Mesilla Park, N. Mex., June, 1909, C. N. Ainslie.
Sacaton, Ariz., June, 1909, C. N. Ainslie.
Casa Grande, Ariz., June, 1910, V. L. Wildermuth.
Wellington, Kans., August, 1910, E. G. Kelly.
Brawley, Cal., March, 1911, V. L. Wildermuth.
Tempe, Ariz., July, 1911, E. G. Smyth.
Buckeye, Ariz., July, 1912, R. N. Wilson.
Newell, S. Dak., August, 1913, C. N. Ainslie.
Salt Lake City, Utah, September, 1915, T. R. Chamberlin.

Trimeromicrus maculatus was described as a new species by Mr. A. B. Gahan [2] from the specimens reared by the writer from *Bruchophagus funebris* infesting alfalfa seeds at Yuma, Arizona.

HIBERNATION

This species, like the others mentioned, hibernates in the larva stage within the infested alfalfa seeds. It frequently hibernates as early as September. In early spring the larvæ change to pupæ, remain so for about 30 days, and the adults emerge from the seeds by the time the new seed pods are forming.

[1] GAHAN, A. B. DESCRIPTIONS OF NEW GENERA AND SPECIES WITH NOTES OF PARASITIC HYMENOPTERA. *In* Proc. U. S. Nat. Mus., v. 48, p. 155-168. Dec. 16, 1914. p. 161: Trimeromicrus, new genus.

[2] Op. cit., 1914, p. 162.

LARVA

The larva (fig. 7) varies from white to smoky gray, averages 1.6 mm. in length, and is somewhat grublike in general shape. The head is of medium size and faintly shows the eye lobes. Each eye lobe shows a very fine seta.

The thirteen body segments are subequal, the first one back of the head being the largest and the others decreasing in size to the anal segment, which is bilobed, the upper lobe containing three very fine setæ. The dorsal portion of the first twelve body segments also shows indications of very short and fine setæ in some specimens.

PARASITIC HABIT.—The larva of this species was found to attach itself externally upon the larva of its host. In the course of a few days the host larva apparently dies and the parasite makes a rapid growth, feeding upon the body contents of the dead host.

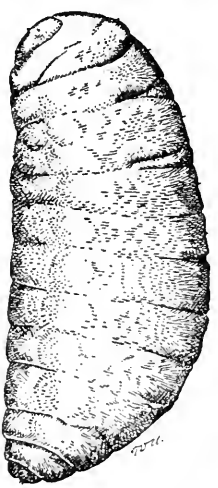


FIG. 7.—*Trimeromicrus maculatus:* Larva.

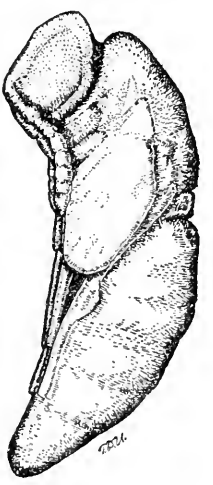


FIG. 8.—*Trimeromicrus maculatus:* Pupa.

PUPA

The pupa (fig. 8) is white when newly formed. It is 1.6 mm. long and about 0.6 mm. wide. The head is placed slightly forward and the appendages are folded close to the body. The entire pupa is enclosed in a thin pupal skin. During the last few days of the pupal period the pupa turns almost black.

PUPATION.—The duration of the longest pupal period observed was 15 days and the shortest was 6 days. The average number of days in the pupa stage as observed in the laboratory was 9.

RELATIVE PROPORTION OF SEXES

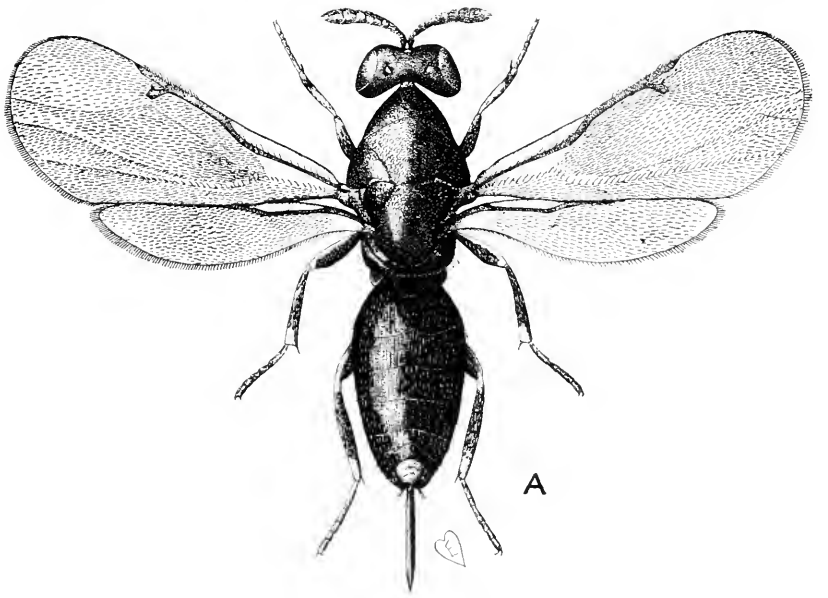
The localities from which this species was reared showed both sexes well represented. A count, made of 322 adults reared from various localities, showed 85 males and 237 females, or a ratio of 1 male to 2.67 females.

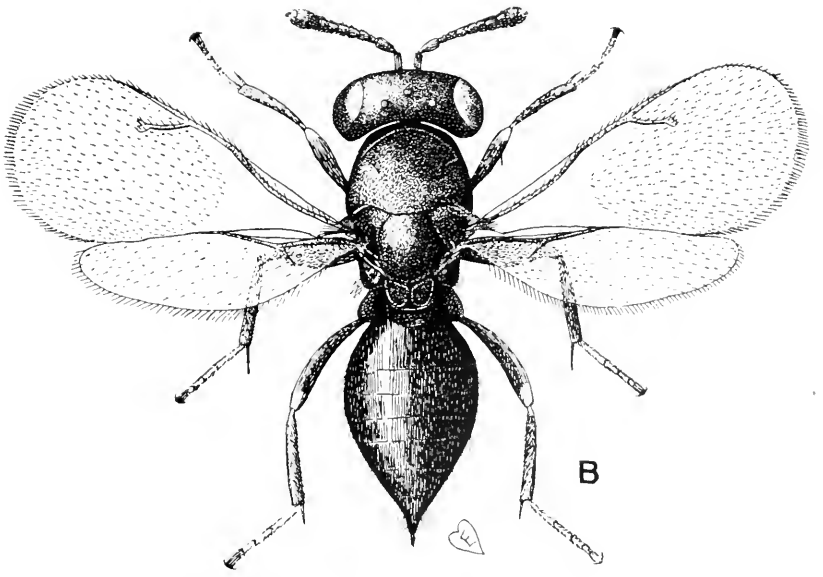
ECONOMIC IMPORTANCE

Trimeromicrus maculatus is apparently well established in the Yuma Valley of Arizona, where it was found to destroy about 7 per cent of the larvæ of *Bruchophagus funebris* infesting alfalfa seeds. Apparently it is also well established in the Honey Lake Valley of northeastern California.

PLATE 22

A.—*Liodontomerus perplexus:* Adult female.
B.—*Eutelus bruchophagi:* Adult female.


A


B

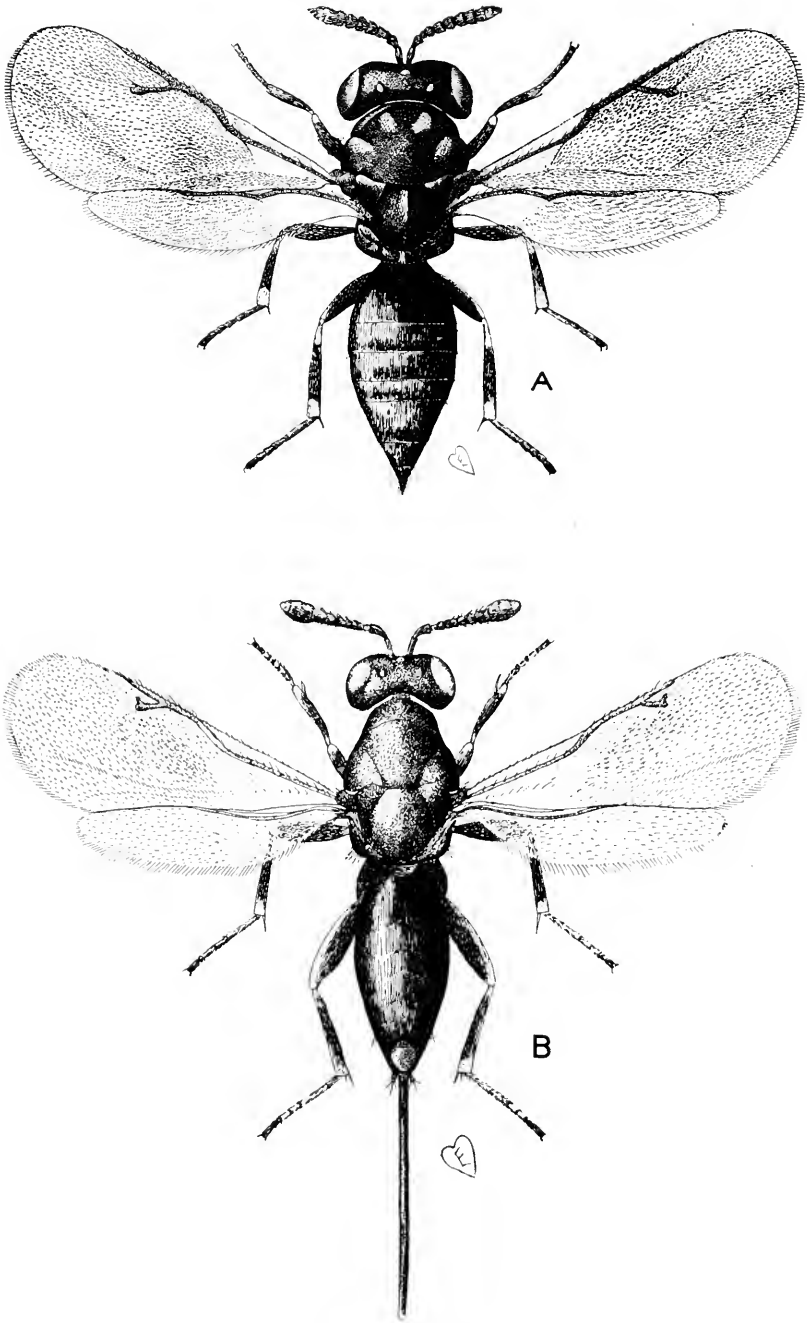

A
B

PLATE 23

A.—*Trimeromicrus maculatus:* Adult female.
B.—*Liodontomerus secundus:* Adult female.

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Vol. XVI FEBRUARY 17, 1919 No. 7

JOURNAL OF AGRICULTURAL RESEARCH

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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., FEBRUARY 17, 1919 NO. 7

CYANOGENESIS IN ANDROPOGON SORGHUM

By C. T. DOWELL

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INTRODUCTION

It is a prevalent belief among farmers and also among certain writers on the subject of sorghums (*Andropogon sorghum*) that when the sorghum is cut and cured it is no longer poisonous to stock. While this is a strong belief among farmers and is stated as a fact by certain writers and investigators, yet there are other writers and investigators who have claimed that curing has no effect on the power of sorghum to poison stock. In fact, the literature on this subject is quite conflicting in its statements. For instance, Churchill (*3*)[1] states that sorghum is rendered safe for feeding by curing. Turrill (*8*) states that in curing the sorghum is rendered harmless. On the other hand, Schröder and Dammann (7) and also Brunnich (*2*) claim that the sorghum is not rendered harmless in the curing process. Furthermore, the well-known fact is recalled in this connection that linseed cake and certain varieties of beans are known to contain hydrocyanic (prussic) acid in the form of glucosid. Peters, Slade, and Avery (6) are not sure whether sorghum is rendered suitable for feeding by curing, and stated that the subject should be further investigated.

During this past summer reports came to this Station through the newspapers of several cases of poisoning caused by sorghum which had been cut for some time. This information, the fact that several inquiries were made by farmers as to whether or not it would be safe to feed sorghum which had been cut during dry weather, and the lack of definite information in the literature caused me to take up the present investigation.

There are several questions that should be investigated. The first and probably the most important is to determine whether or not the glucosid is decomposed and the prussic acid liberated when the sorghum is cured; second, to determine whether or not the enzym becomes inactive in the process of curing as claimed by Peters, Slade, and Avery (6); third, to determine the effect of the presence of substances such as glucose and

[1] Reference is made by number (italic) to "Literature cited, p. 181."

maltose on the liberation of the hydrocyanic acid from the glucosid; and, fourth, to determine whether or not the hydrocyanic acid may be present in more than one form as has been claimed by Willaman (*10*). While these are the main points studied, there are several others, possibly of minor importance, that were studied.

EXPERIMENTAL WORK

Four different samples of sorghum were used. One was a sample obtained from Mr. Ed. Singleton, of Chickasha, Okla., and was a part of a lot of sorghum which had been cut when it was about 2½ feet high and at a time when there was an extreme drouth in the southwestern part of the State. This was a part of some sorghum which had been fed to 12 head of cattle, 10 of which had died within one hour. This will be called sample 1. Sample 2 was cut at the same stage of growth by Mr. P. A. Gould, of Stillwater, but had not been subjected to as extreme drouth as sample 1, as it was cut at the beginning of the dry weather. Sample 3 was a second-growth sorghum which had grown after heavy rains had fallen and there had been plenty of moisture in the ground all during its growth. This sample was cut fresh each time as it was needed and was about knee-high at the time of cutting. Sample 4 was a volunteer sorghum cut from the Experiment Station farm. This sample was in the dough stage when cut, but it had been subjected to the dry weather of the summer and had grown quite vigorously after the rains had fallen.

The method of determining the hydrocyanic acid was a modification of that used by Viehoever and Johns (*9*) and by Knight (*5*). In the case of the dry samples Nos. 1 and 2, the sorghum was cut into fine pieces and then run through a feed mill. Samples 3 and 4 were cut a little at a time, this part being thoroughly wet and bruised in a large iron mortar. The bruised portions were placed in water in the digestion flask. At first each of these samples were kept in the digestion flask in a water bath at 40° C. for two hours, the apparatus being so arranged that any hydrocyanic acid which passed off would be collected in sodium hydroxide. After this period of digestion the water bath was removed, and 100 cc. were distilled as rapidly as possible, the hydrocyanic acid being collected in sodium hydroxid. It was found by two or three trials that all of the hydrocyanic acid was driven over by distilling 100 cc. At first the distillate was evaporated in vacuum as directed by Viehoever and Johns (*9*), but since this required such a long time it was decided to carry on the evaporation by placing the distillate in a flat-form evaporation dish on a water bath which was heated by an electric hot plate. A current of air from an electric fan at a low speed was directed across the evaporating dish. It was found that under these conditions the solution was usually at about 60° C. and in no case did the temperature go above 70° C. With such an arrangement the evaporation could be made easily within two hours. After the distillate was evaporated almost to

dryness, freshly prepared ferrous sulphate was added and acidified with 30 per cent nitric acid as directed by Viehoever and Johns (*9*). Instead of filtering the Prussian-blue precipitate into a Gooch crucible, as was done by Knight (*5*), it was filtered in the ordinary way and washed thoroughly with dilute nitric acid and then with water. The precipitate and filter paper were then placed in a flat-form platinum dish and heated slowly to dryness in an electric muffle furnace, and then heated strongly to burn the precipitate and oxidize the iron. The dish containing the residue, consisting of the ash of the filter paper and the ferric oxid, was weighed. From the weight of the ferric oxid the amount of hydrocyanic acid was calculated, and from this the percentage of hydrocyanic acid in the dry sorghum. The percentage of moisture in the different samples of sorghum was found by drying at 105° C.

No effort was made to determine whether or not this method would give accurate results, but it was thought that the results would be as accurate as those obtained in using Knight's method (*5*); the colorimetric method of Viehoever and Johns (*9*) and of Francis and Connell (*4*) could not be used, since a colorimeter was not available. Moreover, it was thought that this method would give results sufficiently accurate for comparative purposes.

In order to determine whether or not a part of the hydrocyanic acid was lost in the drying, sample 3 was cut and digested as described above, and then some of it was allowed to dry in the laboratory for 2½ days and was then placed on top of a Freas oven overnight. The temperature on top of this oven was 33° C. A part of this sample was used for the determination of hydrocyanic acid and another for the determination of the water still present.

In order to determine the effect of the rate of drying on the loss of hydrocyanic acid, if any, another part of sample 3 was dried at 50° C. within 24 hours. The results obtained here are given in Table I under experiments 1, 2, and 3.

In order to determine the effect of the presence of glucose and maltose on the liberation of the hydrocyanic acid, portions of sample 2 were digested in a solution containing 1 per cent of dextrose and 1 per cent of maltose. The results of two trials here are given in Table I under experiment 5.

To determine whether or not a part of the hydrocyanic acid existed in the form of nonglucosidic acid, as has been claimed by Willaman (*10*), portions of samples 2 and 3 were digested, and 200 cc. distilled off. Then 50 cc. of 10 per cent sulphuric acid were added to the digestion mixture, which had a volume of about 800 cc., and another 100 cc. was distilled. This last distillate was evaporated, and tests were made for hydrocyanic acid, with negative results, as indicated in Table I under experiment 6.

It has been pointed out by Auld (*1*) that with most feedstuffs digestive conditions would be unfavorable for the action of the enzym on the

glucosid, but he points out very correctly that a slight acidity is the best condition for the action of the enzym and that this acid condition might be found in the paunch of ruminants when certain feedstuffs are used. This being true, it was important to know the acidity of the juice of the sorghum. The juice was pressed from portions of samples 3 and 4, and portions of this juice were diluted very much and titrated with sodium hydroxide, phenolphthalein being used as the indicator. These results are given in Table I under experiment 7. The acids present in samples 1 and 2 were not determined, but it is quite probable that all the acids present were nonvolatile and remained in the dry sorghum. Several other determinations of lesser importance were made, these results being also given in Table I.

TABLE I.—*Results of experiments showing the cyanogenesis in dry and fresh sorghum under various conditions*

Experiment No.	Description of experiment.	Percentage of hydrocyanic acid found.
1a	Sample 3. Digested in water at 40° C. for 1 hour	0.0221
1b	Sample 3. Same as 1a	.0228
2a	Sample 3. Dried for 2½ days in the laboratory, then dried for 16 hours at 33° C	.0050
2b	Sample No. 3. Same as 2a	.0069
3a	Sample 3. Dried at 50° C. for 24 hours. Sample thoroughly dried	.0109
3b	Sample 3. Same as 3a	.0070
4a	Sample 2, plus emulsion digested at 40° for 2 hours	.0222
4b	Sample 2. Same as 4a, except no emulsion present	.0130
4c	Sample 1. Same as 4a	.0514
4d	Sample 1. Same as 4b	.0450
5a	Sample 2 in a solution of 1 per cent of dextrose and 1 per cent of maltose	.0038
5b	Sample 2. Same as 5a	.0059
6a	Sample 3. Digested for 1 hour at 40° C., distilled off 200 cc., then added 10 cc. of 10 per cent sulphuric acid and distilled another 100 cc. Test for hydrocyanic acid in last distillate	None.
6b	Sample 2. Same treatment as 6a	None.
7a	Titrated juice from sample 3. Normality found to be *0.013 N*	
7b	Titrated juice from sample 4. Normality equals *0.0507 N*	
8	Sample 4. Digested for 2 hours at 40° C	.0087
9a	Sample 2. Digested for 2 hours in 5 per cent tartaric acid	.0119
9b	Sample 1. Treatment same as 9a	.0018
9c	Sample 3. Treatment same as 9a, except sample was ground under 5 per cent tartaric acid	None.
10a	Sample 3. Kept at 40° C. for 15 minutes	.0281
10b	Sample 2. Same treatment as 10a	.0177
11a	Sample 2. Treated with water at 80° C. and kept at this temperature for 1 hour	.049
11b	Sample 2. Treated with water at 90° C. and kept at this temperature for 1 hour	.0040
12a	Sample 2. Kept in air bath at 70° C. for 1 hour	.0124
12b	Sample 2. Kept in air bath at 115° C. for 1 hour and 30 minutes	.0087
13a	Sample 1. Kept for 1 hour in *N/100* sodium hydroxid made acid and distilled	.0220
13b	Sample 3. Kept in solution of sodium hydroxid (*N/100*) for 1 hour	.0051
13c	Sample 3. Treatment same as 13b, except the sodium hydroxid was *N/50*, and the solution was made slightly acid with tartaric acid and kept for 1 hour at 40° C	.0136

DISCUSSION OF RESULTS

Any discussion of the experimental results will necessarily be of the nature of a summary. A comparison of the percentage of hydrocyanic acid found in experiments 1a and 1b with those in 2a and 2b shows that approximately three-fourths of the acid is set free in the process of drying. This goes to confirm the common belief that sorghum is safe for feeding after it has been dried. At the same time the results show that not all of the hydrocyanic acid disappears. A comparison of experiments 2a and 2b with 3a and 3b shows that the rapidity with which the sorghum is dried determines the percentage of the hydrocynaic acid that is retained by it. This point is of considerable importance in Oklahoma on account of the fact that farmers quite frequently cut their sorghum during drouths after it has been partially dried while yet standing; and after it is cut, being already partly dry, it dries very quickly. Under such conditions a large percentage of the hydrocyanic acid would be retained in the fodder. Sample 1 was cut under such conditions.

A glance at experiments 4, a, b, c, and d, will show that the enzym which is present in the sorghum is still active and that the addition of emulsin does not cause the hydrocyanic acid to be liberated in greater quantity.

A comparison of the amount of hydrocyanic acid found in experiments 5a and 5b with experiment 4 shows that the addition of such a small quantity as 1 per cent of dextrose and 1 per cent of maltose seems to hold back or prevent the liberation of about three-fourths of the acid. This is an extremely important result from the practical standpoint. Dextrose and maltose were selected because of the fact that they are formed by the action of the pytalin on the starches in the paunch. This retention of the hydrocyanic acid in the presence of these sugars may be assumed to be due either to a reaction between the sugars (aldehydes) and hydrocyanic acid or to a lessening of the activity of the enzym by the sugars. This would lead to the suggestion that in case there is any doubt about the poisonous nature of the sorghum one should feed some concentrate before feeding the sorghum. In this way a considerable quantity of dextrose and maltose would be produced by the salivary digestion and would tend to prevent liberation of the hydrocyanic acid of the sorghum which is fed afterwards. At the time of this experiment I had not read Peters, Slade, and Avery's work (*6*), in which they showed that it was possible to give very large doses of hydrocyanic acid without any harmful effects provided at the same time a somewhat proportionate amount of dextrose was given.

It has been claimed by Willaman, as has already been stated, that the hydrocyanic acid exists in the sorghum in two forms—glucosidic and nonglucosidic. It seems natural to suppose that the nonglucosidic acid would not be liberated under the conditions that existed in my

work, that is the digestion was carried on in a very faintly acid solution, the acidity being due to the acids present in the sorghum. If this assumption is made, the results in experiments 6a and 6b seem to show that no nonglucosidic hydrocyanic acid exists in the sorghum. Of course it is possible that the nonglucosidic acid was distilled over in the first 100 cc., but this would not be in harmony with Willaman's supposition that it is the hydrocyanic acid that is obtained in 5 per cent of tartaric distillation that causes the poisonous effect and which is a nonglucosidic acid. Furthermore, the fact that no acid was found in the distillate from sample 3 when it was ground under 5 per cent of tartaric acid and distilled from the acid solution shows that nonglucosidic acid is not present.

The results in experiments 9a and 9b show that when a dry sorghum is digested with 5 per cent of tartaric acid a considerable percentage of the hydrocyanic acid is not liberated, and when this is taken in connection with experiment 9c, one may conclude that the water was absorbed by the dry substance more rapidly than was the acid and that some hydrocyanic acid was set free before the acid came in contact with the glucosid.

It is seen from the acid concentrations as found in experiments 7a and 7b that the contents of the paunch would be faintly acid in reaction when the green or the dry sorghum is eaten. It might be argued that the acidity would be neutralized by the alkalinity of the saliva; but when the acidity as found here is compared with the alkalinity of the saliva it is seen that, when the alkalinity of the saliva is taken into account, and assuming a normal saliva flow, the contents of the paunch would still be slightly acid, a condition most favorable for enzym action. This acid condition would exist until rumination talkes place, when the acid would be neutralized.

A comparison of the results of experiment 10a and 10b with that of experiment 1 shows that all the hydrocyanic acid is liberated within the first 15 minutes of the digestion.

Willaman and West (*11*) and other investigators have shown that hydrocyanic acid gradually disappears from sorghum during its growth, so that but little is present in the mature plant. It was thought that this might not be true if large amounts of the acid had been formed as a consequence of dry weather, in the sorghum at some stage of growth. Sample 4 had been stunted by dry weather, but it is seen from experiment 8 that nearly all of the hydrocyanic acid had disappeared. The percentage of hydrocyanic acid found in this sample should be compared with that of sample 1, which was doubtless greater still before the sample was dried.

No discussion is needed of the experiments 11 to 13b, inclusive. The reason for making experiment 13c, was that it was thought that possibly as shown in my work, the enzym is rendered practically inactive by dilute alkaline solution, and it might be that the hydrocyanic acid would not

be liberated on this account in the paunch; but when it later entered the true stomach, where the solution would become slightly acid, the hydrocyanic acid would be set free. The result under experiment 13c seems to show that this is true. Digestion first with *N/100* sodium hydroxid, as shown in experiment 13b, prevents the liberation of the hydrocyanic acid. Certainly, then, digestion with *N/50* sodium hydroxid would prevent the liberation of this acid, and yet it is seen by acidifying this solution and allowing further digestion that more than one-half of the hydrocyanic acid was given off.

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EFFECT OF CERTAIN COMPOUNDS OF BARIUM AND STRONTIUM ON THE GROWTH OF PLANTS

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INTRODUCTION

Although it has been known for more than a century that plants are able to extract appreciable amounts of the relatively insoluble compounds of barium contained in soils, very little scientific investigation has been made to determine whether or not the compounds of this element have any specific function in the vegetable economy. Because compounds of barium are poisonous when taken into the animal body, there appears to be a general impression that these compounds would exert a similar influence upon plants.

In a former investigation [1] the writer has shown that small amounts of barium can be readily detected and determined quantitatively in the ash of tobacco, corn, potatoes, and a number of other plants grown under normal conditions in the field. Since soils contain only very small amounts of barium, necessarily in the form of relatively insoluble compounds, it is a question of considerable scientific interest how and why it is that notable amounts of this element are absorbed and apparently assimilated by plants, under normal conditions of growth. The object of the present investigation was to determine the effect of some of the well-known compounds of barium and of the closely related metal, strontium, upon the growth of plants.

EXPERIMENTAL WORK

Preliminary experiments consisted in growing plants in nutrient solutions to which were added certain compounds of barium, soluble as well as insoluble. It soon developed that plants could be grown in a nutrient solution containing moderate amounts of barium nitrate or carbonate, whereas an equal amount of the chlorid or sulphate produced a decided toxic effect. After having determined that the plants selected for the water-culture experiments were tolerant of barium carbonate and nitrate, it was decided that a method more nearly approximating the normal conditions under which plants are grown would be a better procedure. Accordingly the plan was adopted of growing the plants in barium-free sand contained in earthenware pots to which the necessary basal plant-food ration could be added, together with the desired compounds of barium.

[1] McHARGUE, J. S. THE OCCURRENCE OF BARIUM IN TOBACCO AND OTHER PLANTS. *In* Jour. Amer. Chem. Soc., v. 35, no. 6, p. 826–834. 1913.

Journal of Agricultural Research,
Washington, D. C.
rh

Vol. XVI, No. 7
Feb. 17, 1919
Key No. Ky.-7

98355°—19——2

COWPEAS

In the first series of experiments twelve 1-gallon earthenware jars were filled with a clean quartz sand that contained very little plant food. To each of the pots of sand was added the following basal plant-food ration: 10 gm. of calcium carbonate, 10 gm. of tricalcium phosphate, 5 gm. of magnesium carbonate, 4 gm. of potassium nitrate, 2 gm. of potassium chlorid, and 2 gm. of sodium thiosulphate. In addition to this plant food, varying amounts of barium carbonate were added to all the pots except the first, which served as a check against any other one pot in this series of experiments. Cowpeas (*Vigna sinensis*) were planted in the sand in the pots, and during the time the plants were making their growth the sand was kept moist with clear hydrant water. Previous to starting the experiment, 100 liters of hydrant water were evaporated to dryness and the residue thus obtained examined for barium, but none was found. In another experment 25 liters of water flowing from the drain tiles on the Experiment Station farm were collected and evaporated. The residue thus obtained was examined for barium compounds, but none were found.

The cowpea plants were allowed to grow until they were about 10 to 12 inches tall. They were then taken up in such manner as to preserve the roots intact, and the adhering sand was washed off as well as possible. The photograph reproduced in Plate 24, A, was taken two weeks after planting; that shown as figure B, after the plants were removed from the sand in which they grew.

Table I shows the amount of barium carbonate added to each pot and also the weight of the air-dry plants that grew in each of the pots.

TABLE I.—*Effect of barium carbonate upon the growth of cowpeas—First series*

Pot. No.	Quantity of barium carbonate added to soil.	Weight of 10 air-dried plants.	Pot No.	Quantity of barium carbonate. added to soil.	Weight of 10 air-dried plants.
	Gm.	*Gm.*		*Gm.*	*Gm.*
1 (control)	None.	9. 15	7	5	11. 40
2	0. 5	12. 00	8	6	10. 90
3	1	11. 20	9	8	11. 15
4	2	10. 15	10	10	10. 80
5	3	9. 50	11 [a]	5	.....
6	4	10. 55	12	5	11. 65

[a] This pot received no calcium carbonate, and all the plants died.

From the results obtained in this experiment it is to be observed that there were appreciable increases in the yields of all the plants grown in the presence of barium carbonate and calcium carbonate over that of the control pot. In the absence of calcium carbonate,

however, the action of the barium carbonate was strongly toxic, as shown by the failure of the plants in pot 11.

The effect of the barium compound upon the growth of the cowpeas is more strikingly shown in Plate 24. In figure A the pot on the right is the control, which received no barium compound. The pot in the middle received the same plant food as the control and 10 gm. of barium carbonate in addition. The pot on the left received 5 gm. of barium carbonate, but no calcium carbonate. It received the same amount of tricalcium phosphate as the other pots. One object in mind in this experiment was to ascertain whether there would be a tendency on the part of the plants in this pot to substitute barium for calcium in their growth. The peas germinated and came through the sand, made a stunted growth for a few weeks, and then died. The difference in the growth of the plants in the pot in the center and the one on the left shows very strikingly the toxic effect of barium carbonate in the absence of calcium carbonate. This experiment affords a very striking example in the plants in the center pot of the protective action of calcium carbonate on plants when grown in the presence of a toxic substance.

Figure B of Plate 24 shows the effect of barium carbonate on the growth of the roots of the cowpea plants grown in pots 1, 2, and 8; the plants on the right were the control and received no barium carbonate, the plants in the center received 0.5 gm. of barium carbonate, and plants on the left received 6 gm. of barium carbonate. It will be observed that the plants which grew in the presence of barium carbonate have made a markedly increased root growth over the control. It is also to be borne in mind that the plants in the center received only 0.5 gm. of the barium compound, whereas the ones on the left received 6 gm. or 12 times as much as the former, thus indicating that a very small amount of barium carbonate produces as great effect on the root growth as much larger amounts.

The compounds of strontium have many chemical and physical properties similar to those of barium and calcium. It was thought that a few comparative experiments showing what effect like compounds of barium and strontium might have upon the growth of plants would be of some interest in this connection. Therefore in the series of experiments that follow plants have been grown in the presence of both barium and strontium compounds and compared with similar plants grown in the presence of calcium compounds.

OATS

In a second series of experiments oats (*Avena sativa*) were grown in sand under conditions similar to those in which the cowpeas were grown in the previous experiment, with the same basal plant food ration as before.

After the young oat plants had reached a height of about 10 inches they were thinned to the same number of plants in each pot and as near equal in size as possible. The oats were brought to maturity and harvested and, after thoroughly air-drying, the grain was threshed and the weights of the air-dry grain and straw produced in each pot were determined. There were two control pots in this experiment, and the average weight of the grain and the straw from these two pots was taken as a check against other pots receiving compounds of barium or strontium in this series.

TABLE II.—*Effect of certain barium and strontium compounds upon the growth of oats—Second series*

Pot No. and treatment.	Weight of grain.	Gain or loss in weight of grain over control.	Weight of straw.	Gain or loss in weight of straw over control.
	Gm.	*Gm.*	*Gm.*	
Control pot 1	19. 25	.....	39. 25	.....
Control pot 2	17. 40	.....	34. 50	.....
Average	18. 33	.....	36. 88	.....
Pot 3+2 gm. of barium carbonate	18. 50	+0. 17	40. 00	+ 3. 12
Pot 4+5 gm. of barium carbonate	20. 65	+2. 32	44. 75	+ 7. 87
Pot 5+2 gm. of strontium carbonate	18. 40	+ .07	37. 15	+ .27
Pot 6+5 gm. of strontium carbonate	21. 10	+2. 77	46. 25	+ 9. 37
Pot 7+2 gm. of barium carbonate and 2 gm. of strontium carbonate	16. 50	−1. 83	37. 25	+ .37
Pot 8+5 gm. of barium sulphate	11. 00	−7. 33	24. 75	−12. 13

TABLE III.—*Comparison of weight and percentage of nitrogen, phosphorus, and potassium per pot—Second series*

Pot No. and treatment.	Nitrogen.		Phosphorus.		Potassium.	
	Gm.	*Per cent.*	*Gm.*	*Per cent.*	*Gm.*	*Per cent.*
Control	0. 3217	1. 755	0. 0752	0. 41	0. 0522	.285
Pot 3+2 gm. of barium carbonate	.3608	1. 95	.0777	.42	.0518	.28
Pot 4+5 gm. of barium carbonate	.4120	2. 00	.0847	.41	.0599	.29
Pot 5+2 gm of strontium carbonate	.3367	1. 83	.0773	.42	.0534	.29
Pot 6+5 gm. of strontium carbonate	.4579	2. 17	.0886	.42	.0570	.27
Pot 7+2 gm. of barium carbonate+2 gm. of strontium carbonate	.2739	1. 66	.0644	.39	.0429	.26
Pot 8+5 gm. of barium sulphate	.1727	1. 57	.0418	.38	.0286	.26

In Table II is given the amount of barium or strontium compounds added to each pot, and the air-dry weights of the grain and the straw produced in each of the experiments. Table III gives a partial analysis

of the grain showing the important constituents contained in the grain produced in each experiment.

Both barium carbonate and strontium carbonate have increased the percentage of nitrogen as well as the total weight of nitrogen when applied separately. Applied together, there is diminution. Barium sulphate has diminished both percentage and total weight of nitrogen.

The weights of grain and straw produced in this series of experiments show increased yields in all pots receiving either barium carbonate or strontium carbonate separately. The pot in which there was a mixture of the two carbonates shows a decrease in the yield of grain, while the yield of the straw is practically the same as that of the control. In the pot receiving barium sulphate there is a very marked decrease in both the grain and the straw, which shows the toxic effect of this compound when compared with the carbonate.

The analysis of the grain produced in each of the pots for nitrogen, phosphorus, and potassium shows a slightly greater content of each of these elements where there was an increase in the yields of the plants over that contained in the control. The last two pots in the series, No. 7 and 8, show a marked falling off in their nitrogen, phosphorus, and potassium content when compared with the controls and the other pots in this series. The maximum increase in protein—that is, $N \times 6.25$—amounts to 2.60 per cent over that of the control, and the grain containing it was grown in the presence of 5 gm. of strontium carbonate. The next highest result was obtained where 5 gm. of barium carbonate were present. The phosphorus and potassium content appears to be less affected by barium and strontium compounds than does nitrogen.

SPRING WHEAT

In the third set of experiments spring wheat (*Triticum aestivum*) was sown in pots containing sand to which was added the same basal plant-food ration as that added to the pots in the experiments with cowpeas and oats. The quantities of barium and strontium compounds added are given in Table IV. In addition to the barium and strontium carbonates certain pots received small amounts of what was claimed to be a very active commercial radio-active fertilizer. The amount of this material added to each pot is given in Table IV and is in accordance with the recommendations of the company marketing this material. After the young plants had reached a height of 6 or 8 inches, they were thinned to the same number of plants in each pot and were brought to a state approaching maturity. Unfortunately, when the wheat grains were in the dough stage, a careless attendant left the ventilators of the greenhouse open over Sunday and the sparrows came in and consumed a part of the grain growing in each pot; hence, the results for the grain in this series of experiments were discarded. The straw was allowed to ripen

and was harvested. When thoroughly air-dried it was weighed. The results appear in Table IV.

TABLE IV.—*Effect of barium carbonate and strontium carbonate on the growth of wheat—Third series*

Pot No. and treatment.	Weight of dry straw.		Gain or loss over control.
	Observed.	Average.	
	Gm.	*Gm.*	*Gm.*
Pot 1 (control), no barium added	41.15	40.95	.....
Pot 2 (control), no barium added	40.75		
Pot 3+2 gm. of barium carbonate	34.25	34.25	−6.70
Pot 4+2 gm. of barium carbonate	34.25		
Pot 5+2 gm. of strontium carbonate	38.50	38.12	−2.83
Pot 6+2 gm. of strontium carbonate	37.75		
Pot 7+2 gm. of barium carbonate+2 gm. of strontium carbonate	32.25	34.00	−6.95
Pot 8+2 gm. of barium carbonate+2 gm. of strontium carbonate	35.75		
Pot 9+2 gm. of barium carbonate+0.7 gm. of radio-active material	37.75	37.25	−3.70
Pot 10+2 gm. of barium carbonate+0.7 of gm. radio-active material	36.75		
Pot 11+0.7 gm. of radio-active material alone	37.35	36.67	−4.28
Pot 12+0.7 gm. of radio-active material alone	36.00		

The results in this series of experiments show a loss in the weight of the straw over the average weight of the straw in the control pots; however, the greater loss occurs in the barium pots. The strontium pots show a loss of one-half of that of the barium pots.

The radio-active fertilizer, when used alone or in combination with barium carbonate, did not affect the yield of the straw greatly, the yield in each case being less than that of the control.

WINTER WHEAT

In a fourth series of experiments winter wneat was sown in pots of sand containing the same basal plant-food ration as in previous experiments. Strontium nitrate was the compound subjected to experimentation in this series. The amounts added are given in Table V, which also gives the yields and average weight of the grains of wheat produced in in each experiment.

The results obtained in this series of experiments show abnormal yields in both grain and straw which probably are due to the large amounts of nitrate radical present rather than to the strontium ion, since strontium carbonate has in no instance given such marked increase in yields.

TABLE V.—*Effect of strontium nitrate on the growth of winter wheat—Fourth series*

Pot No. and treatment.	Number of grains per pot.	Weight of grain per pot.	Average weight per grain.	Weight of straw.
		Gm.	*Gm.*	*Gm.*
Pot 1 (control), no strontium nitrate	372	8.8872	0.0239	34.50
Pot 2 (control), no strontium nitrate	292	7.7650	.0266	27.50
Average	332	8.3261	.0252	31.00
Pot 3+5 gm. of strontium nitrate	369	11.9065	.0323	44.50
Pot 4+5 gm. of strontium nitrate	555	19.6108	.0353	52.00
Average	462	15.7586	.0338	48.25
Pot 5+10 gm. of strontium nitrate	561	17.6505	.03146	62.00

The results obtained in the analysis of the grain for nitrogen, protein, phosphorus, and potassium are interesting (Table VI). It will be seen that with the addition of strontium nitrate there is a decided increase in the nitrogen content of the grain and a decrease in the phosphorus, while the potassium content remains practically constant.

TABLE VI.—*Percentage of nitrogen, protein, phosphorus, and potassium contained in the grain produced in each of the foregoing experiments*

Pot No.	Nitrogen.	Protein (N×6.25).	Phosphorus.	Potassium.
Pot 1 (control)	1.68	10.50	0.36	0.18
Pot 2 (control)	1.71	10.68	.35	.24
Average	1.695	10.59	.355	.21
Pot 3	2.77	17.31	.22	.22
Pot 4	2.73	17.06	.23	.18
Pot 5	3.00	18.75	.23	.21

Having obtained unusual results in the yields and in the nitrogen content of the grain in the previous series of experiments, another, the fifth, series of pot experiments, similar to the ones that have been described, was carried out. This series was planned as a further check on the effect of strontium carbonate on the growth and the nitrogen content of wheat. The amount of strontium carbonate added to each pot and the yields of grain and straw produced are given in Table VII.

The seeds in pots 9 and 10 came up, and the stunted plants struggled for existence for the greater part of the time the other plants in this series were making a complete growth. The plants never reached a height of more than 10 inches, thus showing that strontium can not replace calcium in the growth of plants.

TABLE VII.—*Effect of strontium carbonate on the growth of wheat—Fifth series*

Pot No. and treatment.	Weight of grain.	Weight of straw.	Gain or loss in—	
			Grain.	Straw.
	Gm.	*Gm.*	*Gm.*	*Gm.*
Pot 1 (control)	10. 00	36. 50	.....	.....
Pot 2 (control)	9. 00	34. 50	.....	.....
Average	9. 50	35. 50	.....	.....
Pot 3+5 gm. of strontium carbonate	9. 00	42. 00	.....	.....
Pot 4+5 gm. of strontium carbonate	10. 50	40. 00	.....	.....
Average	9. 75	41. 00	+0. 25	+5. 50
Pot 5+10 gm. of strontium carbonate	11. 50	39. 50	.....	.....
Pot 6+10 gm. of strontium carbonate	13. 00	40. 00	.....	.....
Average	12. 25	39. 75	+2. 75	+4. 25
Pot 7+20 gm. of strontium carbonate	9. 50	36. 00	.....	.....
Pot 8+20 gm. of strontium carbonate	10. 00	35. 50	.....	.....
Average	9. 75	35. 75	+ . 25	+ . 25
Pot 9+10 gm. of strontium carbonate, no calcium carbonate	None.	(a)		
Pot 10+10 gm. of strontium carbonate, no calcium carbonate				

[a] Not weighed.

The results in the fifth series of experiments agree very closely with those of the other experiments in which strontium carbonate was used, both with respect to yields and the results obtained in the analyses of the grain (Table VIII). They also show conclusively that the increased yields obtained in the fourth series of experiments in which strontium nitrate was used were due to the greater amounts of nitrate being present which was assimilated and thus produced grains of wheat that contained 8 per cent more protein than was found in the control experiments, which showed a protein content equivalent to that of wheat grown under normal conditions.

The last two experiments in the fifth series show conclusively that strontium will not replace calcium in the growth of plants. They also show, however, that strontium carbonate in the absence of calcium carbonate is apparently less toxic towards plants than barium carbonate in the absence of calcium carbonate. It will be recalled that in the first series of experiments, in which an attempt was made to grow cowpeas in the presence of barium carbonate without calcium carbonate, all the plants died soon after coming through the sand, whereas in the case of the wheat plants in the presence of strontium carbonate and the absence of calcium carbonate the plants did not die soon after they

were up, but maintained a struggling existence during the greater part of the time other plants in the series were making a normal growth, thus indicating that strontium carbonate is less toxic in the absence of calcium carbonate than barium carbonate.

TABLE VIII.—*Percentage of nitrogen, protein, phosphorus, and potassium in the grain grown in the pots in the fifth series of experiments*

Pot No. and treatment.	Nitrogen.	Protein (N×6.25).	Phosphorus.	Potassium.
Pot 1 (control)	1.69	10.56	0.31	0.20
Pot 2 (control)	1.66	10.38	.27	.18
Average	1.68	10.47	.29	.19
Pot 3	1.68	10.50	.33	.19
Pot 4	1.79	11.10	.35	.19
Average	1.74	10.80	.34	.19
Pot 5	1.61	10.06	.27	.19
Pot 6	1.69	10.56	.31	.17
Average	1.65	10.31	.29	.18
Pot 7	1.64	10.25	.27	.19
Pot 8	1.68	10.50	.31	.20
Average	1.66	10.38	.29	.19

CORN

In a sixth series of experiments corn plants (*Zea mays*) were grown in pots of sand containing the usual basal plant-food ration. To these pots were added varying amounts of barium and strontium compounds as shown in Table IX. Three corn plants were allowed to grow in each pot until the plants had tasseled and bloomed. As was to be expected, the corn plants were dwarfed on account of greenhouse conditions, the plants reaching a height of about 3 feet. After making their maximum growth the stalks were cut from the roots at the top of the sand. The fodder was stripped from the stalks. The roots were taken up and washed as free from adhering sand as possible. The different parts into which the plants were divided were kept separate, and after thoroughly air-drying, the weight of each of the parts determined and from thence the air-dry weights of the entire plants were computed. These results are given in Table IX.

The results in this series of experiments agree in a general way with those obtained in previous experiments with wheat and oats, where the same compounds of barium and strontium have been applied in equal quantities and under similar conditions.

TABLE IX.—*Air-dry weights of the corn plants in each of the experiments*

Pot No. and treatment.	Air-dry weights.				Gain or loss in weight when compared with the controls.			
	Roots.	Stalks.	Fodder.	Entire plants.	Roots.	Stalks.	Fodder.	Entire plant.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Pot 1 (control)	11.50	13.25	17.60	42.35	.....	.....	.....	.....
Pot 2 (control)	8.00	10.30	15.35	33.65	.....	.....	.....	.....
Average	9.75	11.77	16.47	38.00	.....	.....	.....	.....
Pot 3+2 gm. of barium carbonate	10.65	14.25	18.75	43.65	.....	.....	.....	.....
Pot 4+2 gm. of barium carbonate	12.10	8.25	19.30	39.65	.....	.....	.....	.....
Average	11.38	11.25	19.02	41.65	+1.63	−0.52	+2.55	+3.65
Pot 5+2 gm. of strontium carbonate	13.00	15.80	23.50	52.30	.....	.....	.....	.....
Pot 6+2 gm. of strontium carbonate	11.75	14.50	20.80	47.05	.....	.....	.....	.....
Average	12.38	15.15	22.15	49.68	+2.63	+3.38	+5.68	+11.68
Pot 7+2 gm. of barium carbonate and 2 gm. of strontium carbonate	14.75	13.00	20.25	48.00	+5.00	+1.23	+3.78	+10.00
Pot 8+2 gm. of barium nitrate	11.50	13.25	18.75	43.50	+1.75	+1.48	+2.28	+ 5.50
Pot 9+5 gm. of barium sulphate	10.75	8.00	14.25	33.00	+1.00	−3.77	−2.22	− 5.00
Pot 10+2 gm. of barium chlorid	11.50	7.50	19.25	38.25	+1.75	−4.27	+2.78	+ 0.25
Pot 11+5 gm. of barium carbonate	13.5	12.00	20.00	45.50	+3.75	+ .23	+3.53	+ 7.50
Pot 12+5 gm. of strontium carbonate	13.2	14.5	20.7	48.40	+3.45	+2.73	+4.23	+10.40

It will be noted again that the maximum increase in yield occurred in the presence of strontium carbonate, while equal amounts of barium carbonate produced only a very slight increase in the yield of the entire plants.

It is interesting to note that all of the roots in the corn experiment show some increase in yield over that of the controls, while in the weight for the stalks there are an equal number of minus and plus differences. In the weights of the fodders there is only one experiment in which the fodder produced is less than the control. In the weights for the entire plants barium sulphate gave a very decided negative difference. Both the sulphate and the chlorid reduced the yields in the stalks very decidedly.

TABLE X.—*Analyses of the corn fodder from the preceding experiments*

[The results are expressed as percentage of the moisture-free substance]

Pot No. and treatment.	Crude ash.	Insoluble residue (silica, etc.).	Ferric oxid (Fe_2O_3).	Lime (CaO).	Magnesia (MgO).	Potash (K_2O).
Composite sample from pots 1 and 2 (control)	9.71	1.15	[a]0.44	1.03	c.92	3.22
Pots 3 and 4+2 gm. of barium carbonate	9.66	.84	.39	.89	.49	3.88
Pots 5 and 6+2 gm. of strontium carbonate	9.15	.91	.60	.92	.56	3.50
Pot 7+2 gm. of barium carbonate and 2 gm. of strontium carbonate	10.20	1.01	.44	1.00	.67	3.92
Pot 8+2 gm. of barium nitrate	9.15	1.14	.26	1.14	.67	3.61
Pot 9+5 gm. of barium sulphate	10.54	1.38	.58	1.34	.82	3.96
Pot 10+2 gm. of barium chlorid	11.07	1.21	.73	1.30	.58	3.91
Pot 11+5 gm. of barium carbonate	12.10	1.23	.83	.90	.60	4.34
Pot 12+5 gm. of strontium carbonate	9.53	.78	.14	.90	.50	3.86

[a] The irregularities occuring in the iron determinations are probably due to iron-oxid scales which may have come from the paint on the mill hopper, as some such scales were observed in some of the samples.

TABLE X.—*Analyses of the corn fodder from the preceding experiments*—Continued.

Pot No. and treatment.	Soda (Na_2O).	Barium sulphate ($BaSO_4$).	Strontium sulphate ($SrSO_4$).	Phosphorus pentoxid (P_2O_5).	Nitrogen (N).	Protein (N×6.25).
Composite sample from pots 1 and 2 (control)	0.86	.....	.....	0.22	1.16	7.22
Pots 3 and 4+2 gm. of barium carbonate.	.72	.059	.....	.225	.96	6.01
Pots 5 and 6+2 gm. of strontium carbonate	.43	.....	.15	.24	.81	5.115
Pot 7+2 gm. of barium carbonate and 2 gm. of strontium carbonate	.18	.035	.16	.27	.93	5.85
Pot 8+2 gm. of barium nitrate	.29	.044	.....	.27	1.47	9.17
Pot 9+5 gm. of barium sulphate	.24	.....	.....	.32	1.44	9.00
Pot 10+2 gm. of barium chlorid	.24	Tr.	.....	.28	.98	6.06
Pot 11+5 gm. of barium carbonate	.23	.02	.....	.28	1.63	10.02
Pot 12+5 gm. of strontium carbonate	.37	.....	.20	.27	.93	5.84

The results of the analyses of the fodders that were produced in each of the experiments show no very striking differences in the mineral composition of the fodders in any of the experiments (Table X).

It will be observed that only very small amounts of barium and strontium have been taken up by the plants growing in the presence of compounds of each of these elements.

SOYBEAN

In Plate 24, C, are shown four jars of soybean (*Soja max*) plants that were grown in cultural solutions. The plants in the jars on each end have been grown in a cultural solution containing no barium compound, whereas the two pots in the center have been grown in a similar solution containing barium nitrate. The plants in the two jars in the center received their sulphur from a solution containing taurin, while the plants in the end jars received their sulphur from a solution of magnesium and potassium sulphates. The differences to be observed in the growth of the two sets of plants is attributed to the presence of the barium nitrate which appears to have retarded the growth of the roots, stems, and foliage of the two sets of plants in the center.

When the very small amounts of the barium compounds that occur in the soil and the relatively insoluble state in which they occur are taken into consideration, one is led to wonder how it is that plants are able to extract even as much barium as can be determined in the ash of normal plants. Since no barium was found by careful examination of the residue from the evaporation of 25 liters of water flowing from a tile drain on the Station farm, although the presence of barium in the soil of the area drained had been proved by extracting 0.0508 gm. of barium sulphate from the hydrochloric-acid solution from 500 gm. of an average sample representing the first foot of soil from this field, it would appear that the roots of plants do not obtain their barium from the percolating soil water, but rather by some kind of selective

action upon the soil particles. A determination of total barium in the soil of another field near by gave 0.08 per cent of barium sulphate, obtained by decomposing the soil with hydrofluoric and sulphuric acids.

CONCLUSIONS

From the results obtained in the different series of experiments in this investigation the following conclusions are drawn.

(1) Barium compounds in the absence of calcium carbonate are poisonous to plants; but barium carbonate in the presence of an excess of calcium carbonate apparently exerts a distinct stimulating influence on the growth of the plants studied.

(2) There is no tendency for barium to replace calcium in the growth of plants when calcium carbonate is omitted from a plant-food ration under the conditions of these experiments.

(3) Strontium compounds have in most instances given larger increased yields than barium compounds.

(4) Strontium carbonate can not be substituted for calcium carbonate in the growth of plants under the conditions studied, though strontium carbonate is less toxic to plants in the absence of calcium carbonate than barium carbonate.

(5) Neither barium nor strontium compounds can be looked upon as important plant foods, although the presence of small amounts of the carbonate of each of these elements has given increased yields that are noteworthy in most instances.

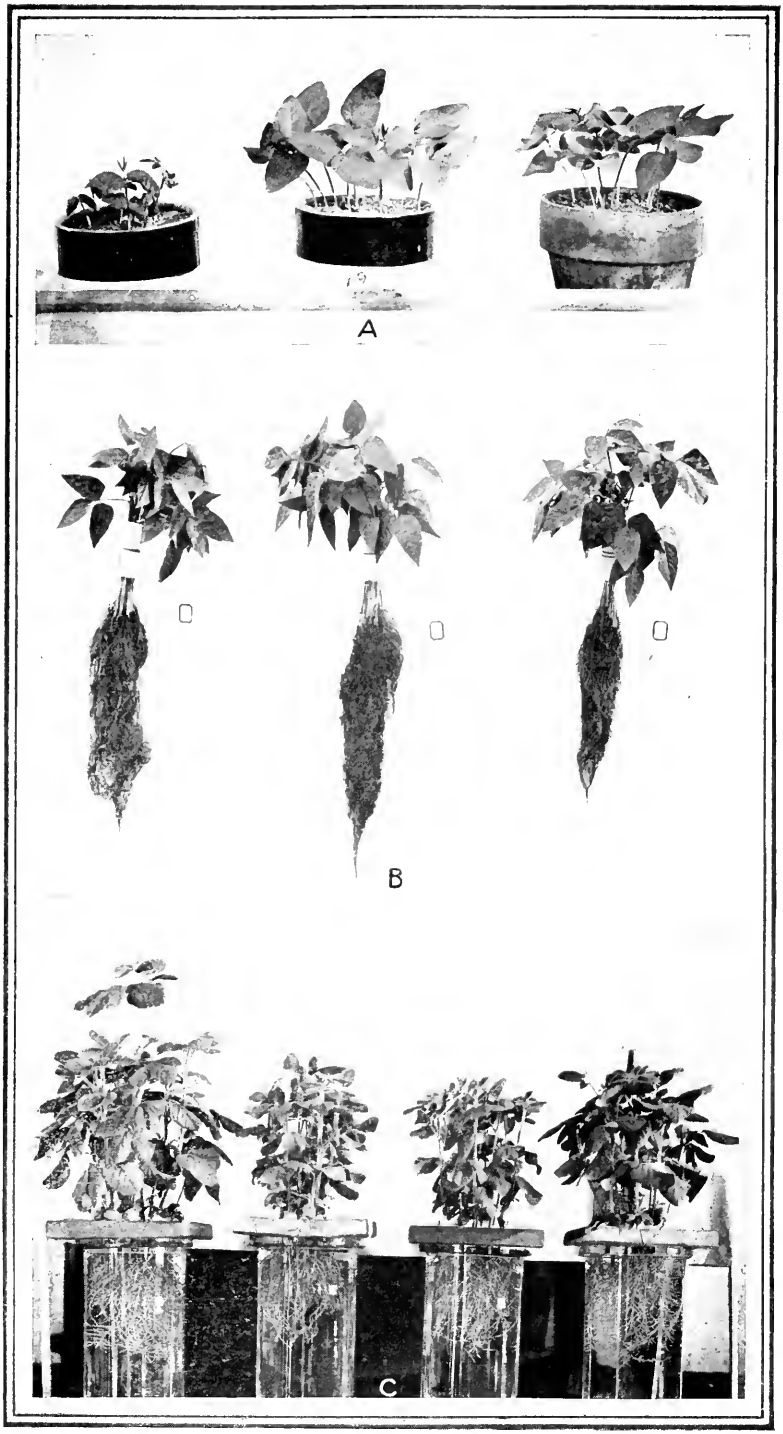
(6) Barium and strontium carbonates accelerated the growth of the roots of such plants as were examined.

(7) Increasing the amount of strontium nitrate gave a corresponding increase in the nitrogen content of wheat.

(8) No barium compounds were found in the residue obtained upon evaporating 25 liters of drainage water collected from the drain tiles on the Station farm, which would indicate that the barium found in plants is taken up in place by the plant roots.

PLATE 24

A.—Effect of barium on the growth of cowpeas with and without calcium carbonate.
B.—Stimulating effect of barium on root growth of cowpeas.
C.—Effect of barium on the growth of soybeans.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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from State Experiment Stations should be addressed to J. G. Lipman, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., FEBRUARY 24, 1919 No. 8

APPLE-SCALD

By CHARLES BROOKS, *Pathologist*, and J. S. COOLEY and D. F. FISHER, *Assistant Pathologists, Fruit-Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The present paper gives a report of studies on the nature and control of apple-scald, including experiments upon the relation of orchard and storage conditions to the development of the disease. The literature upon the subject of apple-scald and the apparatus [1] and methods [2] used in these experiments have been rather fully reported in earlier publications.

RELATION OF CHARACTER OF FRUIT TO SCALD DEVELOPMENT

MATURITY

It is generally recognized that immature apples (*Malus sylvestris*) scald worse than mature ones.[3] A striking example of the fact was obtained in storage experiments at Wenatchee, Wash., in the winter of 1917–18. The apples of the different pickings were from the same trees and were approximately alike in every respect except in maturity. The first picking of the various varieties was made when the ground color of the fruit was very green and when the red varieties had developed but a slight blush, the second picking when the ground color was beginning to show yellow and most of the apples of the red varieties had become deeply colored. The apples were stored in commercial box packages. One or more boxes of fruit were used under each storage condition of every experiment. The final notes for the Rome Beauty and Stayman Winesap were taken on March 19 and for the other varieties on March 12. The Rome Beauty and Stayman Winesap were allowed to stand in cellar storage five days before the notes were taken, and the other varieties were held in a laboratory at 20° C. for four days before note taking. The results are given in Table I.

In all cases there was less scald on the well-colored than on the poorly colored fruit, and in most cases fruit picked at the proper maturity was almost entirely free from scald.

[1] BROOKS, Charles, and COOLEY, J. S. TEMPERATURE RELATIONS OF APPLE-ROT FUNGI. *In* Jour. Agr. Research, v. 8, no. 4, p. 139–164, 25 fig., 3 pl. 1917.

[2] ——— ——— EFFECT OF TEMPERATURE, AERATION, AND HUMIDITY ON JONATHAN-SPOT AND SCALD OF APPLES IN STORAGE. *In* Jour. Agr. Research, v. 11, no. 7, p. 287–318, 23 fig., pl. 32–33, 1917. Literature cited, p. 316–317.

[3] RAMSAY, H. J., MCKAY, A. W., MARKELL, E. L., and BIRD, H. S. THE HANDLING AND STORAGE OF APPLES IN THE PACIFIC NORTHWEST. U. S. Dept. Agr. Bul. 587, 32 p., 7 col. pl. 1917.

TABLE I.—*Effect of maturity of fruit upon susceptibility to apple-scald*

Experiment No.	Variety.	Storage condition.	Date of picking.	Maturity at time of picking.	Percentage showing scald March, 1918.
1	Rome Beauty	Cold storage continously.	Oct. 8	Rather immature	20
			Oct. 24	Well colored	0
2	do	Cold storage 2 months, then in cellar storage.	Oct. 8	Rather immature.	40
			Oct. 24	Well colored	0
3	do	Cold storage till January 25, then in cellar storage.	Oct. 8	Rather immature.	95
			Oct. 24	Well colored	5
4	Stayman Winesap.	Cold storage continuously.	Oct. 9	Rather immature.	65
			Oct. 25	Highly colored	0
5	do	Cold storage 2 months, then in cellar storage.	Oct. 9	Rather immature.	30
			Oct. 25	Highly colored	0
6	do	Cold storage till January 25, then in cellar storage.	Oct. 9	Rather immature.	90
			Oct. 25	Highly colored	5
7	Baldwin	Cold storage till February 11, then in cellar storage.	Sept. 27	Rather immature.	50
			Oct. 30	Well colored	0
8	Bellflower	do	Sept. 22	Immature	90
			Oct. 2	Well colored	40
			Oct. 1	Rather overripe	30
9	Grimes, heavily irrigated.	do	Sept. 22	Color green	95
			Oct. 2	Color yellowing	95
			Oct. 12	Rather overripe	30
10	Grimes, lightly irrigated.	do	Sept. 22	Color green	50
			Oct. 2	Color yellowing	25
			Oct. 12	Rather overripe	10

Scald prevention on eastern-grown fruit is apparently not as readily accomplished. In an earlier report [1] the writers found little contrast in susceptibility to scald on eastern Grimes apples, a part of which were picked on August 11, when the fruit was quite green, a part August 28, when the apples were in condition for commercial picking, and a part on September 21, when the fruit was quite yellow. This experiment was repeated in 1917 on Grimes apples from Vienna, Virginia. The first picking was made on August 21, when the ground color of the fruit was green, and a second picking on September 14, when the apples were becoming yellow and were at their best for commercial picking. The fruit was stored in moist chambers at various temperatures [2] in special storage boxes at Washington, D. C., and notes taken at various times on the development of scald. The results are given in figure 1.

[1] BROOKS, Charles, and COOLEY, J. S. EFFECT OF TEMPERATURE, AERATION, AND HUMIDITY ON JONATHAN-SPOT AND SCALD OF APPLES IN STORAGE. In Jour. Agr. Research, v. 11, no. 7, p. 287-318, 23 fig., pl. 32-33. 1917. Literature cited, p. 316-317.

[2] Temperature equivalents: 0° C.=32° F.; 5° C.=41° F.; 15° C.=59° F.; 20° C.=68° F.; 25° C.=77° F.; 30° C.=86° F.

The results at the higher temperature are in agreement with those of the preceding year, indicating little difference in susceptibility to scald between the well-colored and poorly colored Grimes, but at 0° the latter finally developed about twice as much scald as the former, giving further evidence of the greater susceptibility of green fruit when held at temperatures low enough to prevent ripening.

EASTERN AND WESTERN FRUIT

Experiments were made to determine the relative susceptibility to scald of eastern and western Grimes of practically the same degree of maturity. The western Grimes were shipped from Wenatchee, Wash., to Washington, D. C., in well-iced pony refrigerators. The eastern apples were placed in storage the day after picking. Part of the western apples were from trees that had been heavily irrigated. These apples were large, most of them 3 to 3¼ inches in diameter. The remainder of the western apples were from trees that had received very little irrigation and were small, ranging from 2¼ to 2½ inches in diameter. Most of the eastern apples were from 2½ to 2¾ inches in diameter. All of the apples were held in moist chambers in the storage boxes already mentioned. Ten apples were used in each test. The results are shown in figure 2.

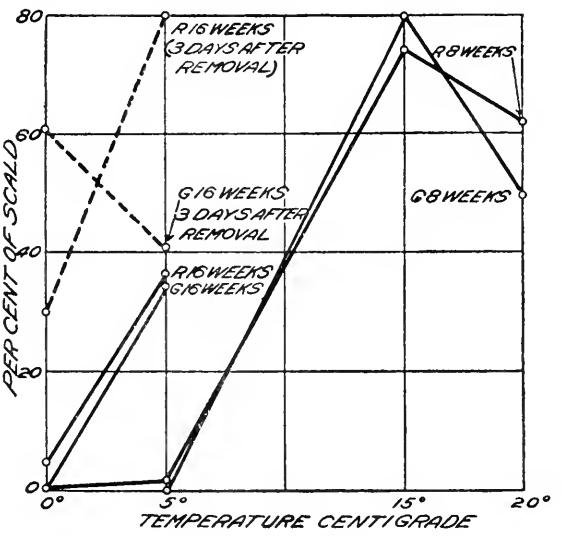

FIG. 1.—Graphs showing the effect of maturity upon susceptibility of Grimes apples to scald. The graphs show the percentage of scald on the two lots of apples at the ends of 8 and 16 weeks, respectively. The ones marked "G" give the results on the fruit picked on August 21 and those marked "R" the results on the fruit picked September 14. The dotted lines show the percentage of scald after the apples had been removed from storage and had stood in the laboratory at a temperature of 20° C. for three days.

The heavily irrigated western apples were somewhat less susceptible and the lightly irrigated ones much less susceptible to the disease than the eastern apples. While the eastern and western fruit did not receive exactly the same treatment, the results as a whole indicate that western Grimes apples from a region of intense sunlight are less susceptible to scald than eastern apples of practically the same maturity.

EFFECT OF IRRIGATION UPON SUSCEPTIBILITY TO SCALD

A study of figure 2 gives some evidence that apples from heavily irrigated trees are more susceptible to scald than those from lightly irrigated ones. In another experiment heavily and lightly irrigated Grimes apples of the same maturity were held in commercial cold storage at Wenatchee, Wash., till February 11, and then in cellar storage till March 12. The apples were stored in commercial box packages, two or more boxes of fruit being used under each storage condition of each experiment. The results are given in Table II.

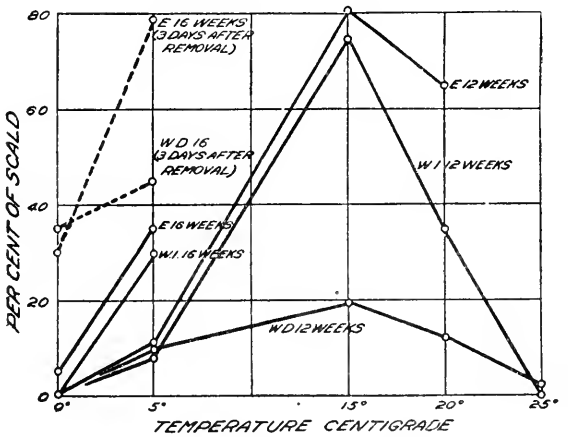

FIG. 2.—Graphs showing the relative susceptibility to scald of eastern and western Grimes apples. The graphs show the percentage of scald at the end of the given week. Those marked "E" give the results on the eastern apples, those marked "WI" the results on the heavily irrigated western apples, and those marked "WD" the results on the western apples receiving practically no irrigation. The dotted lines show the percentage of scald after the apples had been removed from storage and had stood in the laboratory at a temperature of 20° C. for three days.

Under all of the different conditions of picking the heavily irrigated apples showed a greater susceptibility to scald than the lightly irrigated ones, the former averaging about twice as much scald as the latter. There were more large apples in the heavily irrigated lots than in the lightly irrigated ones, but this fact seemed to have but little influence upon the results, as heavily irrigated apples of a particular size were scalded worse than lightly irrigated ones of the same size.

TABLE II.—*Influence of irrigation upon susceptibility of apples to scald*

Experiment No.	Variety and condition.	Percentage scald.	
		Heavily irrigated.	Lightly irrigated.
1	Rather poorly colored Grimes apples picked on September 22..	95	50
2	Fairly well colored Grimes apples picked on October 2.....	95	25
3	Rather overripe Grimes apples picked on October 12.....	30	10

RELATION OF TEMPERATURE TO APPLE-SCALD

A rather full discussion of the relation of temperature to apple-scald has already been published by the writers.[1] The results given in figures 3 to 10, inclusive, of this paper confirm and extend the statements of the earlier report. As in the earlier experiments, the apples were stored in moist chambers and ten or more apples were used in each test. The experiment was started on August 21.

A study of the figures shows that the optimum for scald production is approached at 15° C. and the maximum apparently reached at 25°. With all of the different varieties tested scald failed to develop at either 25° or 30°. This fact gives evidence that scald is not purely an old-age characteristic and that it can not be mainly due to the accumulation of carbon dioxid, for both the aging and respiring of the fruit are accelerated by these high temperatures.

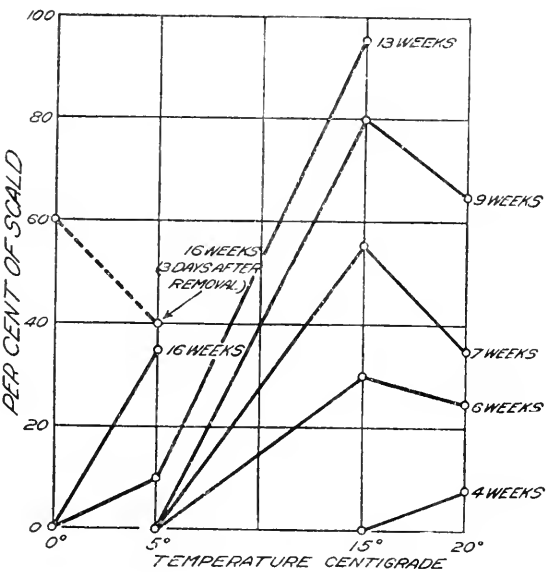

FIG. 3.—Graphs showing the effects of temperature on apple-scald at the end of 4, 6, 7, 9, 13, and 16 weeks. The dotted graph shows the amount of scald that was evident after removal from storage at the end of the given week and holding the apples at 20° C. for 3 days. The apples were Grimes from Vienna, Va., picked on August 20.

A comparison of the results at 15° and 20° shows that in several cases (fig. 3, 4, 5) there was a shift in the optimum as the experiment advanced. Scald appeared first at 20° and for several weeks was worse at this temperature than at 15°, but later became decidedly worse at the lower temperature.

A particular degree of scald usually developed 8 to 12 weeks later at 5° than at 15° and several weeks later at 0° than at 5°. Scald was worse at 5° than at 0° in all cases except with the very green Grimes (fig. 3) and fairly green Rome Beauty (fig. 8).

In all of the above temperature experiments the apples were placed in moist chambers. The relative humidity was practically 100 per cent, the carbon dioxid from 1 to 3 per cent, and there was practically no air movement. In all of the various experiments and at all of the different temperatures similar apples were held in open containers in an atmosphere having less than 0.5 per cent of carbon dioxid, a relative humidity

[1] BROOKS, Charles, and COOLEY, J. S. EFFECT OF TEMPERATURE, AERATION, AND HUMIDITY ON JONATHAN-SPOT AND SCALD OF APPLES IN STORAGE. *In* Jour. Agr. Research, v. 11, no. 7, p. 287–318, 23 fig., pl. 32–33. 1917. Literature cited, p. 316–317.

of 85 to 95 per cent and an air movement of 1/8 to 1/4 mile per hour. With two exceptions, both in the case of very green apples at 0°, the fruit held in the open remained free from scald to the end of the various experiments, indicating that other factors are even more important than temperature, and that a solution of the problem of scald prevention should be found either in the composition or rate of movement of the storage air.

INFLUENCE OF AIR COMPOSITION UPON APPLE-SCALD

HUMIDITY

It did not seem probable that a reduction in the relative humidity from 100 per cent to an average of 90 per cent as mentioned above could be responsible for the complete elimination of scald, but it seemed desirable to have further tests on the point. Table III gives the results of various experiments in which the humidity was varied, with little or no change in temperature or other environmental factors. In cases where it was necessary to introduce outside air this was brought to the temperature of the fruit before being allowed to come in contact with it.

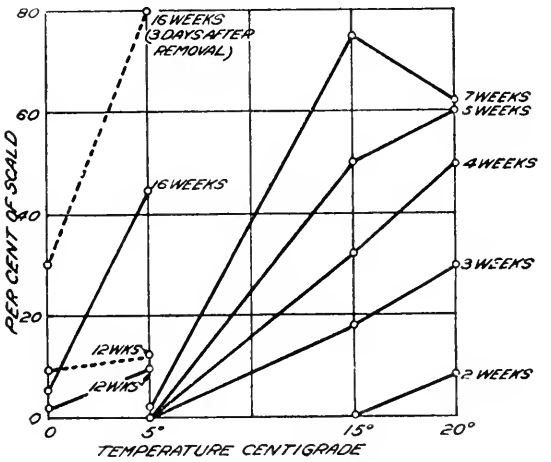

FIG. 4.—Graphs showing the effects of temperature on apple-scald at the end of 2, 3, 4, 5, 7, 12, and 16 weeks. The dotted graphs show the amount of scald that was evident after removal from storage at the end of the given week and holding the apples at 20° C. for 3 days. The apples were from the same trees as those of figure 3, but were picked 24 days later, on September 13, and the experiment was started on September 14.

A study of the results from the various experiments reported in Table III shows that, in general, only about half as much scald developed on apples exposed to dry air as on those exposed to saturated air. It does not seem, however, that high humidity can be the primary cause of the disease, for in no case was scald entirely prevented by dryness, and in every case where the air was stirred, the disease was practically eliminated, even in the presence of the highest humidities. The withering of the apples in the dry air makes this method of partial prevention an impractical one, and the fact that the disease can be prevented without drying naturally raises the question whether the beneficial effects noted from the use of moisture-absorbing agents may not be at least partly due to their power to absorb some substance other than water, or to the fact that the evaporation of the water assists in the elimination of some distinctly harmful substance.

TABLE III.—*Influence of humidity upon apple-scald*

Experiment No.	Treatment.	Percentage of scalds.				
		Grimes at 15° C.	York Imperial.		Arkansas.	
			At 2½° C	At 0° C.	At 2½°C.	at 0° C.
A1	Air saturated, passed slowly over wet filter paper and through wash bottles of water	50	.....	.....	.....	.....
A2	Same as No. 1, but air-dry, bubbled slowly through sulphuric acid and glycerin	23	.....	.....	.....	.....
A3	Same as No. 1, but air in motion at rate of about ⅛ mile per hour	0	.....	.....	.....	.....
B1	Air saturated, wet filter paper in bottom of container and the entering air bubbled through water	10	.....	.....	.....	.....
B2	Air-dry, calcium chlorid in bottom of container and the entering air passed over calcium chlorid and bubbled through glycerin	5	.....	.....	.....	.....
B3	Apples in open, exposed to air having a relative humidity of 85 to 95 per cent and a constant movement of ⅛ to ¼ mile per hour	0	.....	.....	.....	.....
C1	Saturated air, renewed slowly	.....	20	4	60	55
C2	Same as No 1, but air-dry	.....	8	8	32	25
C3	Same as No. 1, but with air circulated by air pump	.....	0	.....	7	.....
C4	Same as No. 2, but air renewed 10 to 15 times more rapidly	.....	0	.....	5	.....
C5	Apples in open package	.....	0	0	0	.....

EXPERIMENT A.—Grimes apples of the lot described in the legend for figure 5 were stored at 15° C. for 7 weeks. In all three cases cited the carbon dioxid of the storage air was held at 3 to 4 per cent by the constant introduction of air containing 3 per cent of this gas. The rate of renewal was such that a volume of air equal to that in the container was carried in once in every 24 hours. In No. 3, however, in addition to this slight air movement, the air was kept in constant motion at a rate somewhat less than ⅛ mile per hour by means of a closed-circuit connection with an air pump.

EXPERIMENT B.—Grimes apples of the same lot as mentioned in Experiment A were used, but they were held in commercial cold storage for eight weeks before the experiment was started. The contrasted results were obtained after three weeks' storage at 15° C. With Nos. 1 and 2 the apples were held in unsealed jars and fresh air drawn in rapidly for about 10 minutes every second day, the volume of air carried through being several times that of the container.

EXPERIMENT C.—The apples used in this experiment were York Imperial and Arkansas of tle same lots as described in the legends for figures 9 and 10, respectively. The contrasted results were obtained after 20 weeks of storage at the temperatures given. With No. 1 the apples were held in a closed container and fresh air introduced continuously at a rate such that a volume of air equal to that in the container was carried in once in 24 hours. The air was kept saturated with moisture by means of wet filter paper in the bottom of the jar and by bubbling the entering air through water. No. 2 was handled exactly as No. 1 with the exception that calcium chlorid was placed in the bottom of the jar and the entering air was passed over calcium chlorid and bubbled through glycerin. No. 3 was treated the same as No. 1 with the exception that the air of the container was kept in motion at a rate somewhat less than ⅛ mile per hour by means of a closed-circuit connection with a rotary air pump. No. 4 had practically the same degree of dryness as No. 2 (evidenced by the withering of the apples), but this was secured by drawing in fresh air at a rate 10 to 15 times faster than in the case of No. 2 without using any drying agent either in the container or with the entering air. With No. 5 the apples were held in the open, exposed to air moving at the rate of ⅛ to ¼ mile per hour, and having a relative humidity at 2½° C. of 70 to 80 per cent and at 0° C. of 85 to 90 per cent.

CARBON DIOXID

Perhaps the most natural assumption in regard to apple-scald is to consider carbon dioxid as the responsible agent. The writers have made numerous experiments looking to the establishment of this hypothesis, but these have resulted in proof that carbon dioxid is not a causal agency in the production of the disease. The nature and results of the experiments are shown in figure 11.

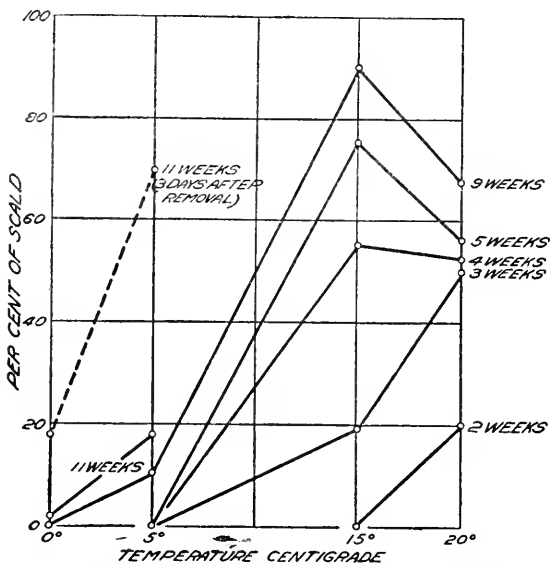

FIG. 5.—Graphs showing the effects of temperature on apple-scald at the end of 2, 3, 4, 5, 9, and 11 weeks. The dotted graph shows the amount of scald that was evident after removal from storage at the end of the given week and holding the apples at 20° C. for 3 days. The apples were of the same lot as those of figure 4 and were picked on the same day, but were held in commercial cold storage from September 14 to October 15, and were transferred to the storage boxes for the above experiment on the latter date. The weeks of storage as given on the graphs are counted from October 15, the time of starting the special experiment.

The results give conclusive evidence that an accumulation of carbon dioxid is not responsible for the production of scald. In 2 of the 10 different tests the amount of scald was slightly decreased with a decrease in amount of carbon dioxid, but with the other 8 it was either unchanged or decidedly increased. The results as a whole indicate that, while an accumulation of the gas may sometimes be an accompaniment of apple-scald, carbon dioxid itself really tends to prevent rather than aggravate the development of the disease.

TABLE IV.—*Effect of storing apples in carbon dioxid for short periods on development of scald*

Experiment No.	Treatment.	Percentage of scald.
A1 . .	Apples in 100 per cent of carbon dioxid at 30° C. for 3 days, then at 15° in moist chamber for 8 weeks.	0
A2 . .	Apples in moist chamber at 15° continuously for the above-mentioned periods without carbon-dioxid treatment.	15
B1 . .	Apples in 100 per cent of carbon dioxid at 15° C. for 6 days, then in moist chamber at 15° for 11 weeks.	0
B2 . .	Same as B1, but continuously in moist chamber without carbon-dioxid treatment.	40

Apples stored in higher percentages of carbon dioxid than those given in figure 11 soon developed a disagreeable alcoholic taste, but if they were removed after a few days' exposure to the gas they were found to have but little, if any, of this objectionable taste and to have developed a decided resistance to scald. The results of two experiments of this sort are given in Table IV. The apples were Grimes of the lot described in the legend of figure 7.

In the first experiment the taste of the apples was slightly affected by the exposure to carbon dioxid, but in the second experiment the apples exposed to carbon dioxid had as good a taste as those held continuously in moist chambers. In both cases the treated apples developed color in storage very much more slowly than the untreated. It would seem from the results that the carbon dioxid had produced a very decided inhibition of the activities of the apple, and thus led to scald prevention.

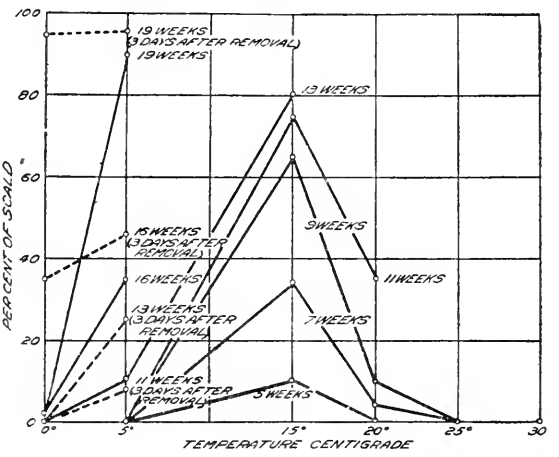

FIG. 6.—Graphs showing the effect of temperature on appel-scald at the end of 5, 7, 9, 11, 13, 16, and 19 weeks. The dotted graphs show the amount of scald that was evident after removal from storage at the end of the given week and holding the apples at 20° C. for 3 days. The apples were from heavily irrigated Grimes trees at Wenatchee, Wash. They were picked on September 27, shipped to Washington, D. C., in iced pony refrigerators, and the experiment started on October 3.

OXYGEN

In the experiments with carbon dioxid reported in figure 11 the oxygen of the air was usually slightly below normal, but with the exception of (c) and (d) under B there was never a deficiency of more than 1 or 2 per cent. With (c) the average carbon dioxid content of the air after the first two weeks of the experiment was 6 per cent and the average oxygen content 8 per cent, while with (d) the average carbon-dioxid content for the period was 14.5 per cent and the average oxygen content 6.9 per cent. In both cases any pressure or suction was prevented by a small U-tube opening closed with oil. The results given in figure 11 give no evidence that these deficiencies in oxygen had any tendency either to increase or decrease the amount of scald. The apples seemed normal at the end of the experiment, with the exception of a very faint trace of an aromatic musty flavor.

In an earlier paper [1] experiments were reported indicating that slight increases (increasing the percentage from 21 to 24) in the oxygen content

[1] BROOKS, Charles, and COOLEY, J. S. EFFECT OF TEMPERATURE, AERATION, AND HUMIDITY ON JONATHAN-SPOT AND SCALD OF APPLES IN STORAGE. *In* Jour. Agr. Research, v. 11, no. 7, p. 287-318, 23 fig., pl. 32-33. 1917. Literature cited, p. 316-317.

of the air also had no appreciable effect upon the development of scald. During the past season this test was repeated, using higher percentages of oxygen. The air was slowly renewed in the manner described in Table III and was not stirred. The temperature was 15° C., except E, which was 0° C. Five apples were used in each test. The results are given in Table V.

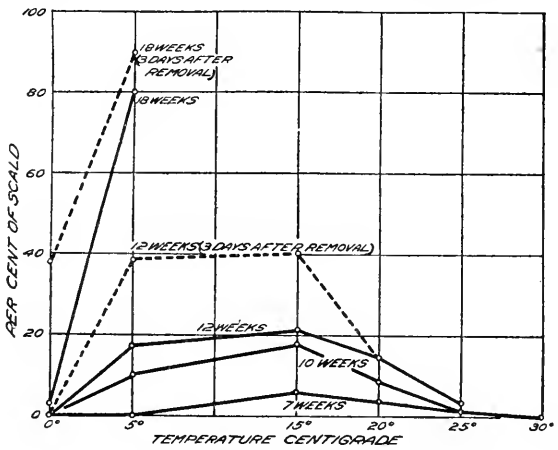

FIG. 7.—Graphs showing the effect of temperature on apple-scald at the end of 7, 10, 12, and 18 weeks. The dotted graphs show the amount of scald that was evident after removal from storage at the end of the given weekand holding the apples at 20° C. for 3 days. The apples were from very lightly irrigated Grimes trees at Wenatchee, Wash. They were picked on October 3, shipped to Washington, D. C., in iced pony refrigerators, and the experiment started on October 9.

The results have not been consistent. An increase in the percentage of oxygen in the air gave a decided decrease in the amount of scald on Newtown, Pippin, and Rome Beauty apples that had been held several months in cold storage before the experiment was started (B and C), but failed to do so on Grimes apples that were exposed in similar atmospheres from the beginning of their storage life (A, D, and E). As a whole, the results are in decided contrast with the uniformly beneficial effects reported later as resulting from air circulation.

TABLE V.—*Influence of increase in oxygen upon the development of apple-scald*

Experiment No.	Variety and treatment.	Composition of air supplied.	Percentage of scald.
A	Grimes apples of lot described in legend for figure 3. Results after 8 weeks.	4 per cent of carbon dioxid, 28 per cent of oxygen.	45
		4 per cent of carbon dioxid, oxygen normal.	40
		Normal air (21 per cent of oxygen). .	65
B	Newtown Pippin from Hood River, Oreg. In cold storage till Jan. 26. Experiment started on this date and ended 12 weeks later.	32 per cent of oxygen.	10
		Normal air (21 per cent of oxygen). .	80
C	Rome Beauty from Vienna, Va. In cold storage till Jan. 26. Experiment started on this date and ended 12 weeks later.	....do	5
			65
D	Grimes apples from Vienna, Va., picked Aug. 26, 1918. The experiment was started Aug. 27 and the results obtained after 8 weeks.	....do	45
			50
E	Same as D, but at 0° C. and the results obtained after 16 weeks.	....do	35
			38

AIR MOVEMENT AS A PREVENTIVE OF SCALD

AIR CIRCULATION

The value of aeration in the prevention of apple-scald was pointed out by the writers in an earlier report[1] and the previous data of the present paper have given confirmatory evidence on this point. Other experiments were made in which the effect of air circulation apart from air renewal was tested. The air movement was obtained by connecting the containers to rotary air pumps. A continuous circulation in a closed circuit was thus secured. The rate of movement was less than ⅛ and probably more than $\frac{1}{16}$ mile per hour. (See Table III for methods used in keeping the composition of the air constant.) The results are given in Table VI.

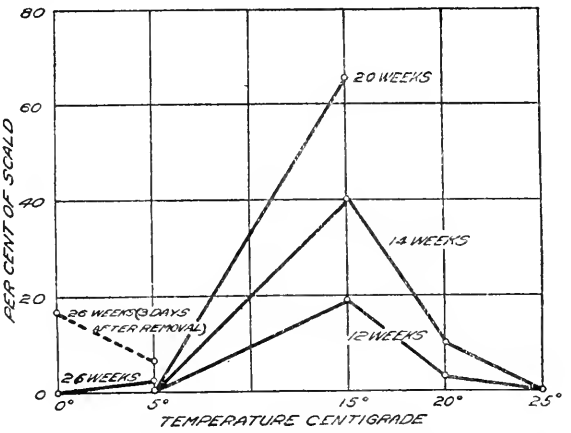

FIG. 8.—Graphs showing the effect of temperature on apple-scald at the end of 12, 14, 20, and 26 weeks. The dotted graph shows the amount of scald that was evident after removal from storage at the end of the given week and holding the fruit at 20° C. for 3 days. The appels were Rome Beauty from Vienna, Va. They were picked on October 3, and the experiment was started October 4.

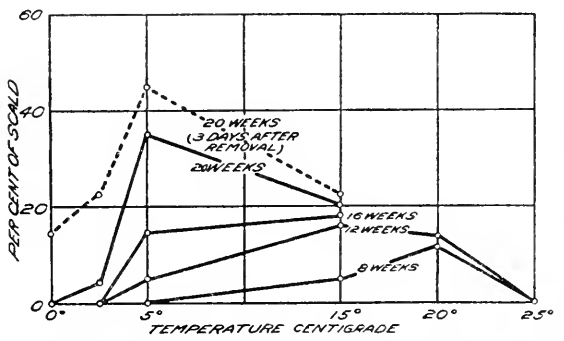

FIG. 9.—Graphs showing the effect of temperature on apple-scald at the end of 8, 12, 16, and 20 weeks. The dotted graph shows the amount of scald that was evident after removal from storage at the end of the given week and holding the fruit at 20° C. for 3 days. The apples were York Imperial from Vienna, Va. They were picked and packed in barrels on October 2 and placed in commercial cold storage the following day. They were removed from storage on December 4 and the above experiment started the same day.

The results are striking. With all of the different air compositions and all of the different lots of apples a gentle air movement practically eliminated scald, while similar apples held in stagnant air of like composition became badly scalded. The writers attribute the beneficial effects of the air movement to the breaking up of layers of dead air adjacent to the skin of the apple, thus disseminating harmful gases that might otherwise hang in the tissues of the apple.

[1] BROOKS, Charles, and COOLEY, J. S. EFFECT OF TEMPERATURE, AERATION, AND HUMIDITY ON JONATHAN-SPOT AND SCALD OF APPLES IN STORAGE. *In* Jour. Agr. Research, v. 11, no. 7, p. 287–318, 23 fig., pl. 32–33. 1917. Literature cited, p. 316–317.

TABLE VI.—*Effect of air movement upon apple-scald*

Experiment No.	Variety and treatment.	Treatment.	Percentage of scald.		
			Grimes.	Arkansas.	York Imperial.
A	Grimes apples of lot described in figure 3 after 8 weeks' storage at 15° C.	With 4 per cent of carbon dioxid; air stirred.	0	.....	.....
		With 4 per cent of carbon dioxid; air not stirred.	40	.....	.....
		With 2 per cent of carbon dioxid; air stirred.	2	.....	.....
		With 2 per cent of carbon dioxid; air not stirred.	60	.....	.....
		Air; air (0.2 per per cent of carbon dioxid and 0.5 per cent of oxygen) stirred.	3	.....	.....
		Air; air not stirred.....	65	.....	.....
		Apples in open; air movement ⅛ to ¼ mile per hour.	2	.....	.....
		Apples in moist chamber; air not stirred.	80	.....	.....
B	York Imperial and Arkansas apples of lots described in figures 9 and 10. Results obtained after 20 weeks' storage at 2.5° C.	Apples in open; air movement ⅛ to ¼ mile per hour.	.....	3	0
		Apples in moist chamber; air not stirred.	.....	80	20
		With 3 per cent of carbon dioxid; air stirred.	.....	15	0
		With 3 per cent of carbon dioxid; air not stirred.	.....	80	5
		Normal air; air stirred.....	.....	7	0
		Normal air; air not stirred.....	.....	60	15
C	Grimes apples of lot described in figure 5 after 6 weeks at 15° C.	Apples in open; air movement ⅛ to ¼ mile per hour.	0	.....	.....
		Apples in moist chamber; air not stirred.	75	.....	.....
		With 6 per cent of carbon dioxid; air stirred.	5	.....	.....
		With 6 per cent of carbon dioxid; air not stirred.	18	.....	.....
		With 3 per cent of carbon dioxid; air stirred.	1	.....	.....
		With 3 per cent of carbon dioxid; air not stirred.	50	.....	.....
		Air (air with 14.5 per cent of carbon dioxid, 6 per cent of oxygen); stirred.	1	.....	.....
		Air (air with 0.6 per cent of carbon dioxid, 0.8 per cent of oxygen); stirred.	3	.....	.....
		Air not stirred; 1 per cent of carbon dioxid.	60	.....	.....

INTERMITTENT AERATION

In the experiments reported in Table VI the air was kept in constant circulation, but this continuity of the movement is apparenty not essential to the prevention of apple-scald. In an earlier paper [1] experi-

[1] BROOKS, Charles, and COOLEY, J. S. EFFECT OF TEMPERATURE, AERATION, AND HUMIDITY ON JONATHAN-SPOT AND SCALD OF APPLES IN STORAGE. *In* Jour. Agr. Research, v. 11, no. 7, p. 287-318, 23 fig. pl. 32-33, 1917. Literature cited, p. 316-317.

ments were reported in which scald was entirely prevented on Grimes apples at 15° C. by drawing the air rapidly through the container for a 10-minute period three times a week. During the past season this experiment was repeated but at 5° C. and with York Imperial and Arkansas apples. The amount of apple-scald developed after 20 weeks is given in Table VII.

TABLE VII.—*Effect of intermittent aeration on apple-scald*

Experiment No.	Treatment.	Percentage of scald.	
		Arkansas.	York Imperial.
1	Air renewed continuously, a volume of fresh air equal to that in the container being passed in every 24 hours.....	85	20
2	Air renewal every second day, a volume of fresh air equal to twice that in the container being passed in in 10 minutes.....	50	30

The control of apple-scald was not as complete as in the earlier experiments, but a limited amount of air had a greater beneficial effect when passed into the container within a period of 10 minutes than when distributed over a period of 48 hours.

With a slow rate of air movement the amount of scald was found to vary with the length of time the movement was continued, as shown in the results given in Table VIII.

TABLE VIII.—*Relation of period of aeration to the development of apple-scald*

Experiment No.	Variety and previous treatment.	Treatment.	Percentage of scald.
A1	Grimes apples of same lot as described in legend for figure 6 after 9 weeks' storage at 15° C.	In moist chamber continuously....	65
A2	.....do.....	In open continuously; air movement 1/8 to 1/4 miles per hour.	0
A3	.....do.....	Alternately 2 weeks with same treatment as No. 1, then 2 weeks as No. 2.	8
B1	Grimes apples of same lot as described in legend for figure 7 after 18 weeks' storage at 0° C.	In moist chamber continuously..	37
B2	.....do.....	In open continuously; air movement 1/8 to 1/4 miles per hour.	0
B3	.....do.....	Same treatment as No. 1 for 8 weeks, then same as No. 2.	20

The rate of air movement was probably but little above the minimum for scald prevention, and the results show a direct relation between the duration of the movement and the amount of apple-scald.

TEMPERATURE CHANGES AS A MEANS OF AERATION

Apples held at a constant temperature have usually scalded worse than those exposed to temperature changes, the beneficial effects of the fluctuating temperature apparently being due to the aeration of the apple tissue thus obtained. Experimental results on this point are given in Table IX.

All of the apples were held in moist chambers and were therefore poorly aerated.

TABLE IX.—*Influence of temperature changes upon apple scald*

Experiment No.	Variety and previous treatment.	Temperature.	Percentage of scald.
1	Rather immature Grimes apples of lot described in figure 3.	At 5° C. continuously for 16 weeks..	38
		At 0° C. continuously for 16 weeks..	05
		At 5° C. for 4 weeks; then at 0° C. for 12 weeks.	6
		At 0° C. for 4 weeks; then at 5° C. for 12 weeks.	20
		At 5° C. for 8 weeks; then at 0° C. for 8 weeks.	10
		At 0° C. for 8 weeks; then at 5° C. for 8 weeks.	60
2	Grimes apples of lot described in figure 7.	At 5° C. continuously for 12 weeks..	40
		At 0° C. continuously for 12 weeks..	0
		At 5° C. for 8 weeks; then at 0° C. 4 weeks.	5
		At 0° C. for 8 weeks; then at 5° C. 4 weeks.	35
3	Grimes apples of lot described in figure 6 after 9 weeks of storage.	At 15° C. continuously.....	65
		Alternately 2 days each at 5° C. and 25° C. (Average temperature, 15° C.)	25

The results in experiments 1 and 2 indicate that the amount of scald was decreased by moving the apples from one temperature to another during the first weeks of storage. The apples were given no aeration at the time of change, and a probable explanation of the beneficial effects resulting from shifting the apples from one temperature to another seems to be some sort of renovation of intercellular air conditions accompanying the temperature changes in the tissues. The apples stored first at 5° and then at 0° had less scald and were of better quality than those stored first at 0° and then at 5° or than those stored continuously at 0°.

In experiment 3 the apples were held part of the time at a temperature (25° C.) that has been proved to be too high for the production of scald. Other experiments have been made in which aeration has been combined with high temperature with decidedly beneficial results in scald prevention. In an earlier paper [1] an instance was reported in which scald

[1] BROOKS, Charles, and COOLEY, J. S. EFFECT OF TEMPERATURE, AERATION, AND HUMIDITY ON JONATHAN-SPOT AND SCALD OF APPLES IN STORAGE. *In* Jour. Agr. Research, v. 11, no. 7, p. 287–318, 23 fig., pl. 32–33. 1917. Literature cited, p. 316–317.

was prevented by one thorough aeration for 24 hours at 20° C. and then by storing at 5° C. In the winter of 1917–18 some striking results on this point were again obtained.

Of two lots of Grimes apples from Wenatchee, Wash., picked from the same trees and placed in commercial cold storage at the same time, one lot consisting of 10 boxes was brought out twice for aeration and note-taking, remaining at a temperature of 20° C., the first time for 4 hours (after 5 weeks' storage), and the second time for 48 hours (after 10 weeks' storage). The second lot consisting of 12 boxes was left in cold storage continuously. At the end of 17 weeks' storage the amount of scald on the fruit in the former lot ranged from 5 to 30 per cent, averaging 15.5 per cent, while that in the latter lot ranged from 50 to 80 per cent, averaging 65 per cent. The two aerations at laboratory temperature were apparently sufficient to reduce the scald to one-fourth that on apples held continuously in cold storage.

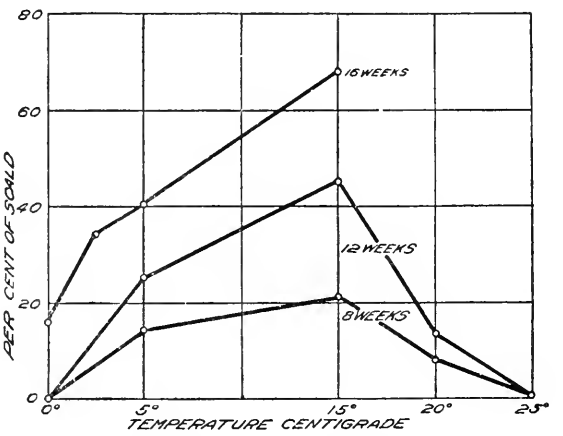

FIG. 10.—Graphs showing the effect of temperature on apple-scald at the end of 8, 12, and 16 weeks. The apples were Arkansas from Middletown, Va. They were picked and packed on October 17 and placed in commercial cold storage the following day. They were removed from storage on December 4 and the above experiment started the same day.

AIR-COOLED CELLAR STORAGE

It has already been pointed out that in the experimental storage boxes apple scald was prevented at all temperatures from 0° to 30° C. by a gentle air movement. Other experiments were made under more nearly commercial conditions, in which air-cooled cellar storage was compared with commercial cold storage. The experiment was made at Wenatchee, Wash. In the fall the door and window of the cellar were kept open at night and closed in the day, and throughout the winter frequent ventilation was given. Hygrothermograph records showed that in October the average temperature of the cellar was 12° C. (53.6° F.) and the average relative humidity 60 per cent; in November the average temperature was 8° C. (46.4° F.) and the average relative humidity 78 per cent. From the first of December to the middle of March the temperature stood fairly constantly at 5° C. (41° F.) and the relative humidity at 86 per cent. In the cold-storage plant the average temperature for November was 2.5° C. (36.5 °F.) and the average relative humidity 84 per cent; for December the average temperature was 0.28° C.

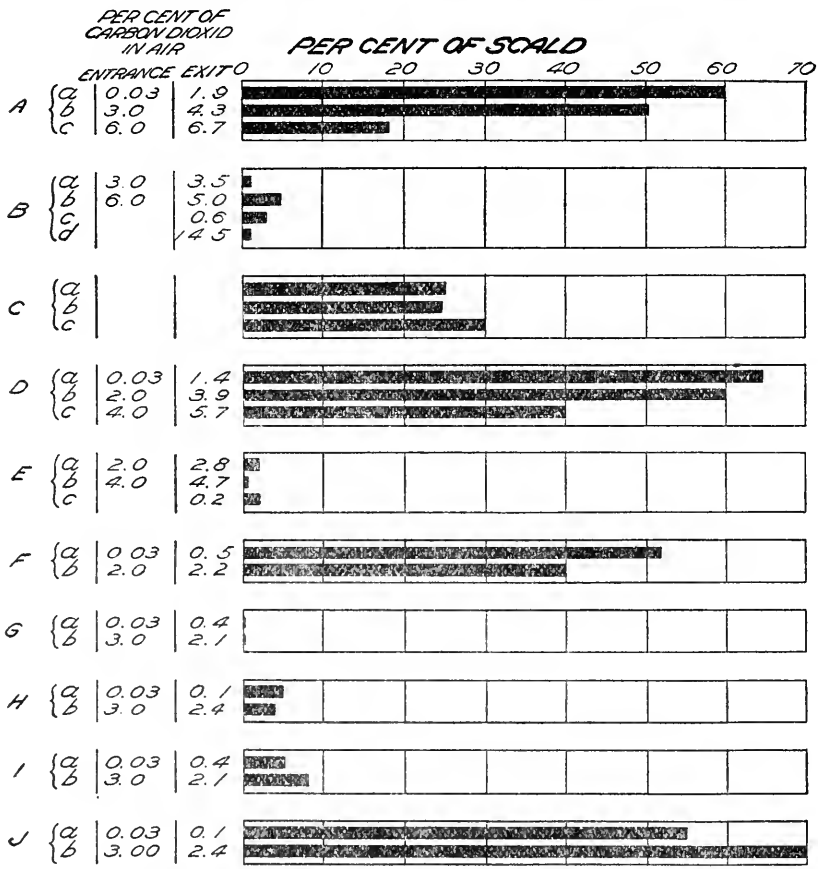

FIG. 11.—Graphs showing the relation of carbon dioxid to apple-scald production. The percentage of scald is shown by the length of the bars, and each group of bars (A, B, etc.) represents a particular experiment. In cases where the air was kept at a practically constant composition by renewal, the fresh air with or without carbon dioxid was introduced continuously at a rate such that a volume of air equal to that of the container was carried in once in 24 hours. In cases where the air was stirred, the circulation was accomplished by means of a rotary air pump. The air was kept practically saturated with moisture in all of the experiments. Five apples were used in each test.

A. The apples used in the experiment were of the lot described in the legend for figure 5. They were held in sealed jars with air slowly renewed. The results were obtained after 6 weeks storage at 15° C.

B. Same treatment as in (A), but the air was circulated constantly with an air pump. In (a) and (b) the air was slowly renewed but in (c) and (d) the circuit was entirely closed. In (c) the air was circulated over soda lime and water and in (d) over water only.

C. Apples of the lot described in the legend for figure 5 but held in cold storage 8 weeks before starting the experiment. The results were obtained after 3 weeks storage at 15° C. (a) Jar as moist chamber with a ½-inch hole in the top. (b) as in (a) but with jar inverted placing the hole at the bottom. (c) As in (a) but with soda lime and water in the bottom of the jar.

D. The apples used in the experiment were of the lot described in the legend for figure 3. [The apples were held in sealed jars with air renewed slowly.] The results were obtained after 8 weeks storage at 15° C.

E. Same treatment as in (D) but air circulated constantly with an air pump.

F. Same treatment as in (D) but at 0° C. for 16 weeks.

G. The apples used in the experiment were of the lot described in the legend for figure 9. They were stored at 2½° C. in sealed boxes with the air slowly renewed and also stirred with an air pump. The results were obtained after 20 weeks storage.

H. Same treatment as in (G) but with the apples held at 0° C. for 20 weeks, stored in sealed jars with air renewed slowly but not stirred.

I. The apples used in the experiment were of the lot described in the legend for figure 10. They were stored at 2½° C. in sealed boxes with the air slowly renewed and also stirred with an air pump. The results were obtained at the end of 20 weeks.

J. Same treatment as in (I) but the apples held at 0° C. for 20 weeks stored in sealed jars with air renewed slowly but not stirred.

(32.5° F.) and the average relative humidity 84 per cent; for January and February the average temperature was 31° F, and the average relative humidity 78 per cent, and for March the average temperature was 34° F. and the average relative humidity 95 per cent. In the cellar there was some daily variation in both temperature and humidity, while in cold storage both were quite constant.

Apples that had been carefully selected as to uniformity in size and maturity were divided into several lots, part of them being placed in cellar storage, part in commercial cold storage, and part moved from one storage condition to the other. In experiment A 2 boxes and in experiment B from 4 to 10 boxes of apples were used in each test. The apples were boxed in the usual manner. The results are given in Table X and show the percentage of scald developed after the given treatment was followed by 5 days' storage at 20° C.

TABLE X.—*Development of apple-scald in air-cooled cellar storage and in commercial cold storage*

Experiment No.	Variety and treatment.	Percentage of scald.			
		Rome Beauty.	Stayman Winesap.	Grimes.	
				After 17 weeks.	After 24 weeks.
	RATHER IMMATURE ROME BEAUTY AND STAYMAN WINESAP APPLES.				
A1	In cold storage continuously for 23 weeks	20	65	.....	.....
A2	In cellar storage continuously for 23 weeks	15	0	.....	.....
A3	In cold storage for 9 weeks; then in cellar storage for 14 weeks	40	30	.....	.....
A4	In cellar storage for 9 weeks; then in cold storage for 14 weeks	0	0	.....	.....
A5	In cold storage for 16 weeks; then in cellar storage for 7 weeks	95	90	.....	.....
A6	In cellar storage for 16 weeks; then in cold storage for 7 weeks	40	0	.....	.....
	HEAVILY IRRIGATED GRIMES APPLES.				
B1	Cellar storage for 17 weeks	.....	.....	16.8	.....
B2	Cold storage for 17 weeks; then in cellar storage for 7 weeks	.....	.....	65.0	88
B3	Cold storage for 19 weeks; then in cellar storage for 5 weeks	.....	.....	.....	62
B4	Cold storage for 10 weeks; then in cellar storage for 14 weeks	.....	.....	.....	14
B5	Cold storage for 5 weeks; then in cellar storage for 19 weeks	.....	.....	.....	6

The apples in cellar storage ripened more rapidly than those in cold storage, particularly during the first part of the season, but in all cases there was less scald under the former condition than under the latter. With apples transferred from one condition to the other the amount of

scald varied with the length of time held under cold-storage conditions. Apples shifted in either direction during the first 9 weeks of storage seemed to derive a benefit from the shifting itself, thus furnishing further evidence that temperature changes may aid in removing scald-producing agencies.

VENTILATION IN COMMERCIAL COLD STORAGE

Experiments were made to determine the effect upon the development of apple-scald in cold storage of different kinds of packages and different amounts of air circulation. The results are given in Table XI.

TABLE XI.—*Influence of package and ventilation upon the development of apple-scald in cold storage*

Experiment No.	Variety and treatment.	Percentage of scald.					
		Dec. 19.		Dec. 22.		Tight barrel.	Ventilated barrel.
		Barrel.	Box.	Barrel.	Box.		
	GRIMES.						
A1	At 0°C. (32° F.) Practically no ventilation.	2	0	45	35	.....	.....
A2	At 2.5° C. (36.5° F.) Some ventilation.	35	3	65	8	.....	.....
	ARKANSAS.						
B1	At 2.5° C. (36.5° F.)	.....	.....	.....	.....	80	50
B2	At 0° C. (32° F.)	.....	.....	.....	.....	70	25
B3	At 0° C. (32° F.)	.....	.....	.....	.....	60	30
B4	At 0° C. Room aired a few times	.....	.....	.....	.....	35	.....
	At 0° C. Room not aired	.....	.....	.....	.....	70	.....

EXPERIMENT A.—The apples used were Grimes, from Virginia, picked on September 7, and placed in commercial cold storage September 8. Part were packed in tight barrels and part in boxes. The apples were removed from storage on December 19, the packages opened, and held at a temperature of 18.3° C. (65° F.) for three days.

EXPERIMENT B.—Arkansas apples from Middletown, Va., picked on October 17, and stored in commercial cold storage on October 18, were used in this experiment. Part of the apples were packed in tight barrels of the usual commercial form and the others in similar barrels with holes for ventilation. Fifteen slits ⅝ inch by 4 inches were cut in each barrel. The apples were removed from storage on February 18 and held at a temperature of 20° C. (68° F.) for three days before taking the final notes.

In all cases the open packages had less scald than the tight ones, averaging about half as much. With the Grimes apples held in an unventilated storage room the fruit in the boxes was scalded practically as badly as that in the barrel; but in those held in a poorly ventilated room the box apples were practically free from scald.

Further evidence of the beneficial effects of storage ventilation is found in experiment B4 (Table XI), the apples in the room without ventilation having twice as much scald as those in the room having an occasional airing.

Cold-storage men who make a practice of opening up windows and doors when weather conditions will permit and allowing outside air to sweep through the storage rooms for a short period of time report great benefit in the way of the prevention of apple-scald.

DELAYED STORAGE

From a study of Tables IX and X it is evident that shifting apples from a higher to a lower temperature and from a lower to a higher one were not equally beneficial in scald prevention, the former always giving much better results. In experiments 1 and 2 of Table IX there was much less scald on apples stored first at 5° C. and then at 0° C. than on those stored first at 0° C. and then at 5°. Also the contrast of No. 3 and 5 with No. 4 and 6 in A of Table X shows that there was less scald on the apples stored first in cellar storage then in cold storage than on those moved from cold storage to cellar storage.

In other experiments the effect of delayed storage at higher temperatures was tested. The results are given in Table XII.

TABLE XII.—*Effects of delayed storage upon apple-scald*

Experiment No.	Variety and package.	Treatment.	Percentage of scald.	
			After 17 weeks.	After 26 weeks.
A1	Grimes apples in boxes stored at Wenatchee, Wash.	Stored at once; in cold storage for 19 weeks.	38	.....
A2	.....do.....	Delayed storage [a].....	15	.....
B1	York Imperial apples from Vienna, Va., stored in barrels at Washington, D. C.[b]	To cold storage the day after picking.	.....	25
B2	.....do.....	In shade in headed barrel for 6 days; then in cold storage.	.....	8
B3	.....do.....	In sun in headed barrel for 6 days; then in cold storage.	.....	10
B4	.....do.....	In shade in open boxes (during delay) for 6 days, then in cold storage.	.....	3
B5	.....do.....	In shade protected from wind in headed barrel for 12 days, then in cold storage.	.....	52
B6	.....do.....	In shade in unheaded barrel for 12 days, then in cold storage.	.....	20
B7	.....do.....	In shade in open boxes (during delay) for 12 days; then in cold storage.	.....	20

[a] During the delay the apples were held in boxes in a shaded, well-aired place. The average maximum day temperature was 23° C. and the average minimum night temperature was 5° C. The apples were delayed 2 weeks and then held in cold storage for 17 weeks.

[b] The maximum outdoor day temperatures during the delay averaged 18.7° C., and the minimum outdoor night temperature averaged 3.4° C.

The amount of scald was reduced by delayed storage in all of the different experiments, with but one exception. This exception was with apples held in a tight-headed barrel in a protected place for 12 days. Apples held in boxes for 6 days and then repacked were practically free from scald, and apples delayed in open boxes or in barrels for 12 to 14 days developed less scald than those stored immediately. The results indicate that the effect of delayed storage upon apple scald will depend largely upon the amount of ventilation the apples receive during the delay. The original maturity of the apples would probably also have a modifying influence. The writers wish to be distinctly understood as making no general recommendation in favor of delayed storage. The temperature experiments already reported show the great importance of immediate cooling as a means of scald prevention, and this is the phase of the subject that should receive the greatest emphasis. They are convinced, however, that with any apples lacking the full degree of color and maturity that might be most desirable (this would include a large part of the average eastern crop) scald may be reduced by a few days' delay in open well-aired packages before the fruit is placed in commercial cold storage, and that if during this delay the fruit can be kept as cool as 5° C. (41° F.) or even 10° C. (50° F.) little or no increase in rot will result from it. They consider that the results that have been obtained from the various apple-scald experiments furnish strong evidence of the value of air-cooled storage houses as a supplement to commercial cold-storage plants.

EFFECT OF GAS ABSORBENTS UPON APPLE-SCALD

The results of the foregoing experiments made it evident that apple-scald is not produced by high humidity nor by an accumulation of carbon dioxid, and yet that it is due to something that can be carried away by air currents and possibly partially taken up by absorbents, such as calcium chlorid. In a previous article the writers[1] reported experiments in which scald was reduced from 65 per cent to 10 per cent on York Imperial and Arkansas apples in commercial cold storage by adding excelsior to the usual barrel pack. Powell and Fulton[2] reported that paraffin wrappers reduced the amount of scald on apples, but that ordinary wrappers did not. The trend of the evidence in these experiments and the results already reported in the present paper led to the testing of various gas-absorbing substances. The results are given in Table XIII.

With the apples whose surfaces were only partially covered with wax scald did not occur beneath the coating; yet there was no close correlation between wax and scald patterns. The wax materials having the greatest absorbing powers apparently prevented scald on other parts of the apple, as well as on those with which they were actually in contact. There was nothing unusual in the taste of the apples under any of the conditions. In general the quality varied inversely with the amount of apple-scald.

[1] BROOKS, Charles, and COOLEY, J. S. EFFECT OF TEMPERATURE, AERATION, AND HUMIDITY ON JONATHAN SPOT AND SCALD OF APPLES IN STORAGE. *In* Jour. Agr. Research, v. 11, no. 7, p. 287-318, 23 fig., pl. 32-33. 1917. Literature cited, p. 316-317.

[2] POWELL, G. H., and FULTON, S. H. THE APPLE IN COLD STORAGE. U. S. Dept. Agr. Bur. Plant Indus. Bul. 48, 66 p., 6 pl. (part col.). 1903.

TABLE XIII.—*Effect of gas absorbents upon apple-scald*

Experiment No.	Treatment.	Percentage of scald.		
			Fruit coated with wax.	Fruit with bands of wax.
A1 ..	In sealed moist chamber; air renewed slowly	67	.....	.....
A2 ..	In the open	0	.....	.....
A3 ..	In unsealed moist chamber	64	.....	.....
A4 ..	Same as No. 3, but with ½ inch of cornstarch in bottom of jar.	21	.....	.....
A5 ..	Same as No. 3, but with ½ inch of animal charcoal in bottom of jar.	1	.....	.....
A6 ..	Same as No. 3, but with apples packed in excelsior	10	.....	.....
A7 ..	Same as No. 3, but with apples packed in sawdust	0	.....	.....
B1 ..	In sealed moist chamber; air renewed slowly	81	.....	.....
B2 ..	In the open	0	.....	.....
B3 ..	In unsealed moist chamber; apples not wrapped	70	.....	.....
B4 ..	In unsealed moist chamber; apples wrapped in usual commercial manner.	70	.....	.....
B5 ..	Same as No. 4, but with wrappers impregnated with paraffin.	20	.....	.....
B6 ..	Same as No. 4, but with wrappers impregnated with vaseline.	0	.....	.....
B7 ..	Same as No. 4, but with wrappers impregnated with cocoa butter.	0	.....	.....
B8 ..	Same as No. 4, but with wrappers impregnated with paraffin (50 per cent), vaseline (50 per cent).	8	.....	.....
B9 ..	Same as No. 4, but with wrappers impregnated with beeswax (30 per cent), vaseline (70 per cent).	3	.....	.....
B10 .	Same as No. 4, but with wrappers impregnated with cocoa butter (75 per cent), vaseline (25 per cent).	1	.....	.....
B11 .	Same as No. 4, but with wrappers impregnated with cocoa butter (80 per cent), olive oil (20 per cent).	1	.....	.....
B12 .	Same as No. 4, but with wrappers impregnated with beeswax (30 per cent), olive oil (70 per cent).	0	.....	.....
C1 ..	None	.....	70	70
C2 ..	Paraffin	.....	20	50
C3 ..	Vaseline	.....	0	2
C4 ..	Cocoa butter	.....	0	3
C5 ..	Paraffin (50 per cent), vaseline (50 per cent)	.....	0	5
C6 ..	Beeswax (30 per cent), vaseline (70 per cent)	.....	0	3
C7 ..	Cocoa butter (75 per cent), vaseline (25 per cent)	.....	0	1
C8 ..	Cocoa butter (80 per cent), olive oil (20 per cent)	.....	1	12
C9 ..	Beeswax (30 per cent), olive oil (70 per cent)	.....	0	0

A.—The apples were Rome Beauty of the same lot as described in the legend for figure 8, but they were held in commercial cold storage for 12 weeks. They were entirely free from scald at the time of starting the experiment. They were stored at 15° C. in nine liter jars. The results were obtained after 12 weeks' storage.

B.—Newtown Pippins of the same lot as described in Table V, B were used in this experiment. The results were obtained after 12 weeks' storage in moist chambers at 15° C. The special wrappers were prepared by dipping the usual apple wrappers in hot waxes and oils of the given composition and then allowing them to drain and cool.

C.—All conditions were the same as in B except that part of the apples were practically covered with a thin coating of wax, others had narrow bands of wax, and still others had no wax in any form. All were wrapped with ordinary apple wrappers as in commercial packing and stored in moist chambers.

As a whole, the results in Table XIII give most remarkably clear-cut and complete evidence that apple-scald can be prevented by the absorption of the gases (other than carbon dioxid) thrown off by the apples themselves in storage. The beneficial effects of the substances used in the experiments described under (A) may have been partly due to their water-absorbing power, but this could hardly be true of those used under (B) and (C). One of the particularly striking features brought out is the fact that the various substances have had a beneficial effect in direct proportion to their absorbing power. Excelsior greatly reduced the amount of scald, but sawdust entirely prevented the disease. Paraffin is distinctly the most inactive of all the waxes and oils used, and it was the only one that did not furnish practically complete control for the disease. Apple-scald can evidently be prevented by substances having a comparatively limited capacity for taking up gases if the absorbing surfaces are placed in rather close contact with the skin of the apple.

NATURE OF APPLE-SCALD

The foregoing experiments have approached the apple-scald problem from several different angles, and the results give considerable evidence as to the real nature of the disease. Apple-scald is not necessarily an old-age phenomenon, but is due to the long-continued action of more or less abnormal storage conditions, conditions that cause the production or prevent the elimination of certain waste products. Most varieties of apples may be exposed to such unfavorable conditions for several weeks without developing scald and without showing any tendency to the disease if later stored under more nearly normal conditions; but they finally reach a certain critical period at which time they are not scalded, yet have developed a tendency to scald that can not be eradicated by removing the agencies that were originally responsible for the trouble. In the experiment reported in Table VIII, B, apples that were held under conditions favorable to scald for eight weeks showed no sign of the disease when removed to a warm temperature for a few days, yet these apples developed scald later, under storage conditions that did not produce scald on fruit that had never been exposed to unfavorable conditions. With apples that have been shifted from one storage place to another it is evident that the conditions existing at the time of the development of scald may not be the ones that are responsible for the occurrence of the disease.

Apple-scald seldom, if ever, becomes evident while apples are held continuously at 0° C. (32° F.), but cold-storage apples may be found to be badly scalded after a few days at a higher temperature. As was pointed out in an earlier paper,[1] the real cause of this sudden appearance of the scald is not the sudden change of temperature. The disease already existed, but the cells were unable to carry out their death processes while a temperature of 0° was maintained.

[1] BROOKS, Charles, and COOLEY, J. S. EFFECT OF TEMPERATURE, AERATION, AND HUMIDITY ON JONATHAN-SPOT AND SCALD OF APPLES IN STORAGE. *In* Jour. Agr. Research, v. 11, no. 7, p. 287–318, 23 fig. pl. 32–33. 1917. Literature cited, p. 316–317.

SUMMARY

The foregoing experiments furnish conclusive proof that apple-scald is a preventable disease. The following are some of the more salient facts that have been experimentally established.

(1) Well-matured apples are much less susceptible to scald than immature ones.

(2) Apples from heavily irrigated trees scald worse than those from trees receiving more moderate irrigation.

(3) The rapidity of development of apple-scald increases with a rise in temperature up to 15° or 20° C., the optimum often shifting from 20° to 15° C. during the storage period.

(4) Apple scald has not occurred at temperatures of 25° or 30° C.

(5) It has been found possible to store apples in air saturated with water vapor without the development of scald. In several different experiments scald was considerably reduced by decreasing the humidity, but the beneficial effects were apparently not entirely due to the decreased moisture in the air.

(6) Accumulations of carbon dioxid (1 to 6 per cent) have not favored the development of apple-scald, but tended to prevent it.

(7) Apples susceptible to scald have been made immune by storing for a few days in an atmosphere of pure carbon dioxid.

(8) Increasing the percentage of oxygen in the air has not given consistent beneficial effects upon apple-scald.

(9) A constant air movement of from 1/8 to 1/4 mile per hour has always either entirely prevented apple-scald or reduced it to a negligible quantity. The intensity of the air movement was apparently more important than the continuity and the circulation of the air more important than its renewal.

(10) Scald has been greatly reduced by shifting apples from one temperature to another. The beneficial effects are attributed to the aeration of the apple tissue thus obtained.

(11) Thorough aerations during the first eight weeks of storage have been more helpful than later ones.

(12) Apples have scalded less in air-cooled cellar storage than in unventilated commercial cold storage.

(13) Apples packed in boxes or ventilated barrels have scalded much less than those in tight barrels,especially when the storage room received an occasional ventilation.

(14) Scald was greatly reduced on rather immature apples by a delay in storing, if the fruit was well aerated during the delay, but was increased by the delay if held under conditions that allowed little or no ventilation.

(15) Ordinary apple wrappers have had no effect on apple-scald, and paraffin wrappers but little; but wrappers soaked in various mixtures of olive oil, cocoa butter, vaseline, or beeswax have entirely prevented apple-scald.

(16) Apple-scald is due to volatile or gaseous substances other than carbon dioxid that are produced in the metabolism of the apple. They can be carried away by air currents or taken up by various absorbents.

ANGULAR-LEAFSPOT OF TOBACCO, AN UNDESCRIBED BACTERIAL DISEASE[1]

By F. D. FROMME, *Plant Pathologist and Bacteriologist,* and T. J. MURRAY,[2] *formerly Associate Bacteriologist, Virginia Agricultural Experiment Station*

INTRODUCTION

About the first of August, 1917, the Virginia Experiment Station received a petition from 52 tobacco growers in Halifax County, asking assistance in combating a tobacco disease which threatened serious losses to the crop. Diseased tobacco plants (*Nicotiana tabacum*) were later received from a correspondent at South Boston, the leaves of which were covered with spots which were different from any previously seen by us, the most distinctive feature being the irregularly angular shape. Numerous motile bacteria were found in crushed tissue mounts and freehand sections of spots, and the organism was readily obtained in pure culture from poured plates of beef-peptone agar. The same organism was obtained later from material which the writers collected in the field from five different places in Halifax County.

Several inspection trips were made during the remainder of the season of 1917. On August 10 the disease was found on the tobacco plants in most of the fields along the road between South Boston and Republican Grove, a distance of 30 miles. It was found later in the northern part of Halifax County, at Clarkton and other points, in the southern part of Campbell County, at Brookneal and Naruna, and at Charlotte Court House, in Charlotte County. Inquiries through county agents extended the distribution to include Mecklenburg, Pittsylvania, Henry, and Patrick Counties, involving the greater part of the flue-cured-tobacco belt in Virginia.

FIELD APPEARANCE OF THE DISEASE

The epiphytotic was well advanced by August 10. Many fields were found in which practically every plant was affected, and in some fully 50 per cent of the crop was estimated by the growers to be unfit for harvest. Late plantings were found to be less severely spotted than the early plantings, and the most forward and vigorous plants were invariably more seriously affected than the less vigorous ones.

The distribution of the spots on the plants was a distinctive feature and one that readily separated them from "frogeye" (caused by *Cerco-*

[1] Paper 53 from the Laboratory of Plant Pathology and Bacteriology, Virginia Agricultural Experiment Station.

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Journal of Agricultural Research,
Washington, D. C.
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Vol. XVI, No. 8
Feb. 24, 1919
Key No. Va. (Blacksburg)-2

spora nicotianae E. and E.). The heaviest spotting was found on the top and middle leaves, while the bottom or sand leaves were but slightly affected. Frogeye, on the contrary, is found chiefly on the sand leaves. The field evidence indicated that the vertical distribution of the spots on the plants was determined by their stage of growth at the time of infection, and that leaves which had attained a certain stage of growth were not susceptible to infection. In some fields the infection was heaviest on the top leaves and in others on the middle leaves. Frequently the spots were found to be most numerous on one side of the plants, indicating the probable dissemination of the inoculum by wind-blown rain.

The consensus of the statements of the farmers placed the first appearance of the spotting between the third and fourth weeks in July, following a protracted period of rainfall. The tobacco at this time was at about the stage for topping. No one had seen any of the spotting earlier in the season, and there had been no evidence of it in the seed beds. One farmer who had planted two fields from the same seed bed stated that the disease was much worse in the field on new ground than on old ground. There was no evidence from any source that continuous cropping with tobacco, as practiced on many farms, had been conducive to heavier infection.

Some of the farmers had been troubled with the disease in the previous year, 1916, and a few stated that they had seen it occasionally over a period of 10 or 12 years, but never so generally destructive as in 1917. Prof. T. B. Hutcheson, of the Virginia Experiment Station, informs us that he has known this spotting in Charlotte County for 12 or 15 years.

Opinions among the farmers as to the cause of the disease were centered on the wet weather, the fertilizer, especially the supposed deficiency in potash, and the seed. The disease was found on both the flue-cured and sun-cured types of tobacco, and no differences in the susceptibility of varieties were apparent.

Evidence that the disease is not occasioned by lack of potash and that it is not a fertilizer phenomenon, except in a secondary way, was obtained from a study of the distribution of the spotting on the fertilizer plots at the experimental substation at Charlotte Court House. Early in September the disease was prevalent on all plots which had received applications of acid phosphate alone or in various combinations with sulphate of potash and nitrate of soda. It was the opinion of several observers who went over these plots that they were uniformly spotted. None of the spotting was present, however, at this time, on any plots which had had no applications of phosphorus and which had received only nitrate of soda or sulphate of potash, or both. There was no spotting on the control plots. The tobacco plants on those plots which had received phosphorus were larger, more vigorous, and matured three weeks earlier than those which had had no phosphorus. The superintendent of

the substation informed us that the tobacco on these backward plots became spotted later with the advent of additional rainfall and cool nights, and that the second growth tobacco from the early harvested plots also became diseased.

CHARACTER OF LOSSES

A demonstration acre of tobacco at the substation at Charlotte Court House was severely spotted in 1917, much more so than in any previous years. The yields from this acre were obtained to determine the variation in yield and grade between 1917 and the average of preceding years. The data obtained are included in Table I. The yield of the average year was determined from demonstration acres for the years 1913, 1914, and 1915. Complete data for 1916 were not available, but the total yield for this year, 1,005 pounds, shows a close agreement with the three preceding years. The effect of the disease is shown both in a reduction in yield and in grade. The yield from the acre in 1917 was 212 pounds less than that of the average year, or approximately 80 per cent of the average. The yields in the different grades for 1917 show a loss in weight in all of the three highest grades and a strong increase in the lowest grade. There were approximately 40 per cent more sand lugs in 1917, in proportion to the higher grades, than in the average year, and the percentage of longs was slightly greater. The gain in the sand lugs was made up by losses in the two middle grades, good lugs and shorts. The total loss in weight was 20 per cent, and the loss in grade approximately 40 per cent.

TABLE I.—*Variation in yield and grade between severely spotted tobacco in 1917 and the average of three preceding years of light spotting*

Grade.	1917	Average year.	1917	Average year.	Difference between 1917 and average year.	
	Pounds.	*Pounds.*	*Per cent.*	*Per cent.*	*Pounds.*	*Per cent.*
Sand lugs	495	205	58. 9	19. 4	+290	+39. 5
Good lugs	.....	290	.....	27. 6	−290	−27. 6
Shorts	155	346	18. 5	32. 9	−191	−14. 4
Longs	190	211	22. 6	20. 1	− 21	+ 2. 5
Total	840	1, 052	.....	.....	−212	−20. 2

DESCRIPTION OF THE SPOTS

The spots are found only on the leaves, none on stems or floral parts. They are scattered over the entire leaf surface, between the larger veins, or are crowded in irregular groups. They are usually bordered by viens which act as barriers against further enlargement. More than 500 spots may be counted on the average leaf of a heavily infected plant. They are about equally prominent on the upper and lower leaf surfaces. The

size of the spots varies from a pinhead up to 8 mm. in diameter at the widest part, and the average diameter of the mature spot is 4 mm. The most striking feature is the irregularly angular shape and the uneven jagged outline (Pl. 25-27). The center of the spot is tan or reddish brown, with a darker thin border and often a suggestion of zonation which is never conspicuous. A small, dark center is usually seen. In earlier stages the spots are darker in color, almost black [1] at first, and are circular or only slightly angular (Pl. 25, A). The center of the spot becomes dry and thin with age, paler in color until almost white, and may drop out, leaving irregular holes which are sometimes so numerous with the confluence of spots that a skeleton leaf composed only of the viens remains. Most of the tobacco is harvested before this stage is reached. No sharply defined halo is present, but a narrow clear zone on the border is seen by transmitted light. The tissue bordering the spots is yellowed, but this diffuses gradually into the normal green. Usually the spots are more numerous on one side of the midrib.

In stained sections of fixed material the organism is found in great numbers throughout the shrunken tissue included in the spot. It is found both within and between the cells, and vacuolated cells are completely filled with it.

INOCULATION EXPERIMENTS

Proof of the pathogenecity of the organism isolated from leafspot material was obtained through inoculations on seedling tobacco plants (Warne variety). This work was carried on in the greenhouse during the winter months. In all, some 150 plants were inoculated, and fully 96 per cent developed spots. All five isolations of the organism produced infection. Infection was readily obtained by atomizing the plants with aqueous suspensions of the organism, by swabbing the leaves with the cotton plug of bouillon cultures and by puncture with contaminated needles. It was found necessary to place the plants in moist chambers for 24 hours subsequent to inoculation. Some plants which were inoculated and left in the open greenhouse failed to develop any spots, and none of the many control plants became infected. No leaf injury is necessary for infection. Apparently the organism gains entrance through the stomata on either the upper or lower surface of the leaf. The inoculum has been recovered in pure culture a number of times, and these recoveries have been used for reinfections. Secondary infections have never developed, but this is a common experience with plant pathogens under the dry atmospheric conditions of the greenhouse.

As many as 200 spots were obtained on a single leaf through inoculation with the atomizer, but the average ran much lower than this. Still heavier infections were obtained by swabbing with contaminated cotton plugs. The spots obtained were typical of the spots seen in the field, but were smaller. They averaged about 1.5 mm. in diameter, and

[1] One farmer stated that his first impression of the spot was that someone had spattered ink on the leaves.

many were no larger than 0.5 mm., mere pin pricks. The spots were frequently grouped on a limited area of the leaf and were often found on one side of the midrib only.

The discrepancy in size between the field spots and those developed in the greenhouse is apparently due to the difference in the size and vigor of the plants. The plants did not grow vigorously in the greenhouse and at maturity were not more than half as large as plants in the field. The largest spots in the greenhouse were always found on the most vigorous plants and on the most rapidly growing leaves. The incubation period was also shortest with the same plants and leaves. This varies between 4 and 10 days, with the average about 7 days. When plants which have developed a number of leaves are inoculated, the first spots are seen on one or two leaves near the top and intermediate in age. Later, within a few days, spots may appear on two or three older leaves immediately below these, but no spots develop on old, full-grown leaves, nor on very young ones. Young leaves become infected, however, when the inoculum is rubbed in with the fingers or a cotton plug. The young leaves are closely set with trichomes, and these seemingly serve as a mechanical protection against inoculation with the atomizer; the spray is caught and retained on them. The spots attain their maximum size within a few days after their appearance. They are largest on the younger leaves and may be mere pin pricks or flecks on the older leaves.

TABLE II.—*Results from the inoculation of tobacco plants with the angular-leafspot organism*

Plant No.	Number of spots per leaf on leaf No.[a]				
	1	2	3	4	5
1	.....	2	.....	.....	.....
2	.....	14	13	1	.....
3	1	1	.....	2	.....
4	5	68	19	**33**	.....
5	.....	.....	5	1	.....
6	14	29	17	21	**9**
7	157	8	39	.....	**30**
8	.....	.....	51	14	**6**
9	.....	44	63	23	.....
10	1	1	5	.....	.....
11	1	18	.....	.....	.....
12	.....	.....	.....	.....	.....
13	3	5	4	**41**	.....
14	6	3	4	.....	.....
15	.....	.....	.....	.....	.....
16	1	1	.....	.....	.....
17	**58**	.....	37	.....	.....
18	30	.....	23	14	.....
Total spots	277	193	280	76	.....
Total flecks	.....	.....	.....	**74**	**45**

[a] Leaves are numbered from the top downward. The plants bore from 10 to 14 leaves. Bold-face figures indicate flecks or spots that are very small and are visible only in transmitted light.

The records of one inoculation series are given (Table II) chiefly to show the vertical distribution of the spots with reference to the position of the leaves and their relative ages. These plants were inoculated on January 16, 1918, with an atomizer containing an aqueous suspension of a strain of the angular-leafspot organism isolated from material from Republican Grove, Va., on August 10. The plants were covered with moist chambers for 12 hours subsequent to inoculation. They were at the blooming stage and were topped just before inoculation. The spots were first visible on January 22, and the counts were made on February 1.

Table II shows that the spots were about equally distributed over the first, second, and third leaves, with a few on the fourth leaves and none on those older and lower on the stem. Flecks developed on the fourth and fifth leaves, but not on younger or older leaves.

COMPARISON OF ANGULAR-LEAFSPOT WITH OTHER LEAFSPOTS OF TOBACCO

The angular-leafspot can not be assigned with certainty to any of the previously described tobacco leafspots of bacterial causation. It has some features in common with the "whitespot" of Delacroix (*2*, *3*),[1] caused by *Bacillus maculicola* Del., but the descriptions of this disease and of the organism are too meager to afford an adequate basis for comparison. "Blackrust," a disease of Deli tobacco described by Honing (*4*), differs from angular-leafspot in several important features, and the causative organism, *Bacterium pseudozoogloeae* Honing, is readily distinguished from the angular-leafspot organism.

The spot which Wolf and Foster (7) have recently described under the name "wildfire" from North Carolina appears to differ strikingly from the disease under discussion. The most noteworthy points of difference being found in the broad, distinct halo which borders the wildfire spots, in their circular form and zonated interior, and in size. Wildfire spots are 2 to 3 cm. in diameter, while those of the angular-leafspot are only 4 mm., on the average. Some contrasting features between *Bacterium tabacum* Wolf and Foster, the wildfire organism, and the angular-leafspot organism are given in Table III. Features in common between the two diseases are found in their sudden appearance and rapidity of spread, and in the relation between rainfall and epiphytotics, although this seems a common feature of bacterial leafspots of tobacco. It seems quite probable that the disease to which Wolf and Foster refer as "speck" is identical with our angular-leafspot. They state that speck is caused by a lack of potash.

[1] Reference is made by number (italic) to "Literature cited," p. 227–228.

TABLE III.—*Comparison of Bacterium angulatum and Bact. tabacum*

Bact. angulatum.	*Bact. tabacum.*
1. Size, 0.5×2–2.5 μ.	Size, 1.2×3.3 μ.
2. 3 to 6 polar flagella.	1 polar flagellum.
3. Liquifies gelatin rapidly.	Liquifies gelatin slowly.
4. Forms acid with saccharose and dextrose.	Forms acid with saccharose, dextrose, lactose, and glycerin.
5. No growth in closed arm of fermentation tubes.	Growth in closed arm of fermentation tubes containing dextrose and saccharose.

An unsigned note in the Yearbook of the Virginia Department of Agriculture and Immigration (*5*) contains a report of a field investigation of a spotting of tobacco leaves (evidently angular-leafspot) which was prevalent in Pittsylvania and Mecklenburg counties in 1917. The spotting is assigned to microorganisms, and it is stated that the disease becomes serious only under certain conditions which affect the resistance of the tobacco plant. The most important of these are considered to be rainfall and excess of nitrogen in the fertilizer or soil. A liberal supply of potash is said to decrease the severity of the disease, but considerable damage was noted even with heavy potash applications. The same disease is said to occur in North Carolina and Maryland.

Two other leafspots, the causes of which have not been assigned to bacteria, and which appear somewhat similar to angular-leaf spot, are "rust" of tobacco in Connecticut (*1, p. 366–367, pl. 31, b, c*) and *Pockenkrankheit* (*6, p. 56*) of tobacco in Europe. Clinton's figures of rust, especially that shown in his Plate 31, c, look much like angular-leafspot. He states that rust is usually found on leaves affected with calico and believes it may be caused by scorching of the sun. *Pockenkrankheit* is ascribed to excessive transpiration accompanying decreased water supply.

OCCURRENCE OF THE DISEASE

It seems quite probable that angular-leafspot is a disease of rather general distribution and one of long standing which has not been sufficiently destructive to attract extensive notice, except in seasons unusually favorable for its development. Our experience indicates that rainfall accompanied by subnormal temperatures favors infection by the leafspot organism and that any combination of conditions which promotes a rapid, succulent growth of the host favors the development of the organism within the leaf tissue. It seems quite probable that infection may be common in some seasons, but that in the absence of the predisposing growth factors little development ensues, and no damage results.

DESCRIPTION OF THE ORGANISM

The caustive organism of angular-leafspot appears to be an undescribed species, and a description is therefore appended.

Bacterium angulatum, n. sp.

As it occurs in the plant and also on media, the organism is a short rod with rounded ends, single or in pairs, 0.5 μ wide by 2 to 2.5 μ long. No spores are produced, and no capsules have been demonstrated. It is motile by means of a small tuft of flagella at one pole, demonstrated by the Van Ermengen silver-nitrate method. The number of flagella varies from about three to six, and they are slightly longer than the body of the bacterium. It stains readily with the ordinary dyes, and is Gram-negative and not acid-fast.

TEMPERATURE RELATIONS

The best growth is obtained at temperatures between 17° and 20° C. There was no growth at 37.5° C.

CULTURAL CHARACTERS

AGAR PLATES.—Colonies are visible in 48 hours at 20 to 22° C. They increase slowly in size, being less than 1 mm. in diameter at three days. After seven days the largest are 4 mm. in diameter and the average about 3 mm. The maximum size attained is 8 mm. They are round, smooth, convex, shining, opalescent at first, later becoming dull white with a slight creamy cast and develop an opaque center with a clear margin. They are finely granular under the compound microscope, with a slightly undulate margin. Buried colonies are lenticular.

AGAR SLANTS.—Growth is slight in three days, the line of the stroke being about 1 mm. broad, and is not more than 5 mm. broad after one month. The growth is filiform, slightly raised, shining, smooth, and slimy. Considerable white sediment is formed at the base. The medium attains a slight pale-green fluorescence.

GELATIN PLATES.—Colonies are visible in 48 hours as small points similar to those on agar. Liquefaction is rapid, beginning in cuplike hollows in 48 hours. The cupules are 5 to 10 mm. broad in 3 days. Thickly sown plates are completely liquefied in 48 hours.

GELATIN STABS.—Liquefaction is infundibuliform and begins in 24 hours. As liquefaction progresses, the upper part becomes stratiform, and the lower maintains the blunt funnel form. Liquefaction is complete within 15 days at 18° to 20° C.

BEEF BOUILLION.—Uniform heavy clouding occurs within 48 hours. No surface scum or pellicle is produced, and there are no zoogleæ. A grayish precipitate forms in old cultures.

BEEF BOUILLION WITH SODIUM CHLORID.—Two per cent sodium chlorid produced only a slight inhibition of growth in 48 hours. Heavy clouding was present at seven days with 2 per cent of sodium chlorid, but growth was practically inhibited with 4 per cent of the salt.

POTATO CYLINDERS.—The form of growth is similar to the agar slant but with a slight dull yellow pigment.

MILK.—Inoculated milk clears slowly and without coagulation. The protein is digested. Clearing begins within seven days in definite layers from the top downward, and is complete within three weeks. The liquid is only faintly translucent at this time and is near Ridgeway's pale fluorite green.

LITMUS MILK.—Lavender-colored litmus milk becomes blue from the top downward in definite layers. The color change begins on the second or third day and is complete within 14 days. During two months the medium remained dark blue and liquid.

FERMENTATION TUBES.—The tests were made in basal solutions of 1 per cent peptone, to which was added 1 per cent of the following carbon compounds: Saccharose, dextrose, lactose, maltose, glycerin, and mannit. Clouding occurred in the open ends of all tubes in 48 hours, but the closed ends remained clear with a distinct line across the inner part of the U. Tests with neutral litmus paper gave an acid reaction with saccharose and dextrose, while the others were neutral or faintly alkaline. No gas was formed with any of the compounds.

The tests for acid production with dextrose and saccharose were repeated, with 100-cc. portions of 2 per cent of each in 2 per cent peptone water. After 10 days the reaction was determined with phenolphthalein as the indicator. Both solutions showed acid production in excess of the controls as follows: Saccharose control, +6.6; saccharose inoculated, +11.0; dextrose control, +8.8; dextrose inoculated, +12.6.

REDUCTION OF NITRATES.—Nitrates are not reduced.

INDOL.—A moderate indol production was obtained in Dunham's solution.

USCHINSKY'S SOLUTION.—Clouding was evident after 48 hours and was only moderate at seven days. The medium did not change color and no scum or pellicle was formed.

AEROBISM.—The organism appears to be strictly aerobic.

Following the chart of the Society of American Bacteriologists, the group number is 211.23220 [1] 33.

SUMMARY

A leafspot disease of tobacco which was prevalent in the flue-cured belt in Virginia in 1917 is described under the name "angular-leafspot." The disease has apparently been present to some extent for several years and may have a wide distribution.

The disease is caused by a specific organism, which is described as "*Bacterium angulatum*." Rainfall is an important aid to infection, and the development of the organism within the tobacco leaf is apparently dependent to a marked degree on those predisposing factors which promote a rapid, vigorous growth of the host.

The disease produced losses in both yield and grade. These were calculated in one field as a 20 per cent reduction in yield and a 40 per cent reduction in grade.

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[1] A very slight fluorescence is imparted to the medium with agar stroke.

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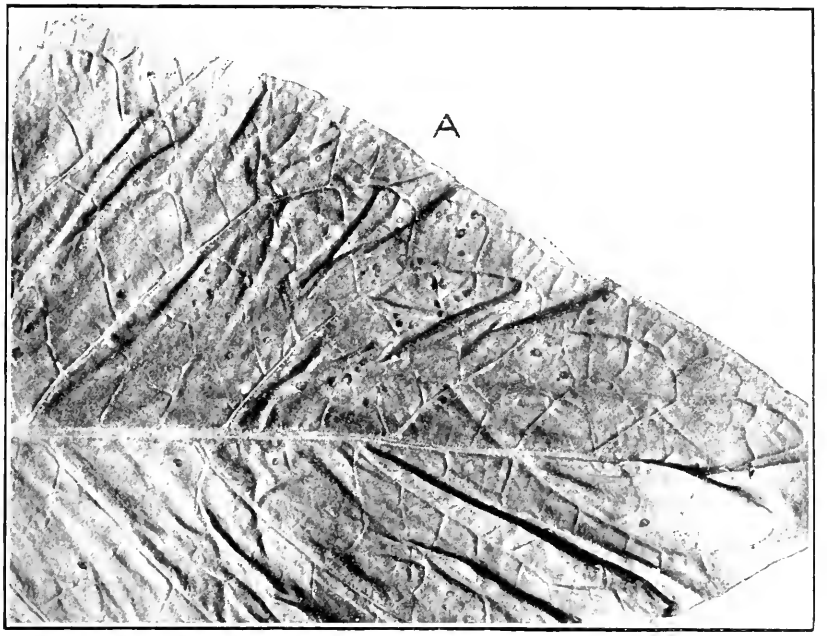
PLATE 25

A.—A tobacco leaf showing an early stage of the angular-leafspot.
B.—Angular leaf spots on a tobacco leaf. About natural size.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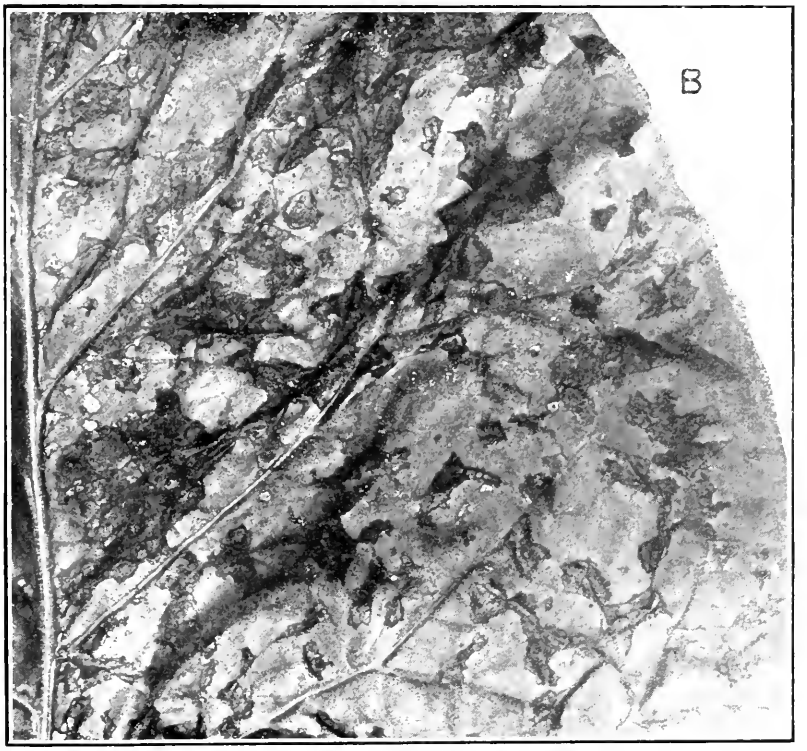
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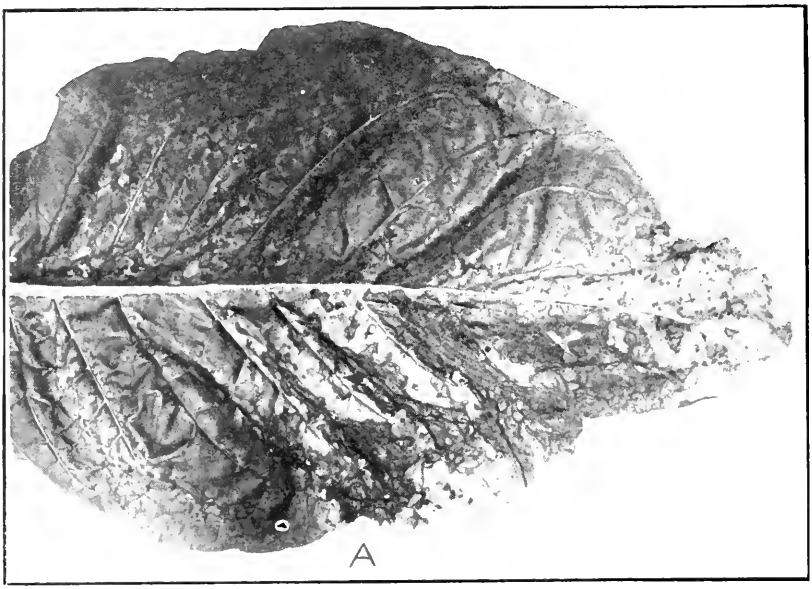
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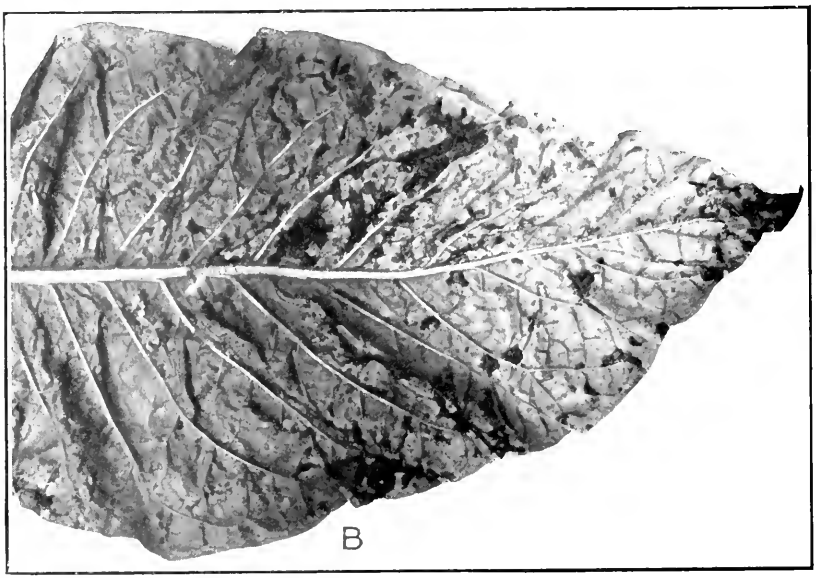
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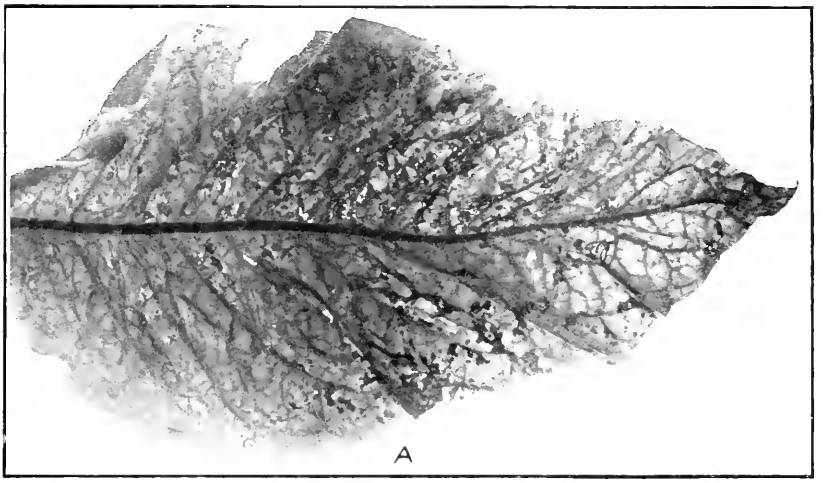
PLATE 26

A.—Upper surface of a tobacco leaf affected with the angular-leafspot.
B.—Lower surface of a tobacco leaf affected with the angular-leafspot.

PLATE 27

A.—Angular leaf spots on a tobacco leaf as seen in transmitted light.

B.—Atypical angular leaf spots on a narrow leaf of tobacco. The spots are blanched and rounded.



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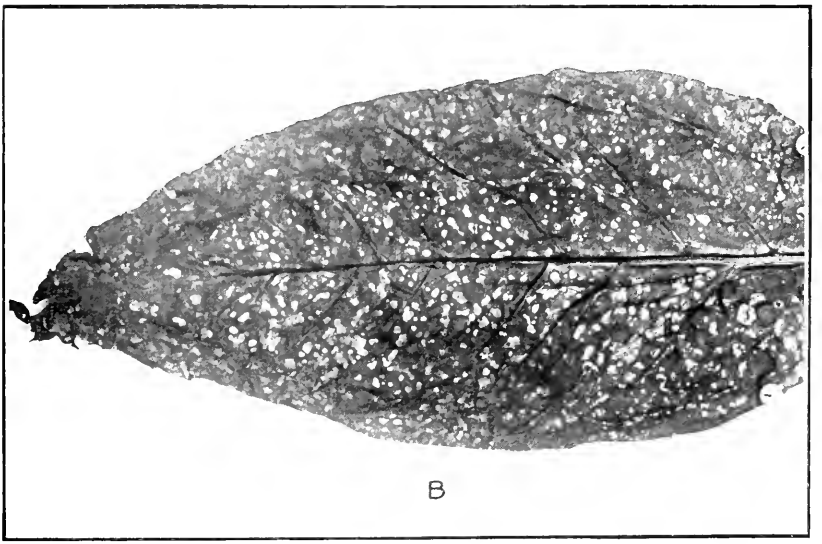

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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from State Experiment Stations should be addressed to H. P. Armsby, Institute of Animal Nutrition, State College, Pa.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., MARCH 3, 1919 NO. 9

TWO SPECIES OF PEGOMYIA MINING THE LEAVES OF DOCK [1]

By S. W. FROST [2]
Instructor in Entomology, The Pennsylvania State College

INTRODUCTION

The life history and habits of two leaf-mining Anthomyids (*Pegomyia* spp.) are presented in this paper for the first time. The docks *Rumex crispus* L. and *R. obtusifolius* L. are extensively mined by several species of Pegomyia. Among these are two [3], *P. calyptrata* Zett. and *P. affinis* Stein., which occur commonly throughout the United States. *P. calyptrata* Zett., is by far the more common of the two species. The adult is readily distinguished by a bluish-gray thorax and a reddish-yellow abdomen. *P. affinis* Stein., on the other hand, is less common. It occurs about Ithaca, N. Y., abundantly in early summer, but later in the season no eggs, larvæ, or adults have been found. This species is distinguished by its inconspicuous gray color.

THE MORE COMMON SPECIES, PEOOMYIA CALYPTRATA

HISTORICAL REVIEW

P. calyptrata Zett. was first described by Zetterstedt (1846).[4] since that time there have been but few references to it. Stein (1897),[5] (1907),[6] and Pandellé (1901)[7] refer to this species, but mention nothing regarding its habits. This species has undoubtedly been noticed by many, but its identity has been unknown. In looking over some unidentified material in the collection at Cornell University the writer found specimens reared by Prof. Comstock as early as 1882. Dr. A. O. Johannsen, also of Cornell, noticed the same species in 1913 mining dock, but did not publish his observations.

[1] Contribution from the Entomological Department of the Cornell University Agricultural Experiment Station.

[2] The writer wishes to acknowledge his indebtedness to Dr. Robert Matheson, of the Department of Entomology, Cornell University, for many helpful suggestions and the criticism of this paper.

[3] There are at least two other species of Pegomyia occurring in this country, *Pegomyia bicolor* Wied. and *Pegomyia winthemi* Meig., which mine *Rumex* spp. These occur in the northern United States and Canada, but do not appear to be common at Ithaca, N. Y.

[4] ZETTERSTEDT, J. W. DIPTERA SCANDINAVLÆ. v. 5, p. 159 Lundae. 1846.

[5] STEIN, P. NORDAMERIKANISCHE ANTHOMYIDEN ... *In* Berlin. Ent. Ztschr., Bd. 42 (1897), Heft 3/4 p. 239–241, 286. 1898.

[6] BECKER, Th., BEZZI, M., KERTÉSZ, K., STEIN, P. KATALOG DER PALÄARKTISCHEN DIPTEREN. v. 3, p. 701. Budapest. 1907.

[7] PANDELLÉ, L. ETUDES SUR LES MUSCIDES DE FRANCE. *In* Rev. Ent. France, t. 20, no. 1, p. 294, 1901.

Journal of Agricultural Research,
Washington, D. C.
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Mar. 3, 1919
Key No. N. Y. (Cornell)-4

DISTRIBUTION

P. calyptrata Zett., occurs in Europe as well as America, although it appears to be exceedingly rare in the former. Stein (1906)[1] neglects to list this species in his paper. Zetterstedt (1846)[2] mentions its occurrence in Sweden, stating that one specimen was taken near Lund by Mr. D. Dahlbom and a second at Vadstena by himself. Two specimens were also taken at Altenburg, Germany. In America it is a common species, especially in New York State, where the author has found it widely distributed. Stein (1897)[3] mentions its occurence in Washington, Minnesota, Illinois, Pennsylvania, and Massachusetts. To these the writer adds New Jersey and New York, having taken the the species at Ithaca, Binghamton, and Tarrytown, N. Y., and Orange, N. J.

HOST PLANTS

P. calyptrata mines exclusively in the leaves of several species of Rumex. Adults have been reared from *R. obtusifolius* and *R. crispus*. Both species of Rumex are equally susceptible to the attack of the miner. *R. acetosa* is evidently a third host plant. Eggs of *P. calyptrata* were found on this plant. They hatched and the young larvæ entered the leaf, but the writer did not succeed in rearing adults. A large number of adults of other species were reared from larvæ mining the leaves of garden beets (*Beta vulgaris*), spinach (*Spinacia oleracea*) and Swiss chard (*Beta vulgaris* var. *cicla*), as well as many weeds, such as *Chenopodium album*, *Amaranthus retroflexus*, and *Atriplex patula;* but P. *calyptrata* was not obtained from any of these.

A number of experiments were performed to induce the larvæ of *P. calyptrata* to mine in the leaves of other plants. Three eggs were carefully removed from a dock leaf and placed on a beet leaf. Two days later the eggs hatched, but the larvæ died without entering the leaf. In a second experiment four second-stage larvæ were dissected from *R. obtusifolius* and placed on *Chenopodium album*. The following day the four larvæ were found dead. In a third experiment two third-stage larvæ from *R. crispus* were placed on *C. album*. The following day one larva had entered the leaf and formed a small blotch mine, but the next day the larva died. These experiments seem to substantiate the fact that *P. calyptrata* mines solely in *Rumex* spp.

LIFE HISTORY AND HABITS

EGGS.—The eggs are glossy white, and in the field are laid usually in groups of three to five, occasionally in groups of six or seven, but

[1] STEIN, P. DIE MIR BEKANNTEN EUROPÄISCHEN PEGOMYIA-ARTEN. *In* Wiener Ent. Ztschr., Jahrg. 25, Heft 2/3/4, p. 47–107. 1906.

[2] ZETTERSTEDT, J. W. DIPTERA SCANDINAVLÆ. v. 5, p. 1775, 1846; v. 12, p. 4751, 1855. Lundae.

[3] STEIN, P. NORDAMERIKANISCHE ANTHOMYIDEN ... *In* Berlin. Ent. Ztschr., Bd. 42 (1897), Heft 3/4 p. 239–241, 286. 1898.

seldom singly. They are laid in neat transverse rows on the undersurface of the leaf. In captivity, however, the eggs are scattered over both surfaces of the leaf and are frequently laid singly instead of in groups.

The number of eggs occurring on a single leaf is surprising. As a rule, one finds only 5 or 6 groups, but it is not uncommon to find more. In one instance the writer found 20 groups of eggs on a single leaf, 65 eggs in all. On another leaf, 16 inches long, he found 18 groups of eggs, 47 eggs in all. It is interesting to note that all the larvæ from these eggs did not mature within the leaf on which they were laid, but migrated and started new mines on other leaves. The length of the egg stage is given in Table I.

TABLE I.—*Incubation period of the eggs of Pegomyia calyptrata*

Experiment No.	Eggs laid.	Eggs hatched.	Length of egg stage.
			Days.
A112	May 7	May 13	6
A111	May 8	May 14	6
A113	...do.....	....do....	6
A117	May 15	May 20	5
A122	May 16	May 22	6
A129	May 23	May 25	2
A132	May 26	May 30	4
A116	May 14	May 19	5
A5	...do.....	May 17	3
A134	May 27	May 30	3
A141	May 31	June 5	5
A15	June 23	June 26	3

LARVÆ.—The eggs hatch in from two to six days, and the young larvæ immediately enter the leaf, making small holes through the lower epidermis. All the eggs of a single group hatch at the same time, and the larvæ feed in a common mine, which is at first linear. The larvæ mine side by side, progressing only in a forward direction. They keep close together, and all change their direction of mining at the same time, leaving behind them a short linear path (Pl. 28, D). In about a day, although no definite time can be set, the larvæ begin to enlarge their mine laterally, forming a blotch. They still remain in a common mine, but separate in different directions. It is not an unusual sight to see several such blotches on a leaf. Each represents a number of larvæ that have hatched from a single group of eggs. These increase in size until they interfere with each other, and a large blotch is produced, covering the entire area of the leaf. Many of the larvæ are naturally forced to abandon their mines and form new ones in other leaves. The presence of nearly mature larvæ in small blotch mines is an indication that they have entered fresh leaves (Pl. 28, H).

The writer has removed a larva from its mine and watched it form a new one. At first the larva cuts a short slit in the epidermis of the leaf. Then by inserting the mouth hooks in this slit and working them back and forth the lower and upper epidermises are separated. The larva then pushes the anterior end of its body into the small opening which it has made. After the first two segments have been forced into the leaf, it is only a matter of a few minutes before the larva works its way completely within it. This operation is accomplished with many vigorous twists of the body as it is drawn into the leaf. Larvæ of the third instar bury themselves completely in the leaf in less than 20 minutes.

The feeding habits of the larvæ are most interesting and can be very conveniently watched under a microscope by means of transmitted light. The pharyngeal skeleton (fig. 1, A–D) bearing the mouth hooks, is loosely joined within the first and second segments and is capable of great freedom of movement. These move very rapidly when the larva is feeding and are very effective tools for tearing away the parenchyma of the leaf. There are two distinct types of movement which the mouth hooks possess. First, a lateral movement; the mouth hooks are turned perpendicular to their normal position and work in the plane of the mine. They strike to the right for a short time, then to the left, separating the upper and lower epidermises but not tearing away any of the tissue. The second movement of the hooks is a vertical one. As the hooks are held in their normal position, they are thrust downward, and pieces of the parenchyma are torn loose from the bottom of the mine. In the first case the two epidermal layers are only separated, while in the second the parenchyma of the leaf is actually removed.

TABLE II.—*Length of the larval period of Pegomyia calyptrata*

Experiment No.	Eggs hatched.	Puparium formed.	Length of larval period.
			Days.
A 126	May 23	June 7	15
A 129	May 25	June 10	16
A 135	May 29	June 8	10
A 35	July 3	July 12	9
A 225	Oct. 8	Oct. 21	13

From Table II it will be seen that the larval period varies somewhat. This variation is undoubtedly due to weather conditions. During warm weather the larvæ mature rapidly, but during cooler weather the miners become inactive, and the larval period may be prolonged several days. Unfortunately the table is not complete enough to show this. However, it shows a tendency for a longer larval period in early May and October than in the latter part of May and July.

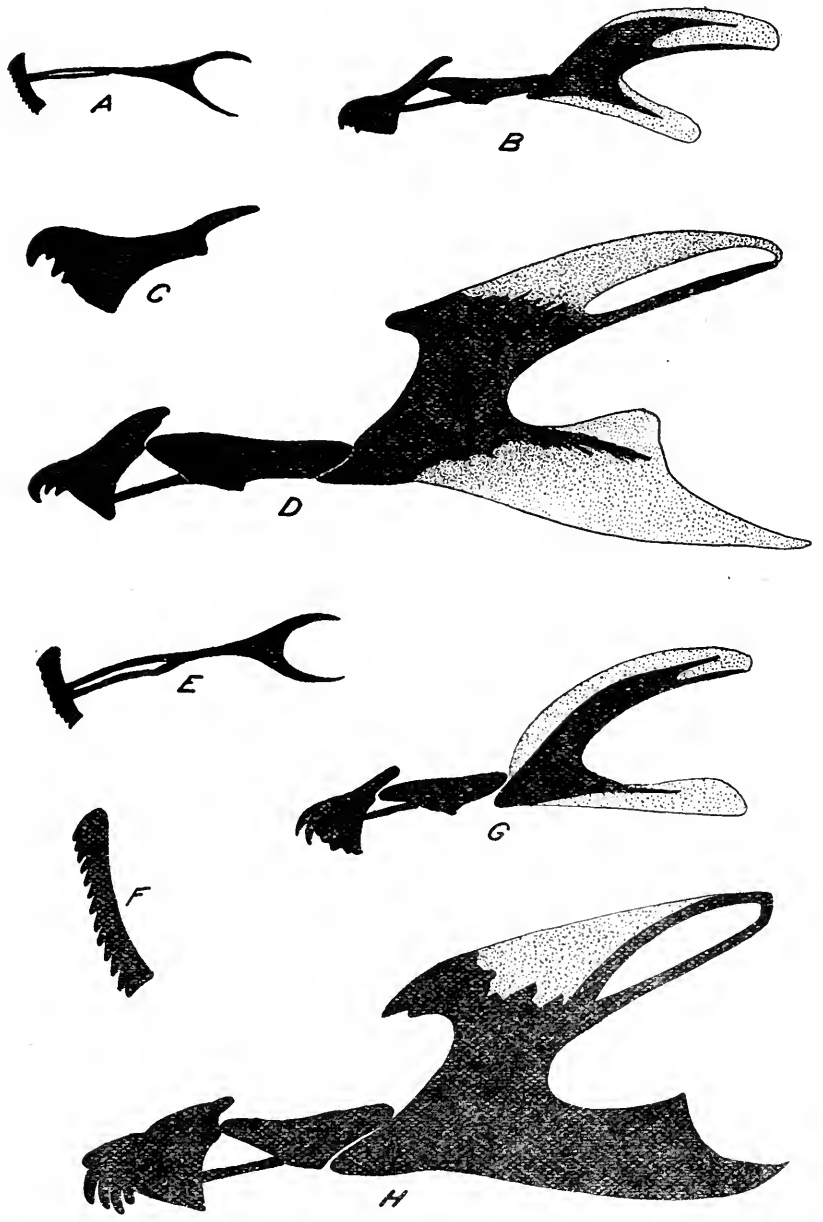


FIG. 1.—*A*, pharyngeal skeleton of first instar, *Pegomyia calyptrata* Zett.; *B*, pharyngeal skeleton of second instar, *P. calyptrata; C*, mandibular sclerite of second instar, *P. calyptrata: D*, pharyngeal skeleton of thjrd instar, *P calyptrata; E*, pharyngeal skeleton of first instar, *P. affinis* Stein.; *F*, mandibular sclerite of first instar, *P. affinis; G*, pharyngeal skeleton of second instar, *P. affinis; H*, pharyngeal skeleton of third instar, *P. affinis*.

FORMATION OF THE PUPARIA.—The mature larvæ escape through the epidermis of the leaf, but seldom make their exit through a definite hole. Usually the upper epidermis become dry and parchment-like, rupturing itself and allowing the larvæ to escape. Sometimes the larvæ cut circular holes through the epidermis. They fall to the ground and ordinarily penetrate the soil to a depth of 2 or 3 inches. If the ground is hard, they do not enter, but form their puparia beneath leaves or other rubbish. The depth to which the larvæ penetrate varies considerably. To determine this, a few experiments were performed with full-grown larvæ. For this purpose a root cage was used, having a space of ¼ inch between the glass and the back. This space was filled with loose sandy soil. The surface of the earth was covered to prevent the larvæ from eacaping. This darkened the top of the soil and slightly altered normal conditions.

Twenty-seven mature larvæ were placed on the surface of the soil. The following day eight puparia were found on top of the earth. The remainder of the larvæ either failed to transform or escaped. In a second experiment 32 mature larvæ were placed on the surface of the soil. Four larvæ transformed on the surface, six were found at a depth of 2 inches, one at a depth of 5 inches, and two at a depth of 6 inches. These experiments, though few in number, give some idea of the depth to which the larvæ penetrate loosely compacted soil.

In captivity many of the larvæ formed their puparia within the leaves. Mined leaves were collected in large numbers and put in receptacles to obtain puparia. In most cases the puparia were found in the leaves or between the leaves from which they had issued. A few of the larvæ wandered about in an attempt to find a more suitable place in which to transform. Evidently many of the larvæ under natural conditions never enter the soil.

ADULTS.—While working with the adults, the writer had an opportunity to experiment with several types of rearing cages. A cage similar to the Riley cage, covered with cheesecloth on three sides and with glass on the fourth, gave good results when large numbers of individuals were used, but proved useless for individual pairs of flies. Glass cylinder cages (Pl. 29, B) yielded better results for this purpose, but were unsatisfactory because the adults escaped when the cylinders were lifted. The most satisfactory type of cage proved to be the Fiske cage (Pl. 29, A, C), used a great deal in the rearing of parasites by the United States Department of Agriculture at Melrose Highlands, Massachusetts. The small opening at the top readily permits one to introduce food, while the ring at the bottom prevents the flies from escaping.

In spite of all the precautions in handling and feeding the adults, they did not live long in captivity. One male was kept alive for 18 days. Table III gives the length of life of the males and females as obtained from pairs that were kept in captivity.

TABLE III —*Length of the life of the adults of Pegomyia calyptrata*

Females.			Males.		
Issued.	Died.	Lived.	Issued.	Died.	Lived.
		Days.			*Days.*
July 2	July 5	3	July 2	July 5	3
July 3	July 19	16	July 3	July 21	18
July 19	July 20	1			
July 20	July 27	7			
Aug. 16	Aug. 23	7	Aug. 16	Aug. 23	7
Do.	Aug. 24	8	Do.	Aug. 22	6
Aug. 22	Sept. 2	11	Aug. 22	Sept. 2	11
Aug. 26	Aug. 30	4	Aug. 26	Aug. 30	4
Aug. 31	Sept. 10	10	Aug. 31	Sept. 7	7
Sept. 1	Sept. 3	2	Sept. 1	Sept. 3	2
Sept. 23	Sept. 28	5			
Oct. 10	Oct. 12	2	Oct. 10	Oct. 12	2
May 6	May 11	5			
			Aug. 16	Aug. 22	6

Copulation frequently takes place a few hours after the female issues. Flies that had issued during the night were observed in several cases to copulate during the following morning. In order to induce copulation, good results were obtained by placing pairs in 6-dram vials. They could be conveniently watched, and mating took place quite readily. Pairs that were in larger cages usually died before copulation took place. Copulation usually lasted about 40 minutes; in one case it lasted for 1 hour and 15 minutes. It is still unknown whether a second or later copulations take place.

The number of eggs laid was obtained only by dissection. About 62 ripe eggs were found in a fertilized female. All these are evidently laid during one oviposition period.

NUMBER OF GENERATIONS.—The writer has been unable to determine accurately the number of generations a year. This is due to the repeated difficulty he experienced in attempts to keep the adults alive for any considerable period of time, as well as the failure to obtain females to oviposit. Only four of many females experimented with laid eggs, and of these none laid more than five eggs. Under such conditions it was impossible to rear the generations through from eggs to adults. However, the number of generations was followed quite accurately by observing conditions in the field and comparing them with conditions under observation at the insectary.

The various generations overlap one another, and it is impossible to tell, from outside conditions alone, when one ends and the other begins. The terminations of the first generation can be plainly seen. For example, in the spring of 1916 the first flies to issue from puparia kept out of doors over the winter appeared on May 4. The adults continued to issue until May 16. The females began laying soon after emerging;

those issuing from puparia in captivity laid in four or five days. This checks with condition in the field, for the first eggs were observed outside on May 7. The larvæ matured in from 9 to 16 days. Toward the end of the larval period they mined rapidly, producing conspicuous blotches on the leaves. The maturing of the first-generation larvæ could thus be easily distinguished. After this it was impossible to follow the number of generations by observation in the field alone. One can go out in the field any time during the summer and find eggs, larvæ, puparia, and adults all at the same time.

From a number of eggs laid in the spring by the females, overwintering as puparia, the writer succeeded in rearing adults of the first generation. These adults laid a few eggs, but none of them hatched. By bringing in eggs from the field at this time the writer reared adults of the second generation. This was continued through the summer, and a fair idea of the number of generations was obtained. During 1915 and 1916 the writer obtained three and a partial fourth generation. The majority of the fourth generation were overtaken by cold weather and perished.

From Table IV it will be seen that some of the puparia formed in June and July, as well as those formed in September, did not give forth adults the same year that they were formed, but overwintered as puparia, and the adults issued the following spring. This seems to be a provision of nature to insure the continuation of the race the following year in case all the individuals of any generation should perish.

TABLE IV.—*Length of the pupal stage of Pegomyia calyptrata*

Experiment No.	Number of puparia.	Puparia formed.	Adults issued.	Number of adults.	Length of stage.
					Days.
A150	6	June 5	July 1	1 ♀	26
A168	4	June 9	July 5	2 ♀	26
		..do....	July 6	1 ♀	27
A165	29	..do....	July 1	6 ♂	22
		..do....	July 3	1 ♀	24
A9	4	June 11	July 6	1 ♂	25
A14	10	..do....	July 3	4 ♂	22
		..do....	July 4	3 ♀	23
A167	29	June 13	July 3	1 ♂	20
A169	10	..do....	July 5	1 ♀	22
A182	17	June 16	July 3	1 ♂ 3 ♀	18
A17	3	June 25	July 16	1 ♂	21
		..do....	May 6[a]	1 ♀	317
A27	3	July 16	Aug. 5	3 ♂	20
A26	5	July 17	Aug. 6	1 ♂	20
		..do....	Aug. 8	1 ♂	22
A28	8	July 23	Aug. 13	1 ♂	21
		..do....	Aug. 14	1 ♂	22
		..do....	May 4[a]	1 ♀	288
A31	6	July 27	Aug. 14	2 ♂	18
		..do....	Aug. 16	1 ♀	20
		..do....	May 5[a]	1 ♀	284

[a] Adults issued the following year from puparia kept out of doors over the winter.

TABLE IV.—*Length of the pupal stage of Pegomyia calyptrata*—Continued

Experiment No.	Number of puparia.	Puparia formed.	Adults issued.	Number of adults.	Length of stage.
					Days.
A32	17	July 29	Aug. 15	2 ♂	17
		..do.....	Aug. 16	1 ♀	18
		..do.....	Aug. 18	2 ♂	20
A34	4	July 30	Aug. 17	1 ♀	18
A36	11	Aug. 1	Aug. 25	1 ♀	24
A37	9	..do.....	Aug. 20	2 ♂	20
		..do.....	Aug. 21	1 ♀	21
A43	6	Aug. 7	Aug. 30	1 ♀ 1 ♂	23
		..do.....	Aug. 31	1 ♀	24
A188	53	Aug. 9	Aug. 29	1 ♀	20
		..do.....	Aug. 30	1 ♂	21
		..do.....	Sept. 1	1 ♀ 1 ♂	23
A190	18	...do.....	...do.....	1 ♀	23
A52	2	Aug. 12	Aug. 30	1 ♀	18
A60	6	Aug. 21	Sept. 11	1 ♂	21
		..do.....	Sept. 13	1 ♀	22
A74	5	Sept. 2	May 6[a]	1 ♀	248
A77	2	Sept. 3	May 5[a]	1 ♀	246
A78	3	Sept. 4	May 4[a]	1 ♂	244
		..do.....	May 5[a]	1 ♀	245
A93	71	Sept. 17	Oct. 9	2 ♂ 4 ♀	23
		..do.....	Oct. 11	2 ♂ 1 ♀	25
		..do.....	May 4[a]	6 ♂ 2 ♀	235
		..do.....	May 5[a]	3 ♀	236
		..do.....	May 6[a]	1 ♂	237
A97	23	Sept. 29	Feb. 14[b]	1 ♂	138[b]
A102	16	Oct. 10	May 4[a]	1 ♂	209
		..do.....	May 5[a]	2 ♀	210
		..do.....	May 6[a]	1 ♀	211
		..do.....	May 7[a]	1 ♀	212
A107	(?)	Oct. 18	May 4[a]	1 ♂	201
		..do.....	May 13[a]	1 ♂	208
		..do.....	May 16[a]	1 ♂	213

[a] Adults issued the following year from puparia kept out of doors over the winter.
[b] Adults issued the following year from puparia kept indoors over the winter.

AN UNUSUAL OCCURRENCE WITH ADULTS.—Twice while working with the adults the writer observed them to issue feet first from the puparium (Pl. 28, F). In one case the fly succeeded in freeing itself from the puparium, but the wings never expanded normally. In the second the feet broke through the puparium, but the fly did not succeed in working itself free. It will be noticed from the illustration that the fly's feet are extending from the posterior end of the puparium, showing that the pupa was formed in its normal position within the puparium.

PREDACIOUS AND PARASITIC ENEMIES

The writer reared two parasites from the puparia of *P. calyptrata*, *Opius quebecensis* Prov., and *Dacnusa scaptomyzae* Gahan. These were kindly determined by Mr. A. B. Gahan, of the Bureau of Entomology, United States Department of Agriculture. Several times the writer observed the latter parasite ovipositing in the larva of the miner.

When the parasite alights on the mine, the larva becomes uneasy and gives several twists in an attempt to avoid the attack. Unfortunately the larva is pinned between the two epidermises of the leaf and has little freedom of movement. Thus, it is a simple matter for the parasite to insert its ovipositor into the larva. The parasites transform within the puparia and issue somewhat later than the flies themselves.

A third parasite was reared from the eggs of *P. calyptrata* and determined by Mr. A. A. Girault as *Trichogramma minutum* Riley. Six parasites issued from three eggs, indicating that more than one parasite develops within a single egg.

In addition to the parasites mentioned above, a larva was attacked by an adult of *Nabis ferus* (L.). The writer also caught a nymph of this species with its beak inserted into the larva of the leaf miner, sucking out the juices from its body. *Nabis ferus* is a predacious species and has been known to attack the larva of *Pegomyia hyoscyami* Panz., the spinach leaf miner.

DESCRIPTION OF THE STAGES OF PEGOMYIA CALYPTRATA

EGG.—The egg (Pl. 28, C) is a dirty white, glossy, elongate, and nearly cylindrical in shape. It has a reticulated surface composed of polygonal areas. The micropyle end is rounded or slightly flattened. The opposite end is distinctly pointed. Length 1.18 mm.; width 0.35 mm.

FIRST-STAGE LARVA.—The newly hatched larva measures 1.3 mm. It is creamy white in color and more or less transparent. The trachea and alimentary canal are visible through the integument for the whole length of the body. The first-stage larva possesses the same number of segments as the mature larva, 12 visible segments, but the segmentation is not distinct. The body is rather smooth, except for the minute fleshy locomotory spines, which are located on the intersegmental areas and the margins of each segment posterior to the second. These fleshy spines are arranged in discontinuous thickly set rows encircling the body slightly oblique to the edges of the segments. The first segment, "pseudo-cephalon" (Henneguy) (Pl. 30, E), bears a pair of sensory papillæ a short distance in front of the mouth opening. On each side of the "pseudo-cephalon" there is a row of 12 to 13 minute button-like areas which extend dorsally from the mouth opening. The pharyngeal skeleton is slender and not as highly chitinized as in the later stages. The mandibular sclerite is elongate and serrated, having about 12 sharp teeth. The anterior spiracles are closed, while the posterior spiracles have single breathing pores. The posterior ends of the trachea are slightly chitinized and appear as two parallel chitinized bars at the posterior end of the larva and are more conspicuous than the spiracles themselves.

SECOND-STAGE LARVA.—The segmentation in the second-stage larva is more conspicuous. The alimentary canal and trachea become obscured by the accumulation of fat and the larva becomes more yellowish in color. The "pseudo-cephalon," as in the previous stage, bears a pair of sensory papillæ. In addition, it has a pair of two segmented antennæ which are located slightly posterior to the papillæ and are not as closely approximated as the latter. The pharyngeal skeleton is stronger and more highly chitinized. The mandibular sclerite is triangular and bears four teeth. The button-like areas on the side of the "pseudo-cephalon" are present but reduced to five in number. Similar to the first-stage larva the intersegmental areas and edges of the segments posterior to the second are encircled by several discontinuous thickly set rows of minute fleshy locomotory spines. These become less pronounced posteriorly. There is an anterior spiracle on each side inserted between the second and third body segments. Each spiracle has from 24 to 26 breathing pores arranged about an oval slightly chitinized, flattened disk. The posterior spiracles project slightly and have two narrow breathing pores. The anus opens between two triangular plates on the venter of the last segment.

THIRD-STAGE LARVA.—When mature the third-stage larva measures from 9 to 9.5 mm. It is yellowish in color, distinctly shiny, and beadlike in shape, especially when viewed from above. There is a reddish area within the anterior end of the larva which disappears when the larva is preserved in alcohol. The "pseudo-cephalon," as before, bears in front of the mouth opening a pair of two jointed antennæ, and in front of these a pair of sensory papillæ. Five minute button-like areas are also present on the side of the "pseudo-cephalon." The pharyngeal skeleton is even more highly chitinized than in the second-stage larva. The mandibular sclerite resembles that of the second-stage larva, but bears three teeth. The intersegmental areas and edges of the third to the last segment are encircled with minute fleshy locomotory spines as in the previous stages. The posterior spiracles are borne on short tubercles and have three breathing pores. The anus opens between two triangular plates (Pl. 30, D). Table V gives the distinguishing characters of the three larval stages.

PUPARIUM.—The puparium when first formed is yellowish with a reddish area at the anterior end similar to that found in the larva. The colored area soon disappears, and in about two hours the puparium becomes reddish brown in color. The divisions of the segments are marked by a grayish powdery substance. The surface of the segments are marked by fine annular striæ. The triangular anal plates of the larva are visible in the puparium. The adult in issuing breaks a piece from the anterior dorsal portion of the puparium, including the first, second, third, and part of the fourth segments. The anterior spiracles of the larva are removed with the cap, which is broken loose. The mouth hooks of the

third-stage larva can then be seen attached to the inner ventral wall of the puparium.

TABLE V.—*Distinguishing characters of the larval stages of Pegomyia calyptrata*

Characters.	First stage.	Second stage.	Third stage.
Reddish area at anterior end.	Absent	Absent	Present.
Sensory papillæ	do	Present	Do.
Button-like areas on side of "pseudocephalon."	12 to 13	5 (Pl. 30, E)	5.
Pharyngeal skeleton.	Slender, weakly chitinized.	Stout, strongly chitinized.	Stout, strongly chitinized.
Mandibular sclerite	Elongate, serrated, 12 teeth.	Triangular with long basal projection, 4 teeth.	Triangular, basal projection short, 3 teeth.
Anal spiracle	One breathing pore.	Two breathing pores.	Three breathing pores.

THE LESS COMMON SPECIES, PEGOMYIA AFFINIS

HISTORICAL REVIEW

This species has almost escaped the keen eye of the systematist. It was described by Stein (1897)[1] as *Pegomyia vicina* Lintner. Later he noticed his mistake and in the same paper [1] named the new species "*Pegomyia affinis*." Since that time there has been no reference to this species. The writer describes for the first time the habits of this interesting anthomyid.

DISTRIBUTION

Little or nothing is known about the distribution of *P. affinis*. Stein[1] records it rather abundant in Pennsylvania, Vermont, and Illinois. The writer has reared and collected specimens at Ithaca and Florida, N. Y., and Arendtsville, Pa.

HOST PLANTS

P. affinis, mines exclusively on *Rumex* spp. Adults have been reared from *R. crispus* and *R. obtusifolius*. It is possible that other species of Rumex are attacked.

LIFE HISTORY

EGGS.—The eggs are much more beautiful than those of *P. calyptrata* and are pure white in color. They are laid in neat transverse rows of three to five, rarely six or seven, on the under surface of the leaf. They have never been found as abundant as those of the preceding species and never more than three or four groups have been found on a single leaf. The incubation period is given in Table VI.

[1] STEIN, P. NORDAMERIKANISCHE ANTHOMYIDEN. *In* Berlin. Ent. Zeitschr., Jahrg. 42, p. 239-241, 286. 1897.

TABLE VI.—*Incubation period of the eggs of Pegomyia affinis*

Experiment No.	Eggs laid.	Eggs hatched.	Length of stage.
			Days.
A115	May 11	May 16	5
A119	May 14	May 21	7
A125	May 18	May 24	6
A130	May 24	May 28	4
A131	May 25	...do.....	3

LARVA.—The eggs hatch in from three to seven days. All the eggs of a single group hatch at the same time, and the young larvæ feed together in a common mine. At first this is linear, but soon the larvæ separate in different directions and a blotch mine is formed which obscures the original linear track. The mines produced on the leaves can not be distinguished from those of P. *calyptrata*.

TABLE VII.—*Length of the larval period of Pegomyia affinis*

Experiment No.	Eggs hatched.	Puparium formed.	Length of larval period.
			Days.
A2	May 11	May 23	12
A124	May 21	June 6	16
A124	...do.....	June 8	18
A127	May 22	June 7	16
A143	May 31	June 13	13

From Table VII it will be seen that the length of the larval period varies from 12 to 18 days. A larger number of records would, perhaps, show even greater variation. This variation is due, as in case of *P. calyptrata*, to weather conditions.

The mature larvæ escape from the leaves through the cracked surface of the dried and parchment-like mine. If the ground is not too hard, they penetrate to a depth of 2 or 3 inches; otherwise, they form their puparia beneath leaves or rubbish.

NUMBER OF GENERATIONS.—The writer is uncertain as to the number of generations. At first he confused the two species mining dock and thought there was but one. Later he noticed his mistake, but it was then too late to make definite observations. The few notes made seem to indicate that there are but two generations a year. A portion of the first-generation adults issued in from 12 to 18 days; the rest overwintered as puparia and issued the following spring. It is believed that all of the second generation overwinter as puparia and issue the following spring. This seems to be the case, because the eggs and larvæ of this species were not found after the end of June.

Table VIII shows the tendency of the first generation to produce some puparia from which adults issue the same year and others that overwinter and from which adults issue the following year. The species is like *P. calyptrata* in this respect.

TABLE VIII.—*Tendency of individuals of the first generation to overwinter as puparia*

Experiment No.	Number of puparia.	Puparium formed.	Adults issued.	Number of adults.	Number of days.
A13	4	June 9	July 3	1 ♂	24
A11	2	...do.....	May 6[a]	2 ♀	333[a]
A10	2	...do.....	July 3	1 ♂	24
A12	9	June 11	May 16[a]	1 ♂	342[a]
A16	4	June 24	May 6[a]	1 ♂ 2 ♀	319[a]
A19	4	July 1	...do.....	3 ♀	313[a]

[a] Overwintered as puparia and issued the following spring.

DESCRIPTION OF STAGES

EGG.—The egg is pure white, waxy, and elongate, with a delicate reticulated surface. This reticulation consists of rectangular areas arranged about the egg in parallel longitudinal rows, giving the egg a very regular and beautiful appearance. The micropyle end is slightly flattened. The opposite end is rounded. Length 1 mm.; width 0.35 mm.

FIRST-STAGE LARVA.—The newly hatched larva measures about 1 mm. It is translucent white in color, and the trachea and alimentary canal are visible through the integument for the entire length of the body. The body is decidedly smooth with the exception of the intersegmental areas and the posterior aspect of the last segment which are covered with many transverse rows of fleshy locomotory spines. Anterior to the mouth opening there are a pair of sensory papillæ. The tubercles on the posterior aspect of the last segment are not distinct. The posterior spiracles are borne on short stalks and have single breathing pores.

SECOND-STAGE LARVA.—At first the trachea and alimentary canal are visible through the integument, but soon they become obscured by the accumulation of fat. The whole body is minutely roughened, especially so on the intersegmental areas and the edges of some of the segments. This roughening is caused by transverse rows of minute fleshy locomotory spines, as described in the first-stage larva. The pharyngeal skeleton is strongly chitinized, and the mandibular sclerite bears two well-defined teeth and several smaller ones. Anterior to the mouth opening are a pair of two-jointed antennæ, and in front of these a pair of sensory papillæ. On each side of the "pseudo-cephalon" are a row of minute button-like areas which extend from the mouth opening dorsad. The posterior spiracles have two breathing pores.

THIRD-STAGE LARVA.—The third-stage larva resembles very much the previous stage, but is a dirty white in color. The locomotory spines on the intersegmental areas are more conspicuous than in the second-stage larva. They are usually rendered visible to the naked eye by means of the dirt which catches between them. As in the previous stage the "pseudo-cephalon" bears a pair of two-jointed antennæ and a pair of sensory papillæ in front of the mouth cavity. The anterior spiracles consist of oval slightly chitinized disks surrounded by about 24 breathing pores. On the posterior aspect of the last segment are six small tubercles surrounding the spiracles in a semicircle on the ventral side. The posterior spiracles have three breathing pores.

PUPARIUM.—The puparium is white or slightly creamy in color when first formed, but in about two hours it turns dark brown or almost black. When the puparium becomes dry, it turns grayish in color. The two anal plates of the larva are visible in the puparium and are surrounded by a ring.

COMPARISON OF THE TWO SPECIES

A comparison of the characters of the two species is given in Table IX.

TABLE IX.—*Comparison of the characters of Pegomyia calyptrata and P. affinis*

Characters.	*P. calyptrata*	*P. affinis*
EGGS.		
Color	White, glossy	White, waxy.
Shape	Pointed at one end	Rounded at both ends.
Reticulations	Polygonal	Rectangular.
LARVA.		
Color	Yellow, shiny	Dirty white, dull.
Mouth hooks	With three teeth	With four teeth.
Sensory papillæ	Close together, smaller than antenna.	Not close together, about same size as antenna.
Posterior end	Not distinctly tuberculate	Distinctly tuberculate.
PUPARIUM.		
Color	Brown	Black.
Segmentation	Beadlike	Not beadlike.
ADULT.		
Color	Abdomen yellow, thorax bluish gray.	Abdomen and thorax gray.

PLATE 28

A.—Eggs of *Pegomyia affinis*.

B.—Parasitized eggs of *Pegomyia calyptrata*.

C.—Eggs of *Pegomyia calyptrata*.

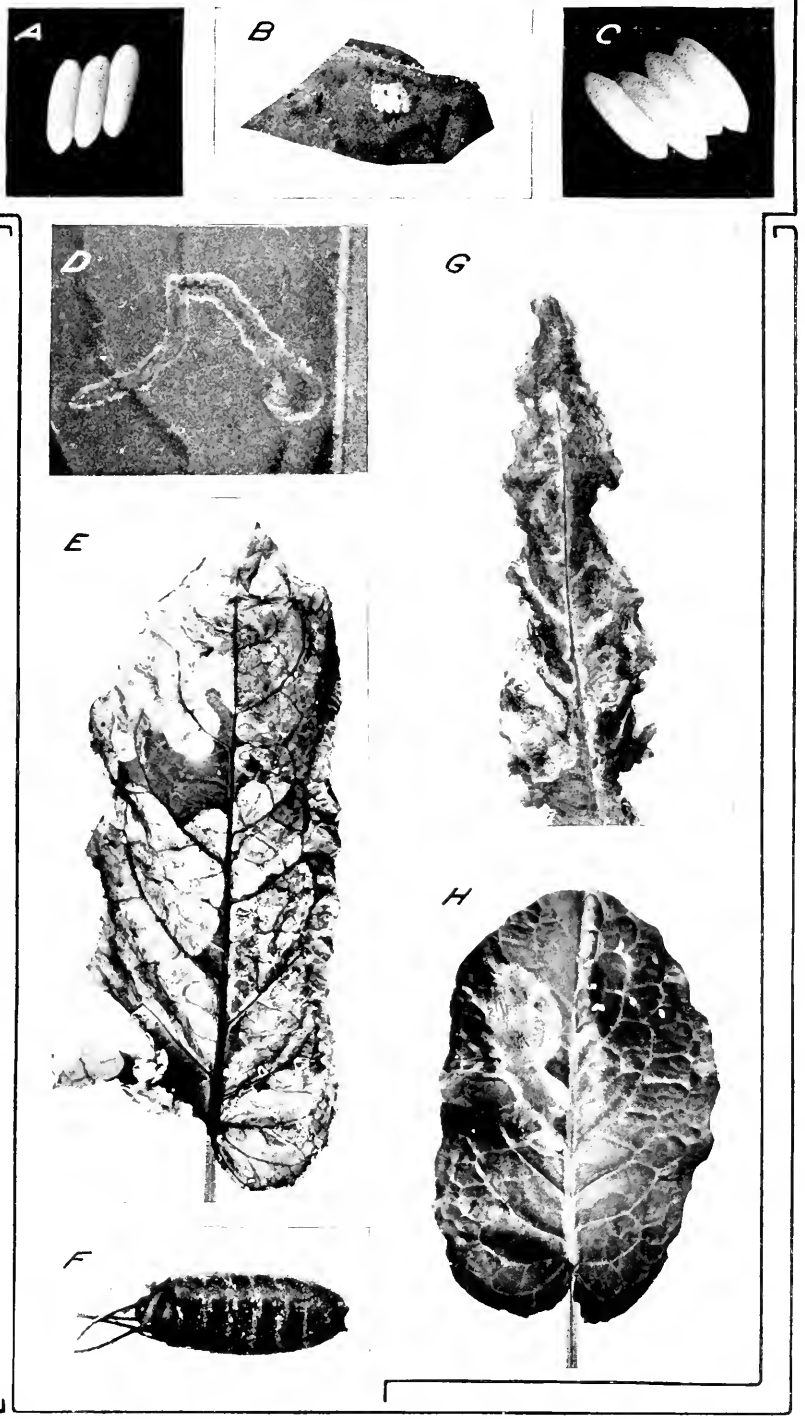
D.—A small mine on Rumex leaf, showing the original linear mine and the beginning of the blotch mine.

E.—A typical mine on *Rumex obtusifolius* produced by the larva of *Pegomyia calyptrata*.

F.—A monstrosity, an adult *P. calyptrata* issuing feet first from its puparium.

G.—A typical mine on *Rumex crispus* produced by the larva of *Pegomyia calyptrata*.

H.—A mine on *Rumex obtusifolius*, produced by a nearly mature larva entering a new leaf to complete its development.


A
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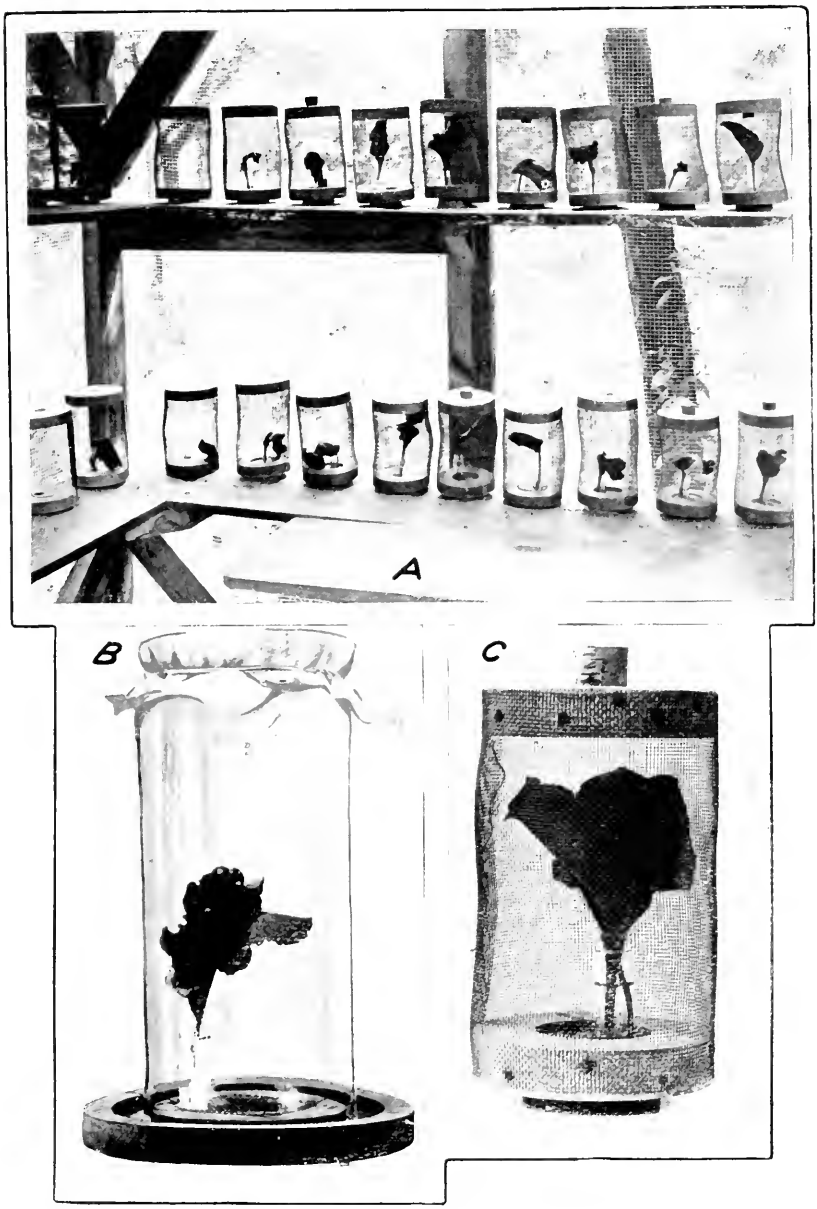
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PLATE 29

A.—Fiske cages, used in studying the adult flies of *Pegomyia* spp., showing the arrangement in outdoor insectary.

B.—Glass cylinder cage, used in studying habits of adult flies.

C.—Fiske cage, used in studying habits of adult flies.

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PLATE 30

A.—Ventral aspect of posterior segment of larva of *Pegomyia affinis*, showing lobes and anal opening.

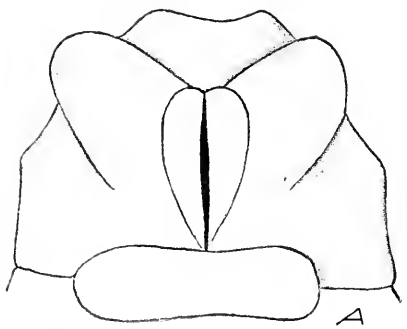
B.—Lateral aspect of posterior segment of larva of *P. affinis*, showing tubercles.

C.—Lateral aspect of posterior segment of larva of *P. calyptrata.*

D.—Ventral aspect of posterior segment of larva of *P. calyptrata*, showing lobes and anal opening.

E.—"Pseudo-cephalon" (Henneguy) of *P. calyptrata*: *a*, button-like areas referred to in text; *b*, sensory papilla; *c*, antenna; *d*, mandibular sclerite; *e*, anterior spiracle.

F.—Posterior spiracle of larva of *P. calyptrata*.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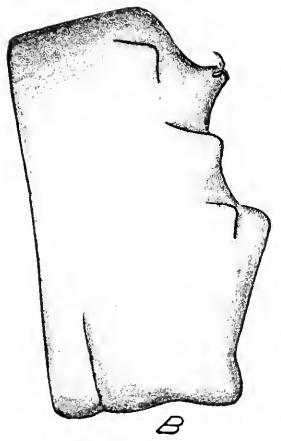
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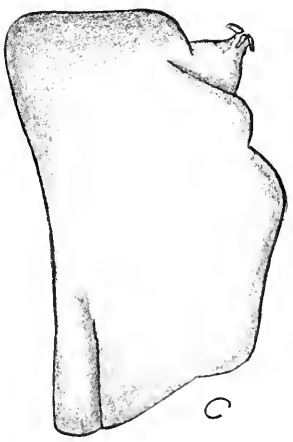
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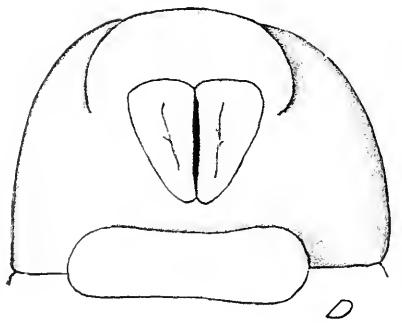
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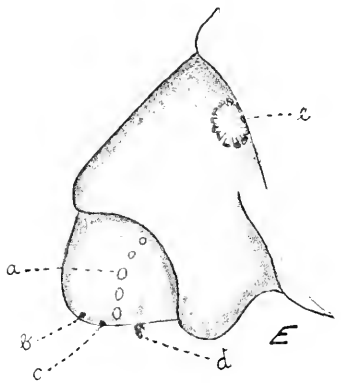
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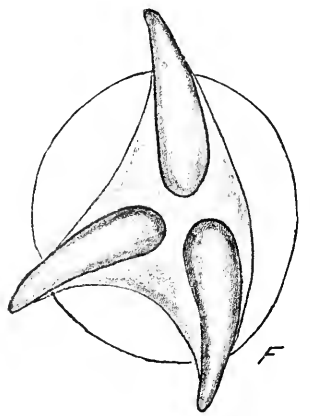
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INFLUENCE OF FOREIGN POLLEN ON THE DEVELOPMENT OF VANILLA FRUITS

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INTRODUCTION

The Agricultural Experiment Station at Mayaguez, Porto Rico, has been conducting experiments for a number of years with a view to the establishment of vanilla growing on a commercial scale in Porto Rico. Material representing many species of Vanilla has been obtained, and the adaptation of these species to Porto Rican conditions is being studied. The questions of pollination, fertilization, yield, and character of the finished product have been given attention. The two types of economic value are *Vanilla planifolia*, a plant having a slender pod of high quality, and the "vanillon" type, which has a shorter, thicker pod greatly inferior in quality and market value to the pod of *V. planifolia*, but which possesses a marked advantage in the ease with which it may be cured, since on ripening it does not split open if allowed to remain several days beyond a certain point in ripening which is difficult to determine, as the change in color is slight.

Since the production of hybrids presented alluring possibilities, various reciprocal crosses were made with a view to the development of valuable strains. It soon appeared that these fruits of these hybrids were noticeably different from the others on the same vines. In order to study this phenomenon, numerous additional crosses were subsequently made. The data presented below show an immediate influence of the foreign pollen on the form of the fruits.

Only a very small percentage of vanilla blossoms are pollinated by natural means in Porto Rico. In a series of blossoms of *V. planifolia* under observation by the writer at the Porto Rico Experiment Station only 1.5 per cent of the blossoms were so pollinated.

In hand pollination the usual method and by far the simplest is to use the pollen of the same flower. Where pollen from another flower of the same species has been applied, no resultant change in the form of the fruit produced has been observed.

The typical well-developed fruit of *V. planifolia* from a close-fertilized blossom is a long slender capsule tapering at the stem end but carrying its fullness well down toward the blossom end. It contains thousands of tiny, oily, black seeds.

[1] The writer wishes to acknowledge his indebtedness to Dr. H. J. Webber, Dean of the Graduate School of Tropical Agriculture, University of California, for suggestions.

The other type of *Vanilla* spp. on which data are here presented is termed "vanillon." Under this name are grouped a number of varieties or species other than *V. planifolia* which, though presenting distinct differences, agree among themselves in having large yellow blossoms in contrast to the much smaller, paler, greenish blossoms of the *V. planifolia*. The fruits are much thicker and shorter than those of the latter and differ from it in being of a more uniform thickness near the two ends, the blossom end frequently being rather tapering.

Where to either the *V. planifolia* or the vanillon stigma pollen of the other has been applied a very decided modification in the form of the fruit has resulted. This modification is in most instances so decided that these fruits can be distinguished from the close-fertilized fruits at a glance.

These conclusions were reached as result of observation and data on crosses made in 1916. Data taken on crosses in 1917 confirmed the work of the preceding season. The accompanying illustrations and tables present the work of the two seasons.

TABLE I.—*Comparison of the girth measurements of the fruit of Vanilla planifolia* ♀ × *V. planifolia* ♂

Length of fruits.	Girth in sixteenths of an inch at 1½ inches from stem end.	Increase or decrease in circumference of pods.	Girth in sixteenths of an inch at 1 inch from blossom end.
Inches.			
6½	22	<	26
6½	18	<	22
7	20	<	24
7	20	<	22
7	22	<	24
7¼	22	<	28
7¼	20	<	25
7½	22	<	24
7½	22	<	25
7¾	23	<	25
7¾	23	<	28
8	22	<	26
8	20	<	24
8½	22	<	28
8½	24	<	28
8¾	20	<	30
8¾	21	<	33
8¾	23	<	30
9	23	<	26
9	24	<	28
9¼	24	<	30
9¼	22	<	28
9¾	22	<	29
Total	501	<	613
Average	21.8	<	26.7

In the tables the measurements of girth are given in sixteenths of an inch expressed as integers for readier comparison. The comparative relation of the two girths is indicated by the symbol between them. "P" indicates *V. planifolia*. "V." indicates species of Vanilla of vanillon type, the accompanying numeral specifying the particular variety. V 13 is a Guadeloupe variety, V 34, V 43, V 51, and V 52 are from Panama, and V 62 is from Mexico.

Table I shows that the typical fruit of *V. planifolia* is considerably greater in girth at 1 inch from the blossom end than at 1½ inches from the stem end. This difference amounted to 22.4 per cent in these fruits. They had been selected to compare in length with the crosses. One hundred unselected fruits which averaged somewhat smaller than these gave the same relative difference of 19 per cent, 93 showing a greater apical girth, with 7 having the two girths equal. The latter is true chiefly in short, poorly developed fruits. These measurements show that the typical, well-developed fruit tapers considerably more at the stem end than at the blossom end.

Table II shows the diametrically opposite development where *V. planifolia* has been fertilized by vanillon pollen. The fruits which developed from the crossed flowers tapered at the blossom end and were well filled at the stem end. The girth at 1½ inches from the stem end measured 25.1 per cent greater than that at 1 inch from the blossom end.

TABLE II.—*Comparison of the girth measurements of the fruit of Vanilla planifolia ♀ × vanillon ♂*

V ♂	Length of fruit in inches.	Girth in sixteenths of an inch at 1½ inches from stem end.	Increase or decrease in circumference of pods.	Girth in sixteenths of an inch at 1 inch from blossom end.
V 52	6½	25	>	18
V 34	6¾	26	>	20
V 34	7	27	>	22
V 52	7	24	>	18
V 52	7	26	>	20
V 34	7¼	26	>	20
V 52	7¼	26	>	20
V 52	7½	26	>	20
V 62	7½	28	>	21
V 62	7½	28	>	20
V 52	7¾	29	>	22
V 34	8	30	>	21
V 52	8	27	>	20
V 52	8¼	30	>	24
V 52	8½	26	>	24
V 52	8¾	30	>	22
V 52	8¾	30	>	28
V 52	9	28	>	26
V 52	9¼	30	>	27
V 52	9¾	32	>	30
Total	.....	554	>	443
Average	.....	27.7	>	22.2

Typical specimens are shown in Plate 31, figures A showing the whole fruits and figure B the same with sections made at the lines of measurements. From left to right the first of the paired fruits shows the development of *V. planifolia* when close-fertilized, while the second shows the results when vanillon pollen has been applied. The sections show that many more ovules have been fertilized near the apex of the ovary by the *V. planifolia* pollen, while the vanillon pollen has fertilized many more near the base than near the apex. This difference in location of the ovules fertilized accounts for the striking difference in form which results from the application of *V. planifolia* or vanillon pollen to the *V. planifolia* stigma.

Table III shows the typical close-pollinated vanillon fruit to be of nearly equal average girth at 1 inch from either end. Of the 54 fruits measured the two girths were equal in 12, the apical girth greater in 18, and the basal girth greater in 24 fruits. The girth at 1 inch from stem end averaged 0.5 per cent greater than at 1 inch from blossom end.

TABLE III.—*Comparison of the girth measurements of the fruit of vanillon ♀ × vanillon ♂*

V	Length of fruit in inches.	Girth in sixteenths of an inch at 1 inch from stem end.	Increase or decrease in circumference of pods.	Girth in sixteenths of an inch at 1 inch from blossom end.
V 51	3	34	>	32
V 13	3 1/2	39	>	36
V 13	3 1/2	37	=	37
V 13	3 1/2	40	=	40
V 34	3 1/2	30	>	29
V 13	3 3/4	41	>	39
V 13	3 3/4	40	=	40
V 51	3 3/4	38	>	36
V 52	3 3/4	40	>	36
V 52	3 3/4	40	>	37
V 52	4 3/4	36	>	34
V 13	4	40	=	40
V 13	4	42	=	42
V 34	4	32	>	31
V 43	4	40	>	32
V 43	4	31	>	29
V 51	4	40	>	36
V 13	4 1/4	42	<	43
V 13	4 1/4	44	<	45
V 13	4 1/4	42	<	43
V 51	4 1/4	41	>	40
V 13	4 1/2	45	=	45
V 13	4 1/2	42	<	43
V 13	4 1/2	43	=	43
V 13	4 1/2	43	<	44
V 52	4 1/2	42	>	38
V 13	4 3/4	46	>	45
V 13	4 3/4	41	<	45
V 52	4 3/4	41	>	40
V 52	5	47	=	47
V 52	5	42	=	42
V 52	5	41	<	44
V 34	5 1/4	35	<	36
V 34	5 1/4	33	=	33
V 43	5 1/4	37	>	34
V 34	5 1/2	35	<	36

TABLE III.—*Comparison of the girth measurements of the fruit of vanillon ♀ × vanillon ♂*—Continued.

V	Length of fruit in inches.	Girth in sixteenths of an inch at 1 inch from stem end.	Increase or decrease in circumference of pods.	Girth in sixteenths of an inch at 1 inch from blossom end.
V 43	5½	43	>	38
V 43	5½	40	=	40
V 34	5¾	34	<	36
V 43	5¾	42	>	39
V 34	6	35	<	38
V 34	6	31	<	35
V 43	6	46	>	45
V 34	6¼	37	<	42
V 43	6¼	43	>	41
V 34	6½	32	<	35
V 43	6½	38	=	38
V 43	6½	39	>	38
V 34	6¾	29	<	34
V 43	6¾	35	<	[a] 42
V 43	6¾	44	<	48
V 43	6¾	46	>	44
V 43	7	45	<	47
V 43	7¼	45	>	43
Total		2, 126	>	2, 115
Average		39. 4	>	39. 2

[a] This fruit which externally resembled a V♀×P♂ cross had ovules fertilized throughout its length which was true of no crosses of V43 ♀×P♂.

TABLE IV.—*Comparison of the girth measurements of the fruit of vanillon ♀ × Vanilla planifolia ♂*

V ♀	Length of fruit in inches.	Girth in sixteenths of an inch at 1 inch from stem end.	Increase or decrease in circumference of pods.	Girth in sixteenths of an inch at 1 inch from blossom end.
V 51	2¾	28	<	33
V 13	3¼	33	<	36
V 34	3¼	24	<	33
V 13	3½	34	<	39
V 13	3½	34	<	40
V 13	3¾	36	<	40
V 13	3¾	35	<	42
V 52	3¾	38	<	43
V 13	4	37	<	44
V 13	4	27	<	43
V 52	4	41	<	44
V 13	4¼	38	<	45
V 43	4½	31	<	45
V 43	4½	29	<	44
V 34	4¾	32	<	41
V 43	4¾	27	<	40
V 34	5	31	<	43
V 34	5	28	<	41
V 43	5	30	<	43
V 43	5¼	35	<	49
V 43	5¼	34	<	47
V 62	5¼	36	<	40
V 43	5½	35	<	50
Total		753	<	965
Average		32. 7	<	42. 0

Table IV shows the decided change in form produced by the application of *V. planifolia* pollen to the vanillon stigma. Without exception the apical girth of the fruits from crossed flowers was greater than the basal girth. The average girth at 1 inch from blossom end was 28.2 per cent greater than that at 1 inch from stem end. Not only was this relative difference evident in the development of the two ends of the crossed fruits but while the development of the base of the crosses fell far below that of the close-fertilized fruits, the development of the apex in the former exceeded that of the latter for the fruits measured.

Plate 32, A, shows V 13 fertilized by *V. planifolia* in the upper row with close-fertilized fruits of V 13 in the lower row. Figure B shows the same fruits with sections cut at the lines of measurement. These sections show the fertilization in the two ends to be much more uniform for the close-fertilized fruits than for the crossed fruits which show a heavier fertilization near the apex than near the base.

In Plate 33, A and B, at the right are shown four fruits of V 34 from blossoms fertilized by *V. planifolia*. The four at the left are from close-fertilized blossoms of V 34. The heavy fertilization of ovules in the apex of the crosses is clearly shown.

In Plate 34, A and B, are shown fruits of V 43, the upper row from blossoms fertilized by *V. planifolia* pollen, the lower row from close-fertilized blossoms. The effect of the *V. planifolia* pollen was more pronounced on this variety than on any other tested, as none of the seven crossed fruits showed any ovules fertilized in the stem end, in some instances none for more than 2 inches from the base, though all showed large numbers of ovules fertilized near the blossom end. All of the close-fertilized fruits examined, however, showed many ovules fertilized near the base, fertilization here being frequently heavier than near the blossom end. In figure B at the top are shown the middle sections of the two fruits at the left in the upper row. The point to which the ovules are fertilized is clearly shown, the ovules appearing as black dots. At the bottom of figure A the middle sections of the two fruits at the left in the lower row show fertilization throughout.

Plate 35, A, shows longitudinal sections of typical fruits, being from left to right V ♀ × V ♂, V ♀ × P ♂, P ♀ × V ♂, and P ♀ × P ♂.

The proportions of the *V. planifolia* and vanillon blossoms suggest a possible reason for the difference in location of the ovules fertilized by the two kinds of pollen with the resultant alteration in the form of the fruit. At blossoming the difference in the length of the ovaries is slight, but the vanillon column is much longer than that of *V. planifolia*, exceeding the length of the latter in some instances by as much as 60 to 70 per cent. Plate 35, B, shows a cleared vanillon column and ovary above with that of *V. planifolia* below, placed to compare the length of ovary at the right and the length of column at the left.

It seems quite reasonable to suppose from the heavy fertilization of ovules near the apex and sparse fertilization or entire absence of fertilization near the base of the ovary when the vanillon stigma has been pollinated with *V. planifolia* pollen that these pollen tubes are unable to reach, or reach in only limited numbers the ovules in the far end of the ovary, which are at a considerably greater distance from the stigma than the farthest ovules of the *V. planifolia* ovary. Even in its own ovary the *V. planifolia* pollen causes a much heavier fertilization near the apex than near the base. This inability of *V. planifolia* pollen tubes to reach the farthest ovules was particularly marked when *V. planifolia* pollen was applied to V 43, which is one of the largest flowered of the vanillon varieties.

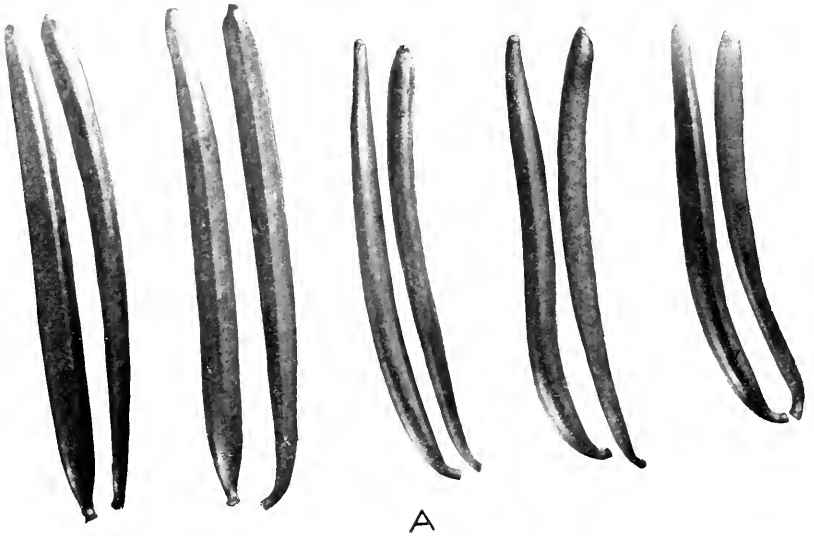
The vanillon pollen tubes, however, reach ovules in the *V. planifolia* ovary at a much shorter distance from the stigma than in their own flower. Many of these first ovules which the *V. planifolia* pollen would fertilize are left unfertilized by the vanillon pollen, the pollen tubes passing by to other ovules which are nearer the normal distance from stigma to ovary in the vanillon flower, and causing a much heavier fertilization in the base of the pod than would the *V. planifolia* pollen.

This might possibly indicate in this instance the necessity for a certain maturity of development of the pollen tube before the ovule can be fertilized.

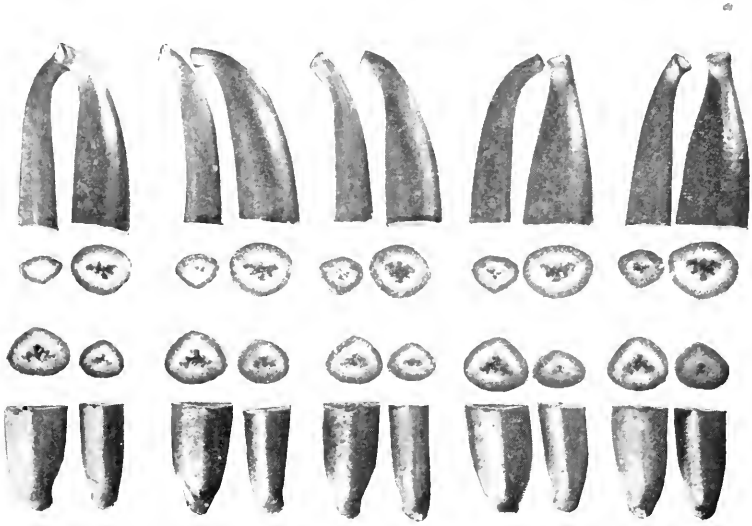
PLATE 31

A.—Left to right, *Vanilla planifolia*, first of each pair close-fertilized, second fertilized with vanillon pollen.

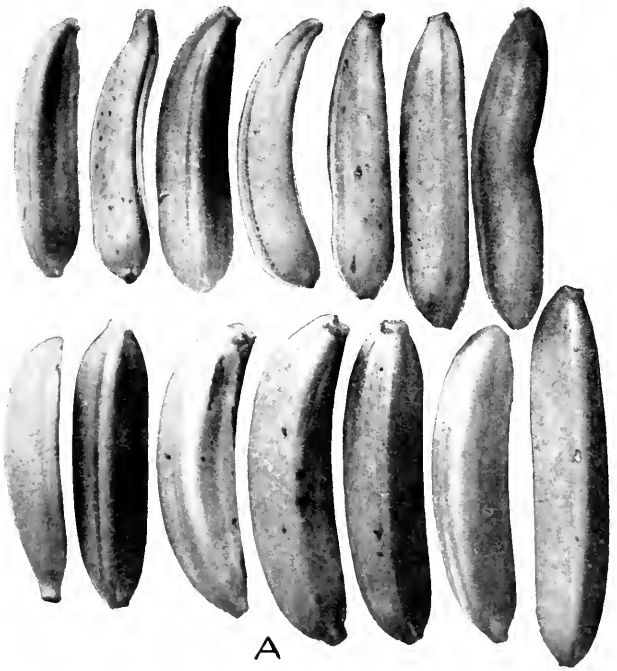
B.—Cross sections of same fruits.

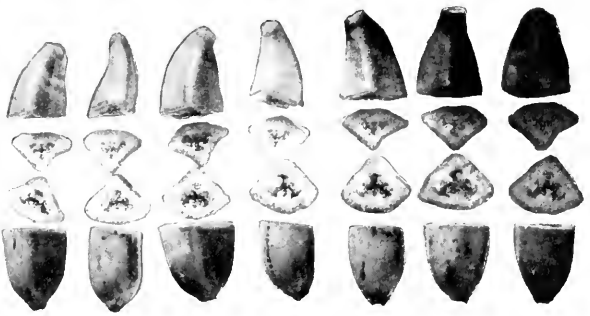


A



B


A


B

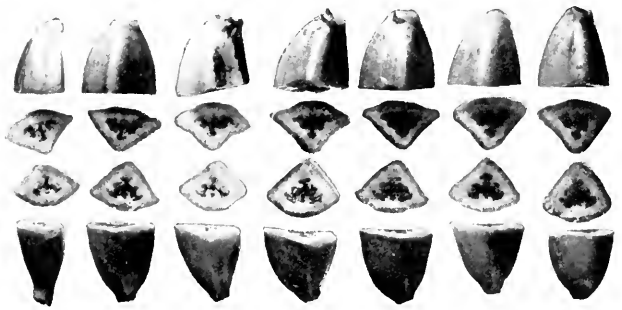


PLATE 32

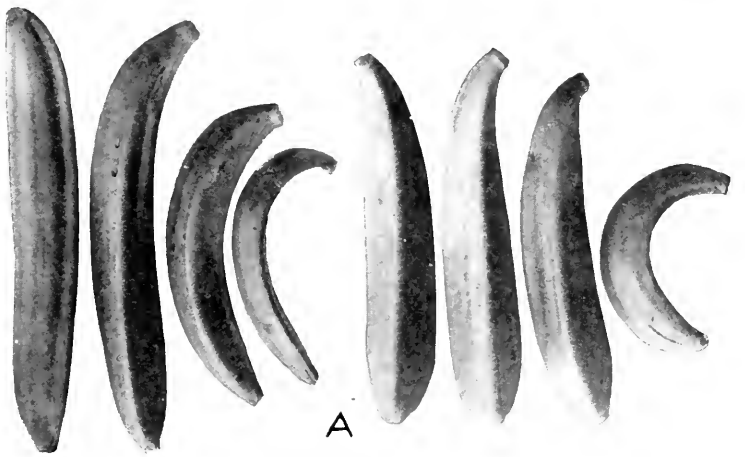
A.—Vanillon No. 13 fruits, close- and cross-fertilized. Lower row close-fertilized, upper fertilized with pollen of *Vanilla planifolia*.

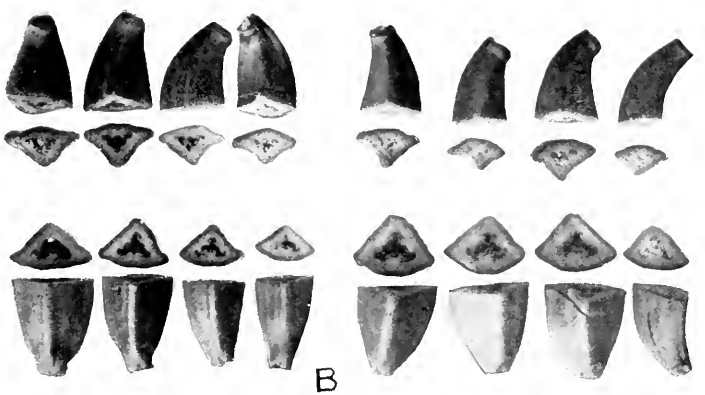
B.—Cross section of same fruits.

PLATE 33

A.—Vanillon No. 34: Fruits at right fertilized with pollen of *Vanilla planifolia*, left close-fertilized.

B.—Cross sections of same fruits.


A


B

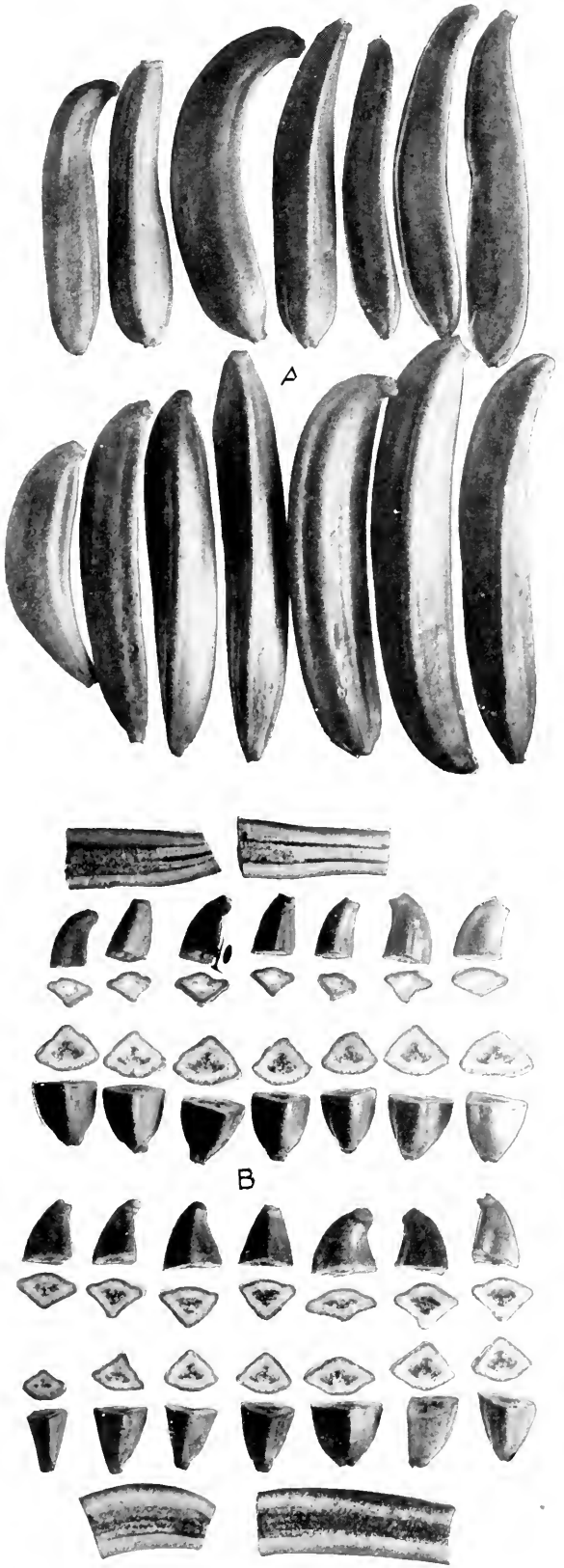

A
B

PLATE 34

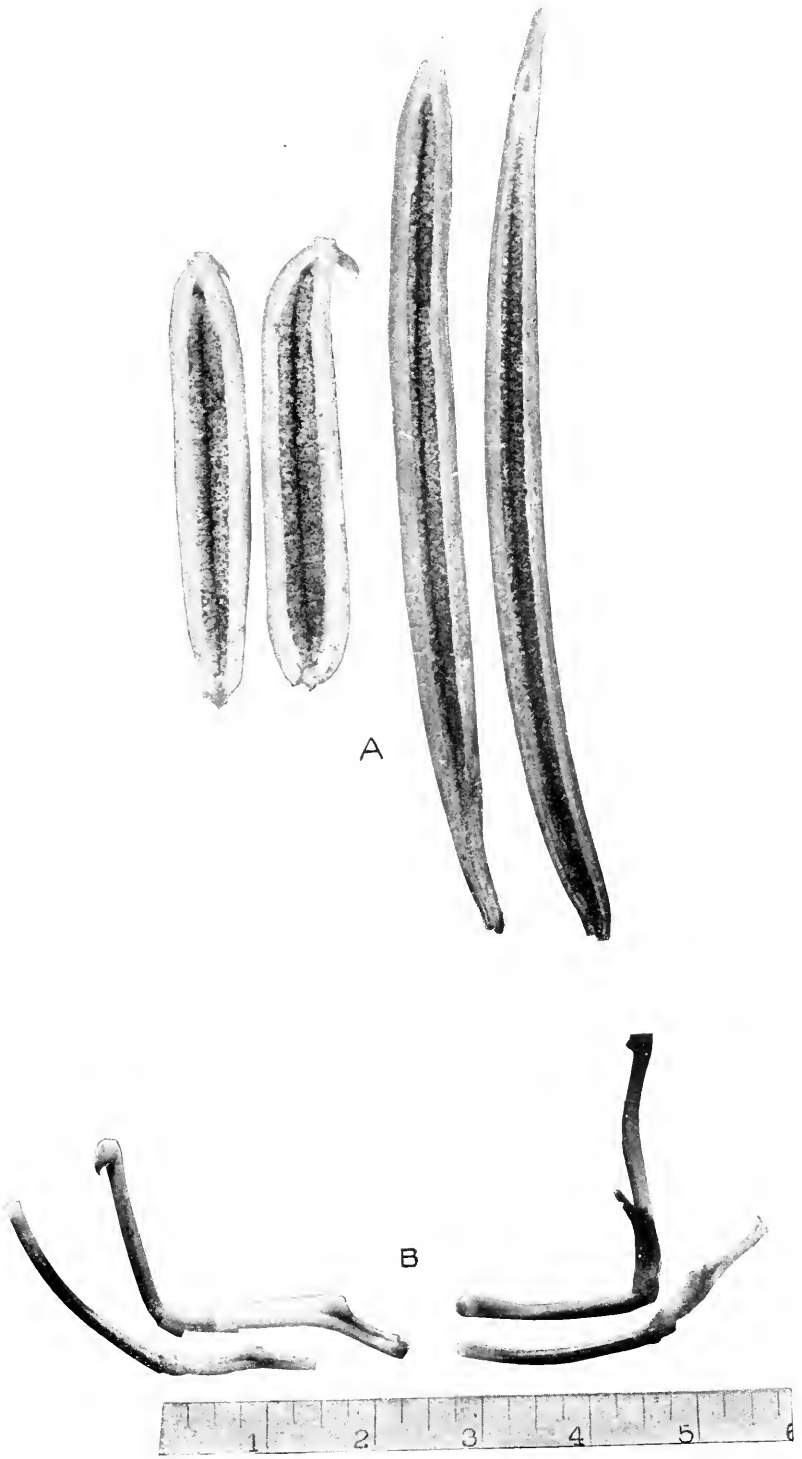
A.—Vanillon No. 43 fruits. Upper row fertilized with pollen of *Vanilla planifolia*, lower row close-fertilized.

B.—Cross sections of same fruits.

PLATE 35

A.—Longitudinal sections of vanilla fruits. Left to right: Vanillon, pistillate, × vanillon, staminate; vanillon, pistillate, ×*Vanilla planifolia*, staminate; *Vanilla planifolia*, pistillate, × vanillon, staminate; *Vanilla planifolia*, pistillate, × *Vanilla planifolia*, staminate.

B.—Comparative length of cleared columns and ovaries. Vanillon above, *Vanilla planifolia* below.


A
B
1
2
3
4
5
6

A BLOOD-DESTROYING SUBSTANCE IN ASCARIS LUMBRICOIDES

[PRELIMINARY PAPER]

By BENJAMIN SCHWARTZ

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RELATION OF ASCARIS INFECTION TO ANEMIA

The view that parasitic worms secrete toxic substances which are absorbed by the host and which are responsible to a considerable extent for the symptoms which often accompany parasitic infections, has received strong support from experimental work with the body fluids and extracts of the various species of Ascaris that parasitize man and domesticated animals. Numerous experiments have shown that the body fluids and extracts of these worms produce decidedly harmful effects on susceptible animals into which they are injected. While it must be admitted that the effects of such injections are far more pronounced than the symptoms usually exhibited by animals that are known to harbor in their intestines numerous ascarids, experiments of this nature have thrown considerable light on the pathology of Ascaris infection, and have also added to our knowledge of the metabolic products of these parasitic worms.

That Ascaris may cause anemia is the opinion of various observers. According to Schimmelpfennig (*9*)[1] and Weinberg and Julien (*14*), the post-mortem appearance of horses infected with ascaris suggests a condition of anemia. As far as man is concerned, a number of clinicians have emphasized the importance of *Ascaris lumbricoides* in relation to anemia. Thus, Demme (*3*) reports two cases of grave anemia in children, resembling pernicious anemia, which he attributed entirely to the presence of *A. lumbricoides* in the intestine. In one case death occurred, the cause of the disease not having been recognized, whereas in the second case complete recovery followed anthelmintic treatment. François (*5*) cites a number of cases of severe anemia resembling that of ancylostomiasis, in which *A. lumbricoides* was evidently the causal factor. Guiart (*8*) considers Ascaris to be a causal agent in anemia, ranking close to Dibothriocephalus and Ancylostoma in importance.

[1] Reference is made by number (italic) to "Literature cited," p. 257–258

In view of the apparent importance of Ascaris as a cause of anemia, the possible relation between the secretions of the parasite [1] and the anemia of the host has a high degree of practical interest. Certain investigators, notably Schimmelpfennig (*9*) and Flury (*4*), attribute hemolytic properties to the body fluids of Ascaris. Flury states, in fact, that the excretions of Ascaris when kept *in vitro* are hemolytic, and inclines to the view that anemia may be caused by the absorption of toxic substances produced by the worms. Weinberg (*12*, *13*), Whipple (*15*), Alessandrini (*1*), and several other investigators, on the other hand, deny the presence of hemolytic substances in Ascaris and state quite emphatically that blood corpuscles of the host in contact with extracts of the worms remain intact. Recently Shimamura and Fujii (*10*), in a report of experiments with extracts of Ascaris on various animals, state that alcoholic and ethereal extracts of the parasites are hemolytic, but that watery extracts of the body substance of the worms previously freed from the ether and alcohol soluble portions produce no effect on red blood cells.

SCOPE AND SUMMARY OF EXPERIMENTAL WORK

For some time past the writer has been studying the problem of the possible absorption of toxic products by animals harboring ascarids. In this work *A. lumbricoides* of swine,[2] of which an abundant supply is easily obtained, has been utilized. The experiments, the results of which are briefly summarized in this paper, were undertaken with a view of determining (1) whether the body fluids of the parasites are hemolytic, (2) whether the excretions of the worms kept *in vitro* contain blood-destroying substances, and (3) the relation which may exist between the anemia of ascariasis and the absorption by the host of toxic substances produced by the parasites. Sufficient data have already been accumulated to warrant certain conclusions, as follows:

(1) The body fluid of *A. lumbricoides* taken from worms shortly after their removal from the host is not hemolytic to the washed erythrocytes of swine, cattle, sheep, rabbits, guinea pigs, and rats.

(2) The fluid from worms which after removal from their host are kept alive in salt solution for a few days acquires hemolytic properties. Fluid from worms kept *in vitro* for 24 hours is only slightly hemolytic if at all, but fluid from worms kept under similar conditions from six to eight days is decidedly destructive to the red blood corpuscles of swine and sheep.

(3) The hemolytic property of the fluid is thermostabile and is not destroyed by boiling.

[1] Several investigators have shown that the fluid and extracts of human, horse, and swine ascaris have indistinguishable chemical and physiological properties.

[2] Ascaris of swine is also referred to as *Ascaris suum* in order to distinguish it from the form which parasitizes man. The two forms are morphologically indistinguishable, however, so far as our present knowledge goes.

(4) There appears to be an inverse relation between the hemolytic property of the fluid and the presence of oxyhemoglobin in it. Fluid from fresh worms contains oxyhemoglobin and is nonhemolytic. When, however, the worms are kept alive *in vitro*, the oxyhemoglobin disappears from the fluid and can no longer be detected by spectroscopic examination one week after the worms have been removed from the host. Meanwhile the fluid becomes hemolytic. Whether oxyhemoglobin in itself is the sole factor in the inhibition of hemolysis or whether other substances are involved which are associated with the oxyhemoglobin and disappear simultaneously with it has not been determined.

(5) Salt-solution extracts of the worms made by grinding up 4 to 10 gms. of the fresh body substance of the parasites and suspending it in 100 cc. of an 0.85 per cent solution of sodium chlorid are hemolytic to the washed erythrocytes of swine and other mammals, the hemolytic potency of the extracts varying directly within certain limits with the duration of the extraction. The reaction is independent of the acidity of the solution, since it is not impaired by neutralization.

(6) Extracts of dried worms in an 0.85 per cent solution of sodium chlorid are decidedly hemolytic to the red corpuscles of various animals.

(7) Salt-solution extracts of the intestine of the worm are more destructive to blood corpuscles than extracts of the body wall, of the reproductive organs, or of the entire worm.

(8) The various salt-solution extracts also do not lose their hemolytic properties on boiling.

(9) The addition of blood serum to tubes containing a mixture of red blood corpuscles and body fluid or extract of the worms usually inhibits hemolysis.

(10) The hemolytic property of the fluid and of extracts of the worms can also be destroyed by the addition of a small quantity of laked blood.

(11) Excretions of the worms absorbed by the solution of sodium chlorid in which the parasites are kept *in vitro* are not hemolytic.

CONCLUSIONS

The failure to demonstrate hemolytic principles in the excretions of the worms when kept *in vitro* appears to favor the view that the hemotoxic substances of ascaris partake of the nature of endotoxins. There is also to be considered the possibility that the death of a worm in the intestine may be followed by a rapid disintegration of its tissues and the liberation of toxic substances before it passes out of the body of the host. Tallqvist (*11*), in fact, has shown in the case of another parasite (*Dibothriocephalus latus*) that the toxic substances are liberated only when the worm disintegrates, which affords a possible explanation why Dibothriocephalus sometimes produces no ill effects on its host, whereas in other instances a severe anemia is present. The fact that in some cases human beings and other animals infested with ascarids remain in apparent good health

while in other cases they show evidences of suffering from such infestation may perhaps be explained in much the same way as the differences observed in cases of infestation with Dibothriocephalus.

The inhibitory effect of the serum on the hemolytic action of the body fluids and extracts of the worms appears to be a direct negation of the view that anemia of animals harboring ascarids is due to the toxic secretions of the worms. It is necessary to remember, however, that a reaction *in vivo* may be very different from a reaction *in vitro*.

Apart from the question of anemia as a result of the absorption of toxins produced by Ascaris, there is the question of anemia as a result of the direct abstraction of blood by the parasite. The opinion that Ascaris is a bloodsucker has been expressed by Schimmelpfennig (*9*), who based his view largely on the fact that the body fluid of ascaris contains oxyhemoglobin, the source of which presumably is the blood of its host. The view that Ascaris may suck blood is also supported by the structure of the mouth parts of the parasite and the lesions observed in the mucosa of intestines of animals harboring ascaris.

It should be remembered that ascaris is provided with strong chitinous lips, denticulated along their edges. That such buccal armature could succeed in lacerating the smaller blood vessels of the intestine is by no means improbable. Blanchard (*2*) states that there can be no doubt that *A. lumbricoides* bites the intestinal mucosa. Guiart (*7*) has shown that *A. conocephala* is often firmly attached to the mucosa of its host; he also states that Leroux found wounds in the intestinal mucosa of man resembling punctures which were apparently produced by *A. lumbricoides*.

Friedberger and Fröhner (*6*) state that the intestinal mucosa of dogs harboring ascarids often shows evidence of punctures.

The above observations, coupled with the presence of oxyhemoglobin in the worms, a substance which apparently is constantly being excreted by the parasites (to judge from their behavior *in vitro*) and which consequently must be as constantly renewed, appear to favor the view that Ascaris probably supplements its food intake by sucking blood from time to time. The hemolytic substance which is particularly abundant in the intestine of the worms apparently serves the purpose of liberating the oxyhemoglobin from the corpuscles some of which passes into the body fluid of the parasites. In this connection it should be recalled that Ascaris is rich in iron and that this substance enters in considerable quantity into the composition of the eggs (Schimmelpfennig, *9*). The significance of the oxyhemoglobin in the body fluid of the worms is not well understood. Whether it merely represents a by-product in the metabolism of the worm and is always excreted as such, or whether it may also first be broken down into simpler compounds with the retention of some of the iron by the tissues of the parasite, still remains to be answered. Whether or not oxyhemoglobin fulfills an important function in the life processes of the worm—perhaps in oxidation—is another question to be solved. In this

connection it is interesting to observe that coincident with the disappearance of oxyhemoglobin from the worms *in vitro* they become sluggish, and that their existence after the complete elimination of this substance is very brief.

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(Contribution from Minnesota Agricultural Experiment Station)

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., MARCH 10, 1919 NO. 10

SOLUBILITY OF LIME, MAGNESIA, AND POTASH IN SUCH MINERALS AS EPIDOTE, CHRYSOLITE, AND MUSCOVITE, ESPECIALLY IN REGARD TO SOIL RELATIONSHIPS

By R. F. GARDINER

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While experiments relating to the solubility of lime, magnesia, and potash in various silicates have frequently been carried out,[1] especially with those minerals in contact with a saturated solution of carbon dioxid, there seems to have been relatively little work done upon the degree of action of soil extracts upon the lime, magnesia, and potash present in epidote, chrysolite, and muscovite, all of which minerals are commonly found in soils.

A knowledge of the solubility of the lime, magnesia, and potash in epidote, chrysolite, and muscovite would also be a means of roughly estimating their availability, so far as a soil extract is concerned, for the soil solution is acting continuously upon the neighboring minerals.

The individual factors involved in the experimental work were as follows: The production of a very slightly acid soil extract by the addition of 500 cc. of distilled water every 24 hours to an acid soil from Auxvasse, Missouri, and the extracts combined. The combined extracts were then analyzed. The next steps were the grinding of selected samples of epidote, chrysolite, and muscovite to pass a screen of 100 meshes to the inch and the analysis of the finely ground minerals. Then two 25 cc. blank solutions of the soil extracts, together with 24 nursing bottles with 25 cc. of the soil extract in each in contact with from 0.1 to 1.0 gm. of epidote, 0.1 to 1.0 gm. of chrysolite, and from 0.1 to 0.4 gm. of muscovite, were placed in a thermostat and kept at a temperature of 25° C. for a period of two months. At the end of the period the soluble material filtered from the minerals by Pasteur-Chamberland filters was analyzed, and a correction made for the composite blank solutions, for lime, magnesia, and potash with the results given in Table I. Preliminary to the results given in Table I, an analysis was made of the water

[1] Storer says that silicates of alumina, lime, magnesia, and potash are "decomposed and dissolved to a certain extent by carbonic acid-water, and also even by pure water." (STORER, FRANK H. FIRST OUTLINES OF A DICTIONARY OF SOLUBILITIES OF CHEMICAL SUBSTANCES. p. 549, 551. Cambridge, [Mass.], 1864.)

extract of the soil, which showed it to contain 0.001 per cent of lime, (CaO), 0.003 per cent of magnesia (MgO), and 0.001 per cent of potash (K_2O). After two months contact with the glass bottles in the thermostat, an analysis of the solution gave 0.01 per cent of lime, 0.002 per cent of magnesia, and a trace of potash.

TABLE I.—*Solubility of the lime in epidote, the magnesia in chrysolite, and the potash in muscovite, in contact with a slightly acid soil extract*

[Minerals ground to pass a screen of 100 meshes to the inch]

Sample No.	Lime (CaO) extracted from epidote 2 months in thermostat at 25° C.	Magnesia (MgO) extracted from chrysolite 2 months in thermostat. at 25° C.	Potash (K_2O) extracted from muscovite 2 months in thermostat at 25° C.	Proportion of total lime extracted from epidote.	Proportion of total magnesia extracted from chrysolite.	Proportion of total potash extracted from muscovite.	Total lime in epidote.	Total magnesia in chrysolite.	Total potash in muscovite.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	1.40	1.60	1.80	8.78	1.45	21.12	15.84	34.60	8.52
2	1.95	.35	1.75	12.25	.29	20.54	.....	.....	.....
3	1.90	.40	1.13	11.93	.49	13.26	.....	.....	.....
4	.25	.23	.95	1.52	.14	11.15	.....	.....	.....
5	.24	.18	.....	1.45	.29	.....	.....	.....	.....
6	.22	.18	.....	1.21	.32	.....	.....	.....	.....
7	.18	.19	.....	1.07	.29	.....	.....	.....	.....
8	.20	.14	.....	1.20	a Trace.	.....	.....	.....	.....
9	.22	.12	.....	1.33	.09	.....	.....	.....	.....
10	.56	.16	.....	3.47	.29	.....	.....	.....	.....

a Probably too low.

The results tend to show that, under the conditions of the experiment, more lime and potash was extracted from the silicates epidote and muscovite, ground to pass a sieve of 100 meshes to the inch, by two months' contact with the soil extract kept at 25°C. in a thermostat, than of magnesia from its silicate in the form of chrysolite similarly ground and subjected to the same conditions.

The removal of such proportionally large amounts of lime and potash from silicates by an acid soil extract would seem to indicate that in time a soil's fertility index, with respect to lime and potash, would under proper conditions of acidity be quite appreciably lowered. That the potash results are truly representative is strengthened by the fact that in percolation experiments tried out by the author a short time ago with dilute acid solutions, such as phosphoric acid and sulphuric acid, in every instance considerable amounts of potash were removed from soil silicates, as was the case also when a peaty soil from Maine intermixed with white mica was shaken up with distilled water and allowed to stand for 24 hours before filtering, the filtrate showing potash to be present in a water soluble condition to the extent of 600 p. p. m.

Because of the small amounts of the samples used in Nos. 1 to 4 in relation to the quantity of solution, it would seem to follow as a natural consequence that the results for those samples would be high and should

not be taken as truly representative, the results including those from samples 4 to 10, taken as a whole, are probably more truly representative of the actual solubility conditions.

(1) In contact with an acid soil extract more potash is removed from muscovite than lime from epidote, or magnesia from chryoslite and that on the whole more lime is extracted from epidote than magnesia from chrysolite.

(2) So far as the solubility of the lime in epidote and the magnesia in chrysolite are concerned, the results are quite constant in samples 4 to 10, with the average for these samples of 0.27 per cent of lime from epidote, and 0.17 per cent of magnesia from chrysolite.

A FIELD STUDY OF THE INFLUENCE OF ORGANIC MATTER UPON THE WATER-HOLDING CAPACITY OF A SILT-LOAM SOIL[1]

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INTRODUCTION

It has long been believed that the increased amount of organic matter in the soil resulting from applications of manure causes an important and beneficial increase in the water supply of crop plants, it both enabling the water to be absorbed more readily during heavy rains and increasing the amount held against downward movement, thus retaining a more generous supply within reach of the roots of the crop plants.

Thorne, in a recent paper (*12*),[2] has raised the question whether organic matter

> possesses a value for soil improvement additional to that of the nitrogen and mineral elements that it may contain

and attributes (*p. 27*) the physical improvement of the soil following the use of manure

> not to the carbonaceous matter of the manure, but to the superior growth of plant roots induced by the nitrogen and mineral elements carried by the manure.

His conclusions are based chiefly upon data from experiments at the Ohio Experiment Station covering a period of 24 years, in which the recovery of nitrogen, phosphorus, and potassium has been higher from sodium nitrate, acid phosphate, and potassium chlorid than from farm manure. As the Ohio experiments

> have been conducted on a soil depleted of its organic matter by a long period of tenant husbandry before the test began and the average yields of the untreated land during the period of the test have been only 7.85 bushels per acre of wheat, 14.70 bushels of corn and 21.76 bushels of oats (*12, p. 26*),

the results would suggest that we have been in the habit of overrating the benefit derived from any increased water-holding capacity of the soil caused by the application of manure, or even that the effect of the added organic matter upon this property may in reality be too slight to have any practical importance.

Entirely satisfactory fields for studies designed to determine the effect of differences in the content of organic matter upon the water-holding capacity of soils are scarce; and with most field soils the bringing about of an appreciable increase through applications of manure as light as those

[1] Published, with the approval of the Director, as Paper 150, of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 277–278.

Journal of Agricultural Research,
Washington, D. C.
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Vol. XVI, No. 10.
Mar. 10, 1919.
Key No. Minn.-37.

used in farm practice requires a rather long time. This is well illustrated at Rothamsted by the Broadbalk Field, which has been continuously in wheat since 1843. On plot 2b, which receives 14 tons of farm-yard manure every year, the first 9 inches of soil gained only 0.098 per cent of organic carbon in the 12-year period from 1881 to 1893 (*9, p. 127*), and in 1893, after having received such an annual application for 50 years, or 700 tons per acre in all, the 9-inch layer of soil contained only 1.342 per cent more organic matter than the adjacent plot 3, which during the same period had been continuously cropped without any manure or fertilizers. By starting with a virgin prairie soil rich in organic matter and putting it under continuous clean cultivation, an appreciable lowering of the organic matter can be induced much more quickly, but even in this case a long period is necessary (*6, p. 136*).

On many fields the great variation in texture from place to place, especially in the portion of the soil mass below the reach of the plow, renders any comparison of the relative amounts of useful moisture a laborious task, the differences shown in moisture retentiveness being more dependent upon differences in texture than upon any differences in the content of organic matter that may have been induced by dissimilar methods of manuring, cropping, or tillage. Detailed studies of the uniformity in texture of the plots or fields under comparison have usually been omitted, and, hence, it may easily be that many of the data published in support of the common belief are due simply to the coincidence that soils of a finer texture, while they retain more water, also usually have a higher content of organic matter. Under natural grassland conditions the heavier soils are the richer in organic matter and in general it appears that when conditions of surface drainage and climate are similar, the finer the texture of the soil the higher will be the organic-matter content when equilibrium between the processes of decay and those inducing an accumulation of organic matter have once been attained. Under the conditions of ordinary mixed farming, arable soils will approach more nearly to grassland than to forest conditions.

An unusual opportunity for such a study is offered by some plots at this Experiment Station, laid out by Snyder (*11*) some 25 years ago. In the summer of 1915, incidental to a study of the effect of different systems of cropping upon the composition, properties, and productivity of the silt loam soil of these plots, we obtained some data upon the water-holding capacity. It so happened that the season was characterized by weather conditions especially favorable for revealing any differences which might exist in the water-holding capacity of the soils of the various plots.

DESCRIPTION AND HISTORY OF PLOTS

The land, originally covered by a heavy growth of deciduous trees, had been cleared about 1856, and during the following quarter of a century formed part of a typical grain farm of that period, oats or wheat being

grown upon it every year without the use of clover or manure. In 1883 the university acquired the farm for experimental purposes and the next year the field was seeded to clover, from that time on being kept in a good rotation until in 1893 Snyder (*11, p. 2*) laid it out in six plots as a fertility experiment (fig. 1, A). All available records indicate that the land included in the plots had been treated alike during the preceding 36-year period (1856–1892). Plots 2 and 3 were to be kept in 4- and 5-year rotations, including clover and receiving manure, and each of the others to be devoted continuously to the same grain crop and to receive

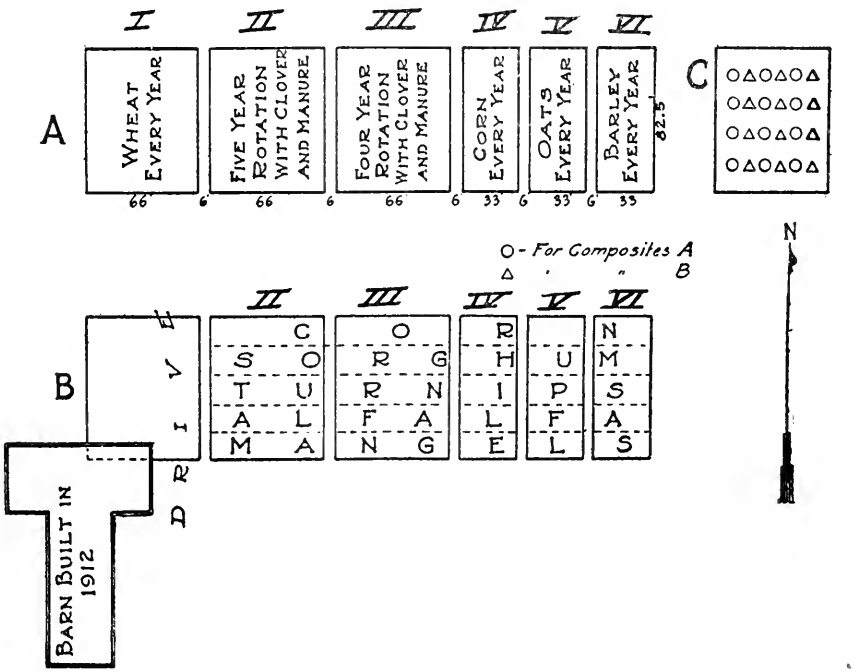

FIG. 1.—Diagram showing arrangement of plots and crops on field J, University Farm, St. Paul, Minn. A is the original plan adhered to from 1893 to 1914, while B shows the cropping plan in 1915. C shows the arrangement of the samples taken for the two composites from a plot.

neither manure nor fertilizer, No. 1, 4, 5, and 6 being planted to wheat, corn, oats, and barley, respectively. The construction of a barn in 1912 upon part of plot 1 has rendered this useless for experimental purposes (Pl. 36).

The present discussion deals chiefly with plots 3 and 4, on the latter of which, beginning with 1893, there had been grown 22 successive crops of corn, without the application of any manure. On the other there had been only 6 crops of corn, but 4 of barley, 7 of oats, and 5 of clover, while 25 tons per acre of manure had been applied, 5 tons with each crop of corn except that of 1897.

Plots 2 and 3 are 5 rods long and 4 rods wide, while the others are of the same length but of only half the width. Each plot is separated from its neighbors by a strip 6 feet wide. The plots have been seeded to the center of this 6-foot strip, the outer edge around each plot being cut away at harvest time.

The soil has been classified by the Bureau of Soils of the United States Department of Agriculture as Hempstead silt loam (*10, p. 26*). The silt loam stratum extends to a depth of from 39 to 50 inches, below which is a thick bed of clean gravel and coarse sand. The surface stratum is very uniform in texture as may be seen from the moisture equivalents reported in Table III.

CULTURAL CONDITIONS

The original plan of the experiment had been carried out until it was interrupted in the spring of 1915, when in order to determine the relative productivity of the different plots we had all five plowed, prepared alike, and planted to the same crops—viz, corn (*Zea mays*), sorghum (*Andropogon sorghum*), turnip (*Brassica rapa*), alfalfa (*Medicago sativa*), and mangels (*Beta vulgaris macrorhiza*)—these being so arranged that each of the five appeared on every plot (fig. 1, B). Weeds were very bad on all the plots except No. 4 but, by frequent use of hoe and horse cultivator, these were kept down in all the crops except the alfalfa. On account of the unusually cool weather, the crops made very slow growth until early in July, and none of the plots at any time appeared to suffer from a lack of moisture.

DISTRIBUTION OF ORGANIC MATTER IN SURFACE FOOT

The ratio of organic carbon to nitrogen in surface soils is so nearly constant that determinations of the latter serve to indicate variations in the content of organic matter. Table I shows the nitrogen content of the successive levels within the first foot of soil on the five plots. The percentages reported in the first part of the table (a) are for composite samples from six borings using a 4-inch plate auger and those in (b) for composites of 24 samples taken by means of a 1.5-inch soil tube of special construction. The latter are really the averages of the data from two sets of samples, A and B, in each of which composites of 12 individual samples were employed, these being distributed as indicated in figure 1, C. The concordance of the data from these two sets, as illustrated by Table II, is such that in the present discussion no purpose would be served by reporting more than their averages. From these data it is evident that any marked differences in nitrogen content, and, hence, in organic matter found between samples from the same level on different plots are to be attributed to differences in crop history and not to the experimental errors of sampling.

TABLE I.—*Nitrogen and organic matter in successive levels of the surface foot*

(A) NITROGEN IN COMPOSITES FROM 6 BORINGS WITH AUGER

Depth of section.	Plot 2.	Plot 3.	Plot 4.	Plot 5.	Plot 6.
Inches.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1 to 3	0.242	0.242	0.176	0.210	0.192
4 to 6	.239	.234	.177	.214	.195
7 to 9	.206	.223	.169	.194	.187
10 to 12	.155	.190	.130	.163	.111
Average	.211	.222	.163	.195	.171

(B) NITROGEN IN COMPOSITES FROM 24 BORINGS WITH TUBE

1 to 6	0.236	0.235	0.180	0.208	0.193
7	.235	.241	.168	.207	.187
8	.212	.232	.163	.203	.170
9	.190	.222	.151	.200	.152
10	.175	.205	.145	.180	.128
11	.160	.192	.126	.158	.116
12	.149	.181	.116	.149	.108
Average	.211	.223	.162	.195	.168

(C) ORGANIC MATTER IN SOME OF ABOVE SAMPLES[a] (=ORGANIC CARBON×1.724)

1 to 6	.....	5.21	3.97	.....	.....
7 to 9	.....	5.02	3.79	.....	.....
10 to 12	.....	4.05	2.41	.....	.....
Averages	.....	4.76	3.39	.....	.....

(D) RATIO OF ORGANIC CARBON TO NITROGEN

1 to 6	.....	12.9	12.8	.....	.....
7 to 9	.....	13.0	13.5	.....	.....
10 to 12	.....	12.4	10.8	.....	.....

[a] The carbon was determined by combustion with copper oxid in a current of oxygen after previous treatment with phosphoric acid.

Plots 3 and 4 show the extremes, the former having the highest and the latter the lowest nitrogen content and at every level plot 3 shows the higher value. The percentages on plot 2 are almost as high as those on 3 while the values for plots 5 and 6 fall between those for 3 and 4. In its low nitrogen content, plot 6 approached 4, the sections below the ninth inch containing even less than the corresponding ones on the latter. The nitrogen content of the first foot on plot 3 was 138 per cent and the organic matter 140 per cent of that on plot 4. These two plots, as they showed the extremes in nitrogen, and, hence, in organic-matter content, while being adjacent, were selected for the more detailed moisture studies.

TABLE II.—*Nitrogen content of soil at different levels on plots 2 and 3, illustrating the degree of concordance of the data from duplicate sets of composite samples*

Depth of section.	Plot 3.			Plot 4.		
	Set A.	Set B.	Difference.	Set A.	Set B.	Difference.
Inches.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1 to 6	0.229	0.240	0.011	0.182	0.178	0.004
7	.237	.244	.007	.166	.170	.004
8	.230	.233	.003	.167	.158	.009
9	.227	.216	.011	.160	.141	.019
10	.212	.197	.015	.147	.142	.005
11	.199	.184	.015	.124	.127	.003
12	.187	.174	.013	.120	.112	.008
13 to 15	.165	.143	.022	.096	.099	.003
16 to 18	.121	.114	.007	.078	.087	.009

UNIFORMITY IN TEXTURE

The moisture equivalents of the soil from the different levels are reported in Table III. Those for the first 12 inches were determined on composites made by combining equal weights of the duplicate samples reported in Table II, and, hence, they represent composites of 24 individual samples from each plot. The data for the second and third feet are from composites from 6 borings on each plot.

TABLE III.—*Moisture equivalents of soil at different levels*

Depth of section.	Plot 2.	Plot 3.	Plot 4.	Plot 5.	Plot 6.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1 to 6 inches	23.9	24.2	22.8	23.9	24.3
Seventh inch	24.0	24.5	23.7	23.6	24.3
Eighth inch	24.2	25.3	23.2	24.1	24.5
Ninth inch	23.4	24.3	23.3	24.0	24.2
Tenth inch	23.5	24.6	22.7	23.1	24.0
Eleventh inch	23.1	24.1	23.0	23.4	23.2
Twelfth inch	22.1	23.8	22.3	22.6	23.0
Second foot	24.1	23.6	24.0	23.2	24.3
Third foot	23.3	23.2	22.5	23.7	22.9

As was to be expected from the content of organic matter, the lowest values within the surface foot are shown by plot 4 and the highest by plot 3. The differences in the moisture equivalent are very slight compared with those in nitrogen and organic matter; while the nitrogen in the surface foot of plot 3 exceeds that in plot 4 by 38 per cent and the organic matter shows a corresponding difference of 40 per cent, the moisture equivalent is only 7 per cent higher. In the case of the second and third feet, the crop history of the plots appears to have exerted no appreciable influence upon the moisture equivalent, the differences shown in these levels being within the limits of error in sampling.

The uniformity in texture of both the surface soil and subsoil from plot to plot makes the field unusually favorable for such a moisture study. The thickness of the silt loam layer overlying the gravel stratum mentioned above varies from about 39 to 50 inches, in nearly all places it being less than 48 inches, and the variations from place to place on the same plots appear as great as those from one plot to another. While we regularly sampled the fourth-foot section along with the second and third, it showed such an extreme range in texture, owing to the varying proportions of its two component layers, silt loam and gravel, that the data on this level are of no use in the present discussion.

WEATHER CONDITIONS

The weather of the crop season of 1915 was favorable for the maintenance of a very moist soil, being abnormally rainy, cool, and cloudy (Table IV). In each of the first three months, May, June, and July, the precipi-

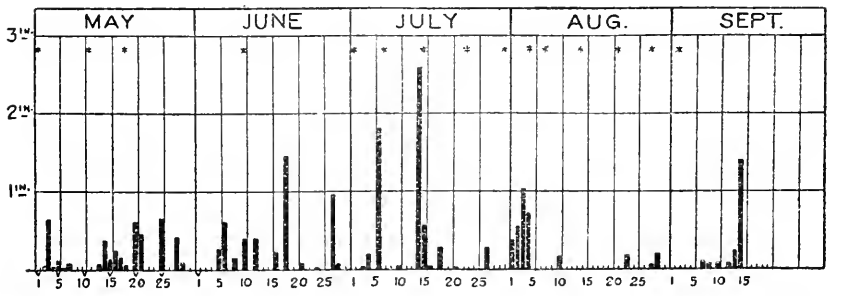

FIG. 2.—Diagram showing the amount and distribution of the rainfall at University Farm, St. Paul, Minn., during part of the season of 1915. The dates of sampling are indicated by asterisks.

tation was somewhat above the normal and in August it was just equal to the normal. For each of the months the mean temperatures varied from 6.1 to 4.2 degrees below normal and the percentage of possible sunshine in the first three months varied from 16 to 26 below normal, while the wind movement was slightly below and the relative humidity slightly above normal.

The precipitation of the autumn of 1914 and the following winter and first two months of spring had been nearly normal, that of April being 2.31 inches. The rainfall of the four months covered by the study came chiefly in the form of slow rains which caused but little run-off; exceptions were provided by two heavy rains, one on July 6 and the other on July 14 (Table V and fig. 2). The dates of sampling happened to be such as to well illustrate the various conditions met with in a wet season. Thus on both July 7 and 15, the samples were taken only a day after a very heavy rain had fallen while on May 18 and August 4 they were taken one day after the last rain in a succession of days with moderate rains.

TABLE IV.—*Weather of the crop seasons of 1915 and 1918 at St. Paul compared with the normal*

PRECIPITATION[a] (INCHES)

Item.	May.	June.	July.	Aug.
Normal	3.34	4.03	3.49	3.36
Departure in 1915	1.01	0.68	2.42	.00
Departure in 1918	.98	−1.18	.41	.20
MEAN TEMPERATURE (° F.)				
Normal	58.3	67.8	72.6	69.6
Departure in 1915	−6.1	−5.4	−5.5	−4.2
Departure in 1918	1.6	−1.1	−2.2	.9
SUNSHINE (PER CENT OF POSSIBLE)				
Normal	61	66	75	67
Departure in 1915	−26	−16	−20	1
Departure in 1918	− 5	− 5	− 7	−3
WIND VELOCITY (MILES PER HOUR)				
Normal	13.0	11.6	9.5	9.5
Departure in 1915	−.9	−1.3	.4	.4
Departure in 1918	−.3	− .2	−.5	.8
RELATIVE HUMIDITY (PER CENT)				
Normal	65	69	67	70
Departure in 1915	9	1	5	1
Departure in 1918	1	−3	−5	−2

[a] At University Farm. From September 1, 1914, to April 30, 1915, 11.86 inches, and for the same period in 1917–1918, 6.89 inches.

TABLE V.—*Daily precipitation at University Farm, St. Paul, during the season of 1915*

Day.	May.	June.	July.	Aug.	Sept.	Day.	May.	June.	July.	Aug.
1	.....	.....	.....	0.39	.....	17	0.16	.....	.....	.....
2	T	.....	.....	.57	.....	18	.07	1.46	0.29	.....
3	0.65	.....	0.04	1.04	.....	19	.....	.....	T	.....
4	.04	.....	.19	.74	.....	20	.62	.....	T	.....
5	.12	0.26	.....	.....	.....	21	.47	.10	.03	.....
6	.03	.62	1.82	.....	.....	22	T	T	.....	.....
7	.09	.....	.....	T	0.10	23	.....	T	.....	0.18
8	T	.17	.....	.....	.07	24	.....	.03	T	.....
9	.....	.....	.....	.....	.....	25	.66	.....	.....	.....
10	.....	.40	.04	.18	.08	26	.....	T	.....	.....
11	T	T	.....	.....	T	27	.....	.96	.29	.....
12	T	.40	.....	.....	.07	28	.44	.09	.....	.06
13	.10	.....	.....	.....	.24	29	.10	.....	.....	.20
14	.38	.....	2.60	.....	1.40	30	.....	.....	.....	.....
15	.15	.....	.57	.....	.....	31	.....	.....	T	.....
16	.27	.22	.04	.....	.....	Total	4.35	4.71	5.91	3.36

T=Trace.

MOISTURE CONTENT OF THE SOIL

Between May 1 and September 2, plots 3 and 4 were sampled 15 times (Table VI) to a depth of 1 foot, the samples being taken in 3-inch sections from three borings in a north and south line across each plot. In the case of each set one boring was in the corn, another in the sorghum, and the third in the mangels, each being close to a crop row. At the time of the first sampling in each month we sampled the second- and third-foot section also, both on these two plots and on the three others (Table VII).

From Table VI it will be seen that on every occasion the surface foot of plot 3 contained more moisture than that of plot 4, and, except on the very last date, the same holds true for the four 3-inch sections. Throughout the first three months the difference ranged between 3 and 5 per cent, being greatest when the sampling occurred soon after the cessation of a rain. During the last half month, at a time when the crops were drawing most heavily upon the soil moisture and there was but little rain, the differences were much less, falling on the average to less than 1 per cent.

There is no evidence that, in general, more water was retained in the second and third foot on plot 3 than on plot 4 (Table VII), although more was found on May 1, which, however, was not long after the frost had disappeared from the subsoil and there had not been time for the downward percolation of the water from the melting snow and the April rains. With the three other plots, the surface foot was intermediate in moisture between plots 3 and 4, the relative moisture content varying roughly with the nitrogen content (Table VII). Only at the time of the first sampling did they, like plot 3, show a higher moisture content in the second and third foot than plot 4.

The above remarks apply directly to the total water content, which includes both the nonavailable and the available. As the portion of the soil moisture available to plants for growth and for the maintenance of life appears to be approximately that in excess of the hygroscopic coefficient (*1, p. 122; 2*), and as the latter value is a little lower for the surface of plot 4, being only 7.7 compared with 8.1 for plot 3 (Table VIII), we regard the differences in useful water as slightly greater than those in the total water reported in Table VI.

INFLUENCE OF ORGANIC MATTER UPON MOISTURE CONTENT

From the above data it would appear that the greater amount of organic matter in the surface foot of plot 3 is responsible for the considerably higher content of both total and free water shown by it throughout most of the summer of 1915.

Any advantage possessed by one plot over another, due to topography, lies with No. 4. The surface of the field is almost level, but after very heavy rains and at the time of the melting of the snow in the spring the last water to disappear from the field is found upon that plot (Plate 36).

TABLE VI.—*Moisture content of the surface foot of plot 4 and the excess of moisture in that of plot 3*

A.—MOISTURE CONTENT OF SOIL OF PLOT 4

Depth of section.	May 1.	May 11.	May 18.	June 10.	July 1.	July 7.	July 15.	July 23.	July 30.	Aug. 4.	Aug. 7.	Aug. 14.	Aug. 21.	Aug. 28.	Sept. 2.
Inches	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1 to 3	24.2[a]	21.4	27.5	24.9	22.6	28.7	28.3	22.3	22.2	28.0	25.1	20.8	19.3	17.1	17.6
4 to 6		26.1	27.7	27.5	24.9	29.1	27.9	24.9	24.7	28.8	26.4	23.5	22.2	20.2	18.6
7 to 9	25.5[a]	25.2	27.7	27.9	25.7	29.1	28.5	25.5	25.2	28.4	26.8	24.3	23.0	21.1	19.7
10 to 12		25.5	26.5	27.2	25.2	28.5	28.0	25.2	23.3	29.2	27.3	24.2	23.5	21.8	20.0
Average 1 to 12	24.9	24.6	27.4	26.9	24.6	28.9	28.2	24.5	23.9	28.6	26.4	23.2	22.0	20.1	19.0

B.—EXCESS OF MOISTURE ON PLOT 3 OVER THAT ON PLOT 4

1 to 3	5.1[a]	6.7	4.9	4.3	2.4	4.4	5.2	4.8	4.3	5.0	3.9	2.5	1.3	0.6	1.5
4 to 6		3.6	5.8	4.9	5.0	4.2	5.1	4.8	3.9	4.6	3.8	3.6	1.5	3.0	3.2
7 to 9	4.4[a]	5.4	5.1	3.3	3.7	3.8	3.1	3.9	2.1	2.5	3.3	2.5	2.1	2.9	.1
10 to 12		4.3	6.7	5.1	3.3	4.3	3.0	2.3	3.2	.7	1.4	2.3	1.0	.9	— .2
Average 1 to 12	4.7	5.0	5.6	4.4	3.6	4.1	4.1	3.9	3.4	3.2	3.1	2.7	1.5	1.9	1.1

TABLE VII.—*Moisture content of soil on plot 4 to a depth of 3 feet, at the first of each month and the excess of moisture at corresponding depths on the other plots, arranged to show any relation of these differences to differences in the nitrogen content of the surface foot*

A—MOISTURE CONTENT

Date and depth of section.	Plot 4.	Excess on other plots.			
		Plot 3.	Plot 2.	Plot 5.	Plot 6.
MAY 1.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1 to 6 inches	24.2	5.1	2.6	3.2	3.3
7 to 12 inches	25.5	4.4	2.8	1.2	.1
Second foot	23.2	3.8	2.2	1.2	2.9
Third foot	20.5	4.9	3.3	2.9	— .7
JULY 1.					
1 to 6 inches	23.7	3.7	4.9	1.7	1.4
7 to 12 inches	25.4	3.5	.6	— .1	.7
Second foot	25.1	1.3	— .4	.5	1.1
Third foot	23.7	.9	.2	.6	1.1
AUGUST 4.					
1 to 6 inches	28.4	4.8	4.0	.8	1.7
7 to 12 inches	28.8	1.6	1.1	— .1	.1
Second foot	28.0	.2	.1	—1.3	.4
Third foot	26.5	—1.3	—1.1	—1.6	—1.3
SEPTEMBER 2.					
1 to 6 inches	18.1	2.3	2.7	1.7	2.3
7 to 12 inches	19.8	.0	—1.0	.8	— .8
Second foot	20.0	1.0	— .7	1.0	1.1
Third foot	21.4	1.0	—2.1	.8	.4

B—NITROGEN CONTENT

1 to 6 inches	0.180	0.055	0.056	0.028	0.013
7 to 9 inches	.161	.071	.051	.042	.009
10 to 12 inches	.129	.064	.032	.033	— .012

[a] A single 6-inch sample used instead of successive 3-inch samples.

It is of interest that the differences in moisture content are as great as would be computed on the assumption that the organic matter of this silt loam has the same water-holding capacity as some of the most absorbent peats, some of these, even when well drained, being able to retain 300 to 400 parts of water to every 100 parts of dry peat. The surface foot of plot 3 carries 1.37 per cent more organic matter than the corresponding level on plot 4 (Table I), from which might be computed a difference of about 5 per cent in water-holding capacity.

TABLE VIII.—*Hygroscopic coefficients of successive levels on plots 3 and 4*

Depth of section.	Hygroscopic coefficient.	
	Plot 3.	Plot 4.
1 to 3 inches	8.1	7.6
4 to 6 inches	8.1	7.6
7 to 9 inches	8.2	7.8
10 to 12 inches	8.1	7.6
First foot	8.1	7.7
Second foot	7.9	8.0
Third foot	7.7	7.5

INFLUENCE OF ORGANIC MATTER UPON PROPORTION OF USEFUL MOISTURE

The nitrogen content of the surface foot of plot 3 is 138 per cent and the organic matter 140 per cent of that on plot 4, while the hygroscopic coefficient is only 5 per cent the higher on the former. As a consequence the proportionate increase in free water is much greater than that in total moisture content, and that in growth water still greater. Thus, the average moisture content of the surface foot for the nine samplings in May, June, and July was 26 per cent on plot 4 and 30.3 per cent on plot 3, the free water 18.3 and 22.2, and the growth water 13.5 and 17.1, respectively, corresponding to increases of 15, 21, and 27 per cent.

Thus, the difference in organic-matter content, owing to differences in the manuring and cropping of the two plots, caused a marked difference in the amounts of useful moisture during the season of 1915 as is well illustrated by a comparison of the ratios of the moisture content to the hygroscopic coefficient (Table IX). The advantages of expressing the moisture condition of soils by such ratios has been discussed in several recent papers (*3, p. 55; 4, p. 453; 5, p. 266*). The expression "hygroscopic coefficient = 10.0; ratio = 1.7" indicates a moisture content of 17.0 per cent, a wilting coefficient of 15.0[1] (*8, p. 65*), 7 per cent of free water, and 2 per cent of growth water. The ratios 1.0, 1.5, and 2.0—2.5 appear to indicate, respectively, the minimum to which crop

[1] The exact figure is 14.7.

plants can reduce the soil moisture (*l*), the point at which root penetration practically ceases (*7, p. 279*), and the water-retaining capacity of well-drained arable mineral soils (*3, p. 69*), and such an expression as the above makes all these relations apparent at a glance. The ratio may be used alone to indicate the *relative moistness*, while its combination with the hygroscopic coefficient expresses the *moisture condition.*

TABLE IX.—*Ratio of moisture content to hygroscopic coefficient at different levels on plots 3 and 4, in 1915*

Date.	Ratio on plot 4.			Excess of ratio on plot 3.		
	First foot.	Second foot.	Third foot.	First foot.	Second foot.	Third foot.
May 1	3.2	2.6	2.7	0.4	0.8	0.6
11	3.2	.....	.....	.4	.....	.....
18	3.6	.....	.....	.5	.....	.....
June 10	3.5	.....	.....	.4	.....	.....
July 1	3.2	3.1	3.2	.3	.2	.0
7	3.8	.....	.....	.3	.....	.....
15	3.7	.....	.....	.3	.....	.....
23	3.2	.....	.....	.3	.....	.....
30	3.1	.....	.....	.3	.....	.....
Aug. 4	3.8	3.5	3.5	.1	.1	—.2
7	3.4	.....	.....	.2	.....	.....
14	3.0	.....	.....	.2	.....	.....
21	2.9	.....	.....	.0	.....	.....
28	2.6	.....	.....	.1	.....	.....
Sept. 2	2.5	2.5	2.9	.0	.1	.0

The high ratios observed in the subsoil of these plots is probably due to the retarding influence which the substratum of gravel and coarse sand exerts upon the downward movement of water (*3, p. 34–41*).

In a study somewhat similar to the one here reported, but made in eastern Nebraska in 1912, one of us (Alway) found a similar influence of the organic matter upon the amount of useful moisture retained (*4, p. 474*), but there the conditions were not so satisfactorily comparable, an exposed subsoil poor in organic matter being compared with an adjacent surface soil.

MOISTURE RELATIONSHIPS AND PRODUCTIVITY IN LATER SEASONS

Plot 3 showed itself far the more productive of the two plots in 1915, as had been the case also in such of the preceding 22 years of the experiment, as the coincidence of the corn crop on plot 3 permitted a direct comparison of yields (Table X). In 1916 spring wheat was sown upon all five plots and the yields were relatively unchanged. However, red clover (*Trifolium pratense*) was seeded with the wheat and while the stand of clover plants was even and moderately thick on all the plots, it was especially fine on No. 4, and in the following year the yield of hay was considerably the greater on this plot and the aftermath also was

heavier than on No. 3. The second growth, being too light to make a fair cutting of hay, was plowed under and the field seeded to winter rye (*Secale cereale*), which gave a good yield in 1918, it being almost as heavy on plot 4 as on plot 3. The yield of hay, straw, and grain combined, for 1917 and 1918, amounted to 9,738 pounds per acre on plot 4, compared with 9,088 pounds on plot 3. Evidently the lessened water-holding capacity on the former had no serious effect upon the crop yields.

TABLE X.—*Relative productivity of plots 3 and 4*

Season.	Crop.	Yield per acre.		Productivity of plot 4.[a]
		Plot 3.	Plot 4.	
1896	Corn:			
	Grain, bush.	61. 7	44. 0	71
	Stover, cwt.	44. 8	.....	.....
1897	Grain, bush.	33. 3	10. 0	33
	Stover, cwt.	18. 8	6. 2	33
1901	Grain, bush.	40. 6	37. 8	93
	Stover, cwt.	20. 4	26. 4	129
1905	Grain, bush.	71. 1	26. 6	37
	Stover, cwt.	20. 8	12. 8	62
1909	Grain, bush.	96. 6	48. 9	51
	Stover, cwt.	25. 6	31. 4	123
1915	Grain, bush.	79. 7	55. 0	69
	Stover, cwt.	56. 0	44. 0	78
	Sorghum, as cut green, cwt.	16. 4	12. 6	77
	Turnips:			
	Roots, tons.	12. 9	7. 4	58
	Tops, tons.	3. 4	1. 5	44
	Mangels:			
	Roots, tons.	20. 7	11. 4	55
	Tops, tons.	2. 9	1. 8	62
1916	Wheat:			
	Grain, bush.	29. 6	20. 5	69
	Straw, cwt.	35. 2	24. 5	70
1917	Clover hay, tons.	2. 0	2. 44	122
1918	Winter rye:			
	Grain, bush.	40. 3	38. 3	95
	Straw, cwt.	26. 7	25. 6	96

[a] Yield on plot 3=100.

The moisture content of the soil on the two plots was determined on four occasions during the past season, and, in general, plot 3 was found the more moist in the surface 6 inches (Table XI). When, as in Table XII, we compare the ratios of moisture content to hygroscopic coefficient with those for 1915 (Table IX) it is evident that there was little difference between the two plots in the moisture content of the whole three-foot section in 1918; the subsoil on both plots was much drier than in the earlier year, so dry in fact that until the rains of November fell (Table XIII), there was no opportunity for loss of water by percolation from this section. The dryness of the third foot indicates that it had been fully occupied by the rye roots and hence that all the water

that entered the surface had been retained within reach of the roots. With forage crops like clover, where a still larger amount of moisture is required for maximum yields, the same would hold true.

TABLE XI.—*Differences in moisture content of soil of plots 3 and 4 in season of 1918*

Date and depth of section.	Plot 4.	Plot 3.	Excess on plot 3.
June 11 (1.82 inches of rain on June 8 to 10):	*Per cent.*	*Per cent.*	*Per cent.*
1 to 6 inches	24.8	27.8	3.0
7 to 12 inches	25.1	26.4	1.3
Second foot	19.9	17.7	−2.2
Third foot	13.0	12.4	−0.6
June 26 (Only 0.15 inch of rain since June 10):			
1 to 6 inches	10.8	11.4	0.6
7 to 12 inches	14.5	13.4	−1.1
July 18 (1.89 inches of rain on July 14–16, 2.78 inches since June 26):			
1 to 6 inches	21.6	23.3	1.7
7 to 12 inches	19.6	19.1	−0.5
Second foot	12.0	10.7	−1.3
Third foot	11.5	12.0	0.5
Nov. 12 (2.32 inches of rain Nov. 1 to 8; 8.63 inches since July 18):			
1 to 6 inches	25.2	27.1	1.9
7 to 12 inches	25.1	27.6	2.5
Second foot	24.4	25.1	1.7
Third foot	16.0	16.5	0.5

TABLE XII.—*Moistness of soil on plots in 1918 compared with that in 1915, showing the much drier condition of the subsoil in the former year*

Plot No. and depth of section.	Ratios in 1918.			Ratios in 1915.		
	June 11.	July 18.	Nov. 12.	July 7.	Aug. 4.	Sept. 2.
Plot 3:						
First foot	3.3	2.6	3.4	3.5	3.9	2.5
Second foot	2.2	1.4	3.2	3.3	3.6	2.6
Third foot	1.6	1.5	2.1	3.2	3.3	2.9
Plot 4:						
First foot	3.2	2.7	3.3	3.2	3.8	2.5
Second foot	2.5	1.5	3.0	3.1	3.5	2.5
Third foot	1.7	1.5	2.1	3.2	3.5	2.9

In general, it appears that percolation causes but little loss of the summer rainfall in the case of soils as fine in texture as the silt loams when these are in grasses or small grains, the portion of the subsoil occupied by the roots intercepting and giving up to the crop any of the moisture that penetrates through the surface foot. On fields with a sharply rolling surface a lowered water capacity, due to loss of organic matter, might be accompanied also by greater difficulty of penetration, and hence by a sufficiently greater loss by run-off to cause a markedly lower crop yield.

The comparatively slight influence that the water-holding capacity of the surface soil alone exerts upon the productivity finds an illustration in the common observation that sandy loams provided with fine-textured subsoils, when properly farmed, produce as heavy yields as clay loams.

The moisture available to a crop, in so far as the character of the soil determines the amount, depends upon the water-retaining capacity of the whole soil section penetrated by the roots of the crop, and not chiefly upon that of the surface stratum, and while cultural methods which lessen the organic-matter content of this stratum lower its water-retaining capacity, it forms such a small part of the whole moisture-retaining section that any change in the moisture supply thus induced may be too slight to have any distinct influence upon the productivity.

SUMMARY

The paper reports a detailed study of the moisture conditions found on two adjacent Minnesota plots, both of which had a silt loam soil, very uniform in texture, but differing widely in content of organic matter as the result of great differences in cultural treatment.

During the cool, wet summer of 1915, when cultivated crops were grown, the surface foot, and this alone, showed a very marked difference in the moisture content, especially in the available portion, the soil the richer in organic matter retaining the more water; but in the warmer and somewhat drier summer of 1918, when winter rye was used, much smaller differences were found.

It is concluded that in the case of a finer-textured soil, with a fine-textured subsoil and a comparatively level surface, the differences in the watery capacity that may be caused by differences in manuring or in cultural operations exert but little influence upon the productivity.

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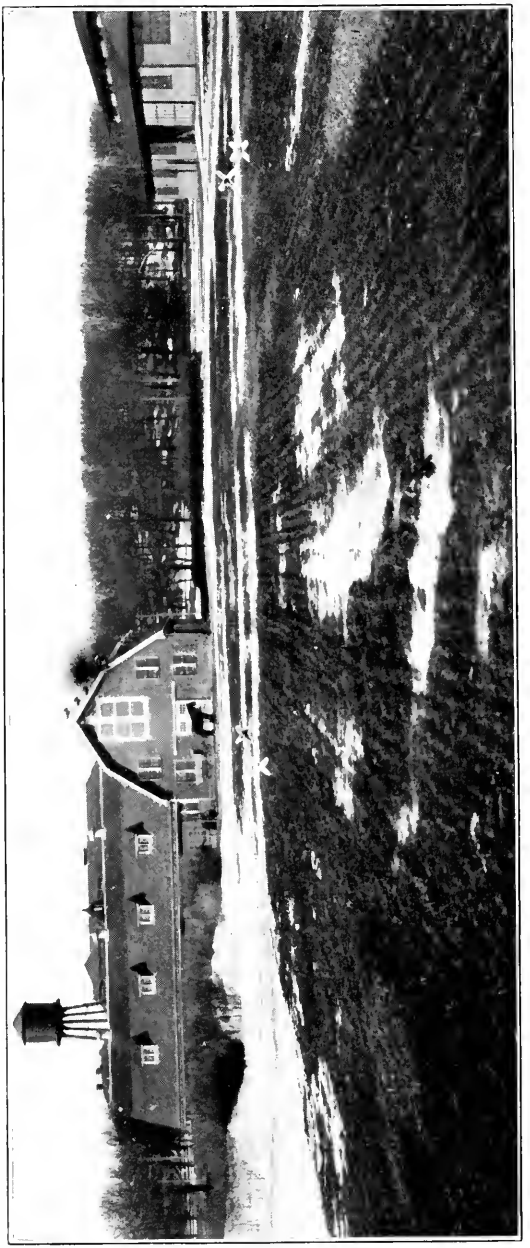

PLATE 36

View of Field J., Minn. Agr. Experiment Station Farm, showing topography and surroundings, looking from plot 6 to the barn which occupies part of plot 1. The photograph, taken on the morning of February 27, 1918, as the snow was disappearing, shows plot 4 to be slightly the lowest, the two streaks of ice from north to south, marked by crosses, both being on the plot. The water, held back by the snow bank at the left, had frozen during the night.

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JOURNAL OF AGRICULTURAL RESEARCH

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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON: GOVERNMENT PRINTING OFFICE: 1919

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

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JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., MARCH 17, 1919 NO. 11

FUSARIUM-BLIGHT OF POTATOES UNDER IRRIGATION

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HISTORY OF FUSARIUM-BLIGHT

The Irish potato (*Solanum tuberosum*) has been one of the most profitable crops grown in the Greeley district of northern Colorado. In this fertile, irrigated section, one of the oldest in the country, the potato gave large yields for many years before any serious setbacks occurred. From 1908 to 1912 the inroads of disease threatened the industry severely, and in 1911 and 1912 the crops were failures. In 1915 a laboratory was established at Greeley for the study of potato troubles, but since that year the yearly losses have been light. Fusarium-blight has been present, however, as a conspicuous malady.

Fusarium-blight, or potato-wilt, is well recognized in nearly all the potato-growing regions of the country, except in the extreme Northeastern States. It is caused most frequently by the fungus *Fusarium oxysporum* Schlect., though other species of this genus have been found involved. In 1899[1] Smith (*15*)[2] first proved that species of this genus would cause plant-wilts, and in 1904 Smith and Swingle (*16*) described a potato-wilt and tuber-rot due to *F. oxysporum*. It was believed by them that the *F. solani* of Pizzigoni (*10*) and of Wehmer (*17*) was identical with their species. The confusion which existed over the definition of species was largely removed by the taxonomic investigations of Appel and Wollenweber (*1*), Carpenter (*2*), Sherbakoff (*14*), and Wollenweber (*20*). Manns (*6*), Orton (*9*), Pratt (*11*), Wilcox (*18*), and others have continued to reveal the great losses which species of Fusarium cause in the potato industry and have suggested methods of control.

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[2] Reference is made by number (italic) to "Literature cited," pp. 301–303.

PREVALENCE AND LOSS

Fusarium-blight has not appeared to persist in any one locality. Its visitations are sporadic. The losses are, therefore, not to be estimated in concrete terms. In Colorado in 1917, a favorable season for the crop, about 46,000 acres of potatoes were grown. It is estimated that, owing to disease, 10 per cent of the acreage planted gave either reduced yield or no yield. In a good field most of the diseased plants soon pass out of sight and make no impression on the casual observer. Yet these occasional diseased plants probably resulted in a loss of 500,000 bushels in Colorado alone. At other times the disease spreads more generally, and whole fields go down and are lost. Since the part of *Fusarium* spp. in the creation of disease depends largely on environmental factors, it is important to note that the conditions which prevail in Colorado do not exist in the same way in other places. The descriptions of diseases caused by species of Fusarium already published do not, therefore, closely apply to Colorado, because the climate, coupled with the soil conditions and irrigation practice existing there, creates a condition unknown in the East, where most of the work on *Fusarium* spp. has been done.

DESCRIPTION OF DISEASED PLANTS

The common manifestations of the disease are a wilting and rolling of a few leaves, followed quickly, slowly, or intermittently by the wilting of the remainder and usually the premature death of the foliage. Occasionally a single leaf will wilt, turn yellow, and die, whereas the remainder of the plant may continue healthy throughout thes eason. Frequently one of the two or more stems of a hill wilts and dies, while the others remain turgid and healthy. The time of appearance varies greatly. It may be noticeable when the first leaves appear or at any time throughout the season up to maturity. A faint lightening in the color of the plant may indicate a gradual attack, and when the attack is severe, a large plant may pass from health to complete collapse in two days. Late in the season, three or four weeks before frost, plants turgid and unwilted, are found upon which all the upper leaves are rolled. They are a little lighter in color than normal plants, and have often been designated by the uninitiated as being diseased by leafroll. Plate 41,B, shows a plant of this type; the leaves are rolled, but show no wilting. These plants may continue to lighten in color, the leaves to roll, and gradually to die. These conditions are due to the presence of Fusarium mycelium in the vascular tissues of the stem. Plate 40,D, shows a plant on which the leaves have rolled and are gradually dying. What has actually happened in this plant is that the fungus has created a physiological drouth which has extended over a long period. Upon examining plants in the earlier stages of wilt, the stem and roots are usually found clean and apparently healthy, but the seed piece has rotted, and remains

as a wet, jelly-like mass. Later in the season the seed piece practically disappears, and the roots and stem may become blackened and decayed. At first the stems may be wet and slippery, but in time they become dry, brittle, and friable. The main and lateral roots of plants having rolled leaves late in the season are often normal in external appearance.

The term "Fusarium-blight" is preferred for the disease, because it is more applicable to all its stages. The name "Potato-wilt" has been used elsewhere, and so has "Fusarium-wilt," but their use is likely to cause confusion with other diseases, and are not accurately descriptive, as they limit the picture to wilt. As will be seen later, the term "Fusarium-blight" covers the diseases much more accurately.

INOCULATION EXPERIMENTS

The cause of Fusarium-blight is *F. oxysporum*. Other species of Fusarium are capable of producing similar phenomena in the potato plant, but *F. oxysporum* is the species commonly found in isolation cultures from diseased material.

At various times cut potato tubers have been inoculated with spore susspensions of *F oxysporum* and kept in moist chambers in the laboratory. These tubers were always destroyed by the action of the fungus, while controls made in the same manner with sterile water remained normal.

On July 17, 1917, six smooth Early Ohio tubers were given surface sterilization. Each tuber was cut into two equal parts, one half being reserved for control and the other half for fungus inoculation. Glass rings of the Van Tieghem cell type were smeared on the edges with petrolatum and put down on the center of the cut surface of the tuber after it had dried slightly from cutting. Two drops of a heavy spore suspension from an authentic pure culture of *F. oxysporum* were placed within the ring under aseptic conditions, and the cell closed with a cover glass. The controls were prepared the same way with sterile water. These seed tubers were taken to the field and planted in a row at a depth of 3 inches, where they were subjected to natural field conditions rather than to the artificial environment of a flat. On August 13, 1917, twenty-seven days after inoculation, the ground was carefully scraped away and the plants taken up. The six seed pieces inoculated with the fungus were badly decomposed. Four of them had disintegrated to a completely rotten mass. The other two had sprouted, and a small firm area remained by the stem. *F. oxysporum* was recovered from these in pure culture. The controls were uniformly healthy throughout. Plate 37, A, illustrates a control and a diseased seed piece of this experiment.

On August 10, 1917, fifteen tubers were cleaned and sterilized. They were cut as before, and one half of them were inoculated with a spore suspension of *F. oxysporum* dropped into glass rings, the other half being treated as controls. Five inoculated and five control seed pieces were planted together in sterilized soil in each of three flats. They were

watered with sterile water. The flats were set on the edge of a field, where ordinary weather factors would act as normally as possible. These flats were noted finally on September 8, 1917. The control plants were healthly throughout, with normal foliage and stems 10 inches long. In the first flat only one tuber of the inoculated seed sent up a pair of sprouts. One of these was dead and the stem blackened for 2 inches above the ground. Although the seed piece was thoroughly decayed, the other sprout appeared healthy, as shown in Plate 37, B, It is characteristic in every respect of the field blight. A closer view of the seed piece, together with a control seed piece from the same flat, is shown in Plate 37, C. In the second flat two seed pieces failed to germinate, owing to decay. One was dead, and the stem was blackened. In the third flat one seed piece failed to germinate, while four sent up sprouts. These had all wilted thoroughly, though death had not yet occurred. None of these plants had been subject to frost. Isolation cultures were made from all the diseased plants from the three flats, and *F. oxysporum* was recovered in pure culture in each case.

On September 9, 1917, three flats were prepared for inoculation. Sterile soil was used. Seed pieces of the Pearl variety were inoculated with a spore suspension of *F. oxysporum* in the usual way. The spore suspension was made from cultures taken from the diseased seed pieces of the inoculation experiment of July 17. No controls were made, because of lack of space. Owing to the lateness of the season, these flats were taken to Fort Collins, Colo., where, through the courtesy of the Horticultural Department of the Agricultural College, they were placed in the greenhouse. The plants were not seen until November 3, at which time all were dead with the exception of three plants, then about to die.

On August 13, 1917, nineteen whole tubers which had been planted in the field for three weeks and which had sent up sprouts were inoculated. Fourteen of them were inoculated with a spore suspension of *F. oxysporum* poured into glass tubes entering the epidermis of the tuber; five were treated with sterile water as controls. In each case the plant had a healthy, vigorous start. On September 9 the plants were taken up and examined. The seed pieces of the controls were sound and the plants healthy; seven inoculated plants were healthy, though the seed piece was decayed; the other seven were wilted, the stem was blackened, and the seed piece was thoroughly decayed.

On August 10, 1918, sixty-one plants planted in sterile soil in flats were inoculated with *F. oxysporum*. Sixteen plants in flats in sterile soil were treated as controls. The method of handling was different from that used before. The seed pieces had been planted with the cut surface turned up, about a month previously, and the flats left in a cool place. They were watered periodically with sterilized water. By August 10 the

plants had germinated and sent up strong, vigorous sprouts. The seed pieces were solid and unusually well calloused. For the purpose of inoculation the soil was scraped away, and a small core about 1 cm. long was taken out of the upper surface of the seed piece with a small coring tool. The pit made by the removal of the core was filled with a spore suspension of *F. oxysporum* and closed with a cover glass smeared with petrolatum. The controls were treated in the same manner, except that the pit was filled with sterile water. For the next 20 days the flats were exposed to approximately field conditions. The experiment was discontinued on August 30. At that time the inoculated plants had wilted to the ground, while the control plants were normal. Upon examination of the underground parts of the inoculated plants the seed pieces were found to be wholly decayed, and the main root was infected. The roots were not destroyed nor was the main root decayed, but the vascular tissue was woody and filled with mycelium. Isolation cultures made from 40 of the inoculated plants gave *F. oxysporum* in pure culture.

INOCULATIONS ON MATURE PLANTS

On August 13, 1917, inoculations were made on Early Ohio potato plants in the field for the purpose of approximating the disease in its mature stage. The plants were in good soil and had shown no signs of blight from natural infection, though the seed pieces had been attacked. The method employed was very simple. The soil was carefully scraped away from the stem to a depth of 3 or 4 inches, and a slit was made with a scalpel lengthwise through one stem of the plant. A wedge of melilotus stem upon which *F. oxysporum* had been cultured, and which bore mycelium and spores plentifully, was inserted in the slit, and the whole being covered with soil. Forty-six plants were inoculated with the fungus, and 26 were treated as controls. On September 18 these plants were taken to the laboratory for examination. All of the plants had two or more stems, but in the case of the plants inoculated with *F. oxysporum* only the treated stem showed any injury. The plants treated as controls recovered from the mechanical injury, and the wound healed. Plate 38, B, shows a control plant and the method of inserting the wedge. Of the inoculated plants 3 were lost, 4 showed no infection, 2 showed weak or doubtful infection, and 37 showed positive infection. Stems showing infection were typical of the natural blight in every respect. The stems were dead, blackened, and shattered in most cases. Plate 38, A, illustrates two stems of the same plant; the stem at the left was inoculated; the one at the right was not, and shows no injury. Of the 37 stems showing positive infection 20 were selected at random and isolation cultures made. These yielded pure cultures of *F. oxysporum* in 18 cases, the 2 others being badly contaminated.

MODE OF INFECTION

Hitherto infection of potato plants by *F. oxysporum* through the root hairs and small rootlets has been accepted as the usual method. Smith and Swingle (*16, p. 13*) said this occurred, and Manns (*6, p. 306*) reasserted the fact. In case of the cowpeas and cotton, Orton (*7, p. 10; 8, p. 8*) found this manner of infection in both cases. Cromwell (*3, p. 425*) supposed root infection to be the means of entrance of *Fusarium trachephilum* Smith, causing the wilt disease of soybean. Jones and Gilman (*4, p. 7*) found the roots of cabbage to be attacked by Fusarium. These numerous instances would call for careful examination of the roots of infected plants. During the years 1916 and 1917, in only 6 plants out of many hundreds examined was this method of infection determined as probable in the case of fusarium-blight of the potato in the Greeley district. In 1918 the soil temperature at a depth of 6 inches was 6° F. above the average for the month of June of the preceding two years. Plants of the Charles Downing variety, planted during the last of May or early June, were badly diseased in some fields by being attacked through the fine roots and root hairs by *F. oxysporum*. This one variety was more severely attacked than any other, even in fields containing several varieties. Most other varieties were not assailed in this manner at all, except a few scattering Early Ohio plants. Higher temperatures seem to be necessary for root infection.

Infection from seed tubers containing the Fusarium organisms in the vascular bundles has been very seldom found. Wollenweber (*19, 20*) has shown that *F. oxysporum* overwinters in potato tubers, where it causes the familiar vascular discoloration. With the sprouting of the eyes when the seed piece is planted the organism infects the new plants and presumably causes wilting and death. One of the most extensively advocated control measures has been aimed to avoid this kind of infection. No trouble has been experienced with this method of infection in the last three years. Field experiments tending to show the nonseverity of this method of infection will be given below.

SEED-PIECE INFECTION

In the Greeley district and in other parts of Colorado potato seed pieces become infected with the Fusarium organism from the soil. The cut seed is vastly more liable to attack than the whole seed, and the decay following infection will begin two or three days after planting. It is justifiable to assume that in the average field nearly all cut seed pieces are infected. Fields have been examined in which hundreds of seed pieces were dug a few days after planting, and less than 5 per cent were found to be free from infection. The infection occurs through the large open wound of the cut surface, lightly protected by callus. The interior loose parenchyma at the center of the tuber, farthest from the active tissue of the vascular

region, is the weakest and least protected. There is a difference in the susceptibility of seed of different varieties, but what seems more important is that seed of the same variety from different sources varies greatly in its power of resistance. The rot following infection may be swift, and the fungus will destroy the seed piece before germination begins.

Plate 37, E, illustrates a seed piece upon which no eye has germinated, though the piece is nearly destroyed by rot. In this illustration two dark spots are to be noted in the vascular region, denoting vascular infection; yet no decay originated at that point. When the decay is slower, the seed germinates and sends up a vigorous shoot. Plates 37, D, and 38, D, illustrate cases of germination followed by seed-piece rot. The region adjacent to the active tissue is the last to decay because it is more resistant and because the decay begins in the loose parenchyma and advances toward the germinating point or place of attachment of the shoot. Where decay is delayed sufficiently to allow germination to take place, the decay works slowly through the active region, or it may stop temporarily. Plate 37, D, illustrates how the decay is delayed nearest the growing part and how it advances evenly toward this region. Plate 39, D, illustrates the base of a plant the seed piece of which had decayed thoroughly. The stem is cut away, showing the healthy tissue within and the absence of the parasite. The general good health of the roots should also be noted.

Some plants appear to grow normally for a few weeks, after which symptoms of disease begin to appear. The color may or may not change, and the leaves may show curling, rolling, or wilting. One lower leaf may turn yellow, wilt, and fall, while the remainder of the plant is a picture of health. In a single hill containing two or more sprouts the tip of one may wilt and the other remain healthy. Plate 40, C, represents a plant consisting of two stems, one of which is healthy, the other wilting. The stem at the left will die, while the stem at the right may live through the season and yield normally. Upon taking up such a plant the decay of the seed piece will be shown to have advanced toward and into the wilted stem, while in all cases the root system is healthy in every branch. Plate 40, B, shows the top of a plant consisting of three stems. The top leaf and the one below it on the same stem are wilted. Neither the other leaves nor the color of the plant indicated anything abnormal. Plate 39, C, shows the seed piece and the three stems of the tops illustrated in Plate 40, B. The wilted leaf shown in Plate 40, B, is on the middle stem pictured in Plate 39, C, which is at the center of the decay. The stem at the left, healthy on Plate 40, B, is here shown with a slight sound area remaining in the seed piece. A stem which shows these symptoms in early summer when conditions are favorable may not at once succumb, but is usually doomed to an early death. Plate 39, B, shows a young plant in which the decay advanced continuously from the seed piece into

the stem. Many plants are to be found which show the violent symptoms, wilting, drooping, and death, within a few days.

The great majority of plants in a field may advance to late maturity with no visible signs of Fusarium-blight. Entire fields have been observed which showed natural wilting caused by delayed irrigation; wet periods may occur, owing to excessive rain following an irrigation; and either of these conditions are conducive to the increased activity of the fungus, though recovery is possible and often occurs. In an entirely healthy field at any period of the season conditions may arise in which the blight gains the ascendancy, the plants wilting and dying in the course of a week. This may happen as late as September, yet infection did not occur immediately before the appearance of wilt, as the fungus had been present since the time of planting.

Whole seed is protected by a sound epidermis underlain by an active vascular tissue, the best protection the seed may have. Whole seed germinates quickly and establishes a sound, vigorous plant weeks before the seed piece has been destroyed by fungi, and the plant becomes liable to attack. It is not unusual for whole seed to remain sound through the growing season, though the ultimate death of the nongerminated eyes, the worn-out vitality of the vascular region, and the dead epidermis make infection possible. Injuries in handling or planting, such as are received from picker planters, render infection comparatively easy. Clipping the stem end to inspect for vascular infection is a most reprehensible practice, as it breaks the epidermis and makes a wound in that part of the tube, tissue which is lowest in vitality and least in the power of self-protection.

OCCURRENCE OF THE CAUSAL FUNGUS

In the case of seed pieces which obtained a favorable start and sent up sprouts the decay is slow. In individuals where it takes weeks to decay, the decayed watery portion leaches away and a callus forms when the growing tissue is reached. Where field conditions are right, the fungus will continue its slow advance into the foot of the stem, causing no decay and slight or no discoloration. As conditions unfavorable to the plant arise, the fungus grows in the vascular bundles and causes discoloration. Plate 39, B, shows a 6-weeks'-old stem to the left portion of which the decomposed and dried piece clings. Discoloration of the pith is found only at the very foot of the stem, and the upper vascular bundles are free from any trace of the fungus. In the field this plant would be regarded as healthy. In plants of this type the lowest roots on the stem are cut off from supplying water, and thereby cause some of the temporary queer symptoms to be noted in the foliage. The plant may recover, draw on the roots above more heavily, and continue growth. Nothing more may happen throughout the season; harvest may arrive, and the plant yield normally. In other cases where the soil is wet and compact the fungus is more active; it decays the foot of the stem, cuts off stolons, decays new potatoes, and finally kills the plant. Plate 39, A, pictures a

plant taken from a field where irrigation water got beyond control and flooded a portion of the field. The fungus advanced rapidly, decayed the stem, and caused the death of the plant. The advance was so rapid that the roots were killed for only a short distance, remaining uninfected 2 inches from the stem. In severe cases action is rapid and universal; whole fields succumb, causing the well-known epidemics. When stems of rapidly killed plants are pulled up, they are black, soft, and wet, as is illustrated in Plate 38, D. This plant, naturally infected by *F. oxysporum*, is strikingly like the plant shown by Link (*5*, *fig.* 7), as caused by artificial infection with this organism. Other organisms follow closely behind the species of Fusarium and complete the decay of any tissue not thoroughly invaded by that fungus.

ISOLATION OF CAUSAL ORGANISMS

During the growing seasons of 1916, 1917, and 1918 more than 1,500 cultures have been made in attempts to isolate the causal organisms. Plants in every condition, from newly planted diseased seed to new tuber infection at harvest, were used as sources of culture. The material was selected in the field, and taken at once to the laboratory. It was carefully washed under slowly running water and patted to comparative dryness between damp towels. The material was prepared for culturing by breaking it open and, with a sharp chisel-pointed platinum needle, transferring small pieces from the desirable areas to tubes containing sterile melilotus stems.

In making these cultures too many precautions can not be taken to keep within very small areas with the needle. The line of demarcation between apparently firm tissue and diseased tissue is definite and narrow. Cultures made from the firm tissue immediately before the line, and on the line, were usually pure, and sporulated readily. Tissue back of the line gave many contaminations; too far in advance gave no growth at all in culture. In decaying seed pieces it is well to keep within 2 mm. of the line of decay. In the green tissue of growing stems little trouble will be experienced if the stems are not broken or torn before culturing; and, as infected stems soon become woody, a stiff sharp needle is necessary in culturing from them. Any blackened tissue will usually yield a culture. These infected tissues invariably yield species of Fusarium, though, if decay has advanced to the point of disintegration, contaminating organisms will be present. *Stysanus stemonitis* is frequently found in both attacked stems and seed pieces. Bacteria are rarely found in firm or semifirm tissue.

FIELD EXPERIMENTS

A series of experiments were performed with several lots of potatoes to determine as nearly as possible the origin of the disease developing during the growing season, basing the deductions upon the conditions

of the seed at planting time and the symptoms displayed during growth. These experiments were carried out in the field under conditions approximating commercial field practice, and no methods of culture or treatment were used at any time after planting which would not have been used by a commercial grower. For the purpose of this experiment it was conceded that parts of the same seed potato, grown under like conditions, would follow within reasonable limits pretty nearly the same course of procedure in growth, disease symptoms, and general appearance. A difference in two plants from twin seed pieces must be accredited to different conditions encountered during the growing season after planting. Various lots of seed were assembled in 1916 to test out this assumption. Among others, they consisted of one lot of certified Wisconsin Pearl, one lot of certified Wisconsin Rural, two lots of Early Ohio from the Red River Valley in Minnesota, one lot of Rural from the Carbondale District of Colorado.

All tubers were cut from bud to stem end, dividing the tuber into two equal parts. All tubers above 6 ounces were cut into four pieces. These were cut in the field and planted immediately side by side in parallel adjacent rows. They were given as good care as possible during the growing season. The summer was excessively warm until July 30, 1916, at which time 3.09 inches of rain fell. The remainder of the summer was comparatively cool. Notes were taken four times during the summer: Once when the plants were about 6 inches high, then when they averaged 12 inches high, again when they were full grown, and finally when no normal change was to be expected. No reference was made to any previous note; other members of the force were asked to assist in the work, and every method employed by which an impartial diagnosis could be made.

KEY TO TABLE I.

For the purpose of summarizing and presenting the performance of these lots of tubers, a new form of table, known as an aggregation table, has been constructed. This table must not be confused with a correlation table, which it resembles in general appearance, but not in context. In any single row of potatoes six different ultimate types of plant were recognized. These have been designated as "H," "HD," "DH," "D," "A," and "O." The meaning of these symbols are as follows: "H" denotes a plant which appeared healthy throughout the growing season; "HD" denotes a plant which was healthy during the first part of the season, but finished by being diseased; "DH" denotes a plant which gave manifestations of disease during the first part of the season, but finished by being healthy; "D" denotes a plant which was diseased throughout the season; "A" denotes a plant so badly diseased as to be merely existing, with no hope of progeny; "O" denotes no germination, or a case in which the seed piece suffered the maximum of disease and rotted in the ground. All plants in a row fell into one of these divisions. In comparing the plants from twin seed pieces in the two adjacent rows at the same time it is seen that in classification certain coincidences, or lack of them are significant. In the case of some plants classed as "H" in one row, the twins in the adjacent row were "H" also; in more cases they differed for all the

other five groups. Originally in numbering rows in the field the even and odd followed naturally, so that in the tables the parallel adjacent rows are most easily designated by the terms "even" and "odd." In the tables the "even" rows are read vertically—that is, the designating letters are placed across the top, and the aggregate totals for each class across the bottom; the "odd" rows are read horizontally, the letters at the left and the aggregate totals at the right. However, as noted before, many of the twins differ individually in their performance, so that in order to express this deviation the total of one class in one row is split up according to the numbers required to express the reciprocal in the other row. A concrete example is given in Table 1 A. In the even row there are 352 H, 4 HD, 5 DH, 1 D, 13A, and 12 O plants, a total of 387. In the odd row there are 320 H, 5 HD, 7 DH, 1 D, 23 A, and 31 O plants, a total of 387. In comparison with 352 H plants in the even row, twin for twin in the odd row, 296 plants are also H, while 4 are HD, 7 DH, 1 D, 20 A, and 24 O. Turning about, it is seen that, of the 320 H plants in the odd row, twin for twin in the even row, 296 are H, 3 HD, 3 DH, 1 D, 11 A, and 6 O. The same system follows for the other classifications. It is revealed, then, how nearly alike twin seed pieces perform, for where they are alike the numbers appear in either the HH, HDHD, etc., squares down to OO. Differences are shown when they appear elsewhere.

TABLE I.—*Aggregation of seed piece performance of Irish potatoes*

A.—WISCONSIN PEARL

[1 equals 0.258398 per cent of 387]

	H		HD		DH		D		A		O		Total.	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
H	296	76.49—	3	0.78—	3	0.78—	1	0.26—	11	2.84+	6	1.55+	320	82.69—
HD	4	1.03+	1	.26—	....	.....	....	.....	....	.....	....	.....	5	1.29+
DH	7	1.81—	....	.....	....	.....	....	.....	....	.....	....	.....	7	1.81—
D	1	.26—	....	.....	....	.....	....	.....	....	.....	....	.....	1	.26—
A	20	5.17—	....	.....	2	.52—	....	.....	....	.....	1	.26—	23	5.94+
O	24	6.20+	....	.....	....	.....	....	.....	2	.52—	5	1.29+	31	8.01+
Total	352	90.96—	4	1.03+	5	1.29+	1	.26—	13	3.36—	12	3.10+	387	100.00

B.—INFECTED WISCONSIN PEARL

[1 equals 3.70370 per cent of 27]

H	25	92.60—	....	.....	....	.....	....	.....	....	.....	....	.....	25	92.60—
HD	1	3.70+	....	.....	....	.....	....	.....	....	.....	....	.....	1	3.70+
DH	1	3.70+	....	.....	....	.....	....	.....	....	.....	....	.....	1	3.70+
D	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....
A	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....
O	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....
Total	27	100.00	....	.....	....	.....	....	.....	....	.....	....	.....	27	100.00

C.—CERTIFIED WISCONSIN RURAL NEW YORKER

[1 equals 0.225225 per cent of 444]

H	89	20.05—	1	0.23—	24	5.41—	1	0.23—	19	4.28—	30	6.76—	164	36.94—
HD	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....
DH	16	3.60+	....	.....	32	7.21—	2	.45+	14	3.15+	16	3.60+	80	18.02—
D	....	.....	....	.....	2	.45+	....	.....	....	.....	....	.....	2	.45+
A	18	4.05+	....	.....	11	2.48—	2	.45+	2	.45+	11	2.48—	44	9.91—
O	67	15.09+	1	.23—	19	4.28—	4	.90+	23	5.18+	40	9.01—	154	34.68+
Total	190	42.79+	2	.45+	88	19.82—	9	2.03—	58	13.06+	97	21.85—	444	100.00

TABLE I.—*Aggregation of seed piece performance of Irish potatoes*—Continued

D.—INFECTED CERTIFIED WISCONSIN RURAL NEW YORKER

[1 equals 6.25 per cent of 16]

	H		HD		DH		D		A		O		Total.	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
H	6	37.50	....	.....	1	6.25	....	.....	1	6.25	1	6.25	9	56.25
HD	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....
DH	1	6.25	....	.....	1	6.25	....	.....	....	.....	1	6.25	3	18.75
D	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....	....	.....
A	1	6.25	....	.....	....	.....	....	.....	....	.....	....	.....	1	6.25
O	2	12.50	....	.....	....	.....	....	.....	....	.....	1	6.25	3	18.75
Total	10	62.50	....	.....	2	12.50	....	.....	1	6.25	3	18.75	16	100.00

E.—RED RIVER VALLEY EARLY OHIO.

[1 equals 0.259067 per cent of 386]

H	141	36.53−	2	0.52−	25	6.48−	.....	.....	14	3.63−	37	9.59−	219	56.74−
HD	2	.52−	.....	.....	.....	.....	.....	.....	.....	.....	1	.26−	3	.78−
DH	29	7.51+	.....	.....	11	2.85−	.....	.....	1	.26−	6	1.55+	47	12.18−
D	2	.52−	.....	.....	.....	.....	1	.26−	.....	.....	.....	.....	3	.78−
A	7	1.81+	.....	.....	.....	.....	.....	.....	1	.26−	4	1.04−	12	3.11−
O	62	16.06+	1	.20−	16	4.14+	.....	.....	3	.78−	20	5.18+	102	26.42+
Total	243	62.95+	3	.78−	52	13.47+	1	.26−	19	4.92+	68	17.62−	386	100.00

F.—INFECTED RED RIVER VALLEY EARLY OHIO.

[1 equals 2.857142 per cent of 35]

H	16	45.71+	.....	.....	4	11.43−	.....	.....	1	2.86−	2	5.71+	23	65.71+
HD	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
DH	2	5.71+	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	5.71+
D	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
A	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	2.86−	1	2.86−
O	4	11.43−	1	2.86−	2	5.71+	.....	.....	.....	.....	2	5.71+	9	25.71+
Total	22	62.85−	1	2.86−	6	17.14+	.....	.....	1	2.86−	5	14.29−	35	100.00

G.—CARBONDALE RURAL NEW YORKER

[1 equals 0.3125 per cent of 320]

H	29	9.06+	.....	.....	20	6.06+	1	0.31+	5	1.56+	27	8.44+	91	28.44−
HD	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
DH	10	3.12+	.....	.....	40	12.50	1	.31+	3	.94−	16	5.00	70	21.88−
D	.....	.....	.....	.....	2	.62+	.....	.....	.....	.....	.....	.....	2	.62+
A	5	1.56+	.....	.....	8	2.50	1	.31+	1	.31+	9	2.81+	24	7.50
O	29	9.06+	1	.31+	29	12.10	1	.31+	10	3.12+	53	16.50+	133	41.56+
Total	73	22.81+	1	.31+	118	36.87+	4	1.25	19	5.94−	105	32.81+	320	100.00

H.—GREELEY LATE OHIO.

[1 equals 1.111111 per cent of 90]

H	45	50.00	1	1.11+	7	7.78−	.....	.....	3	3.33+	5	5.56−	61	67.78−
HD	1	1.11+	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	1.11+
DH	6	6.67+	.....	.....	6	6.67+	.....	.....	.....	.....	2	2.22−	14	15.56−
D	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
A	1	1.11+	.....	.....	1	1.11+	.....	.....	.....	.....	.....	.....	2	2.22+
O	7	7.78−	.....	.....	2	2.22+	.....	.....	.....	.....	3	3.33+	12	13.33+
Total	60	66.67−	1	1.11+	16	17.78−	.....	.....	3	3.33+	10	11.11+	90	100.00

TABLE I.—*Aggregation of seed piece performance of Irish potatoes*—Continued

I.—INFECTED GREELEY LATE OHIO

[1 equals 1.960784 per cent of 51]

	H		HD		DH		D		A		O		Total.	
	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.
H	26	50.98+	1	1.96+	2	3.92+			2	3.92+	2	3.92+	33	64.71−
HD														
DH	4	7.84+			5	9.80+							9	17.65−
D														
A					1	1.96+							1	1.96+
O	6	11.76+			1	1.96+					1	1.96+	8	15.69−
Total	36	70.59−	1	1.96+	9	17.65−			2	3.92+	3	5.88+	51	100.00

J.—GREELEY PEARL

[1 equals 0.7246377 per cent of 138]

H	63	45.65+	6	4.35−	4	2.90−			4	2.90−	11	7.97+	88	63.77−
HD	1	.72+	2	1.45−							1	.72+	4	2.90−
DH	4	2.90−									2	1.45−	6	4.35−
D											1	.72+	1	.72+
A	5	3.62+	1	.72+					2	1.45−	3	2.17+	11	7.97+
O	10	7.25−	2	1.45−					3	2.17+	13	9.42+	28	20.29−
Total	83	60.14+	11	7.97+	4	2.90−			9	6.52+	31	22.46+	138	100.00

K.—INFECTED GREELEY PEARL

[1 equals 1.8867924 per cent of 53]

H	22	41.51−	3	5.66+	3	5.66+			4	7.55−	6	11.32+	38	7.170−
HD			1	1.89−									1	1.89−
DH														
D											1	1.89−	1	1.89−
A	3	5.66+							2	3.77+	1	1.89−	6	11.32+
O	3	5.66+							1	1.89−	3	5.66+	7	13.21−
Total	28	52.83+	4	7.55−	3	5.66+			7	13.21−	11	20.75+	53	100.00

L.—INFECTED COLORADO PEARL

[1 equals 0.6666666 per cent of 150]

H	115	76.67−	14	9.33+							2	1.33+	131	87.33+
HD	14	9.33+	5	3.33+									19	12.67−
DH														
D														
A														
O														
Total	129	86.00	19	12.67−							2	1.33+	150	100.00

M.—IDAHO-GROWN IDAHO RURAL

[1 equals 0.826446 per cent of 121]

H	105	86.78−	9	7.44−							2	1.65+	116	95.87−
HD	5	4.13+											5	4.13+
DH														
D														
A														
O														
Total	110	90.91−	9	7.44−							2	1.65+	121	100.00

WISCONSIN PEARL (TABLE I, A–B)

Aggregation Table I, A, illustrates the performance of the certified Wisconsin Pearls potatoes in 1916. There was a total of 774 seed pieces planted, the result of dividing 387 tubers. The striking thing to be noted in this section of the table is the fact that with the majority of diseased plants in either row the twin was healthy. In only 11 cases did both twins fall outside of a healthy square, five of these being in the square OO. If vascular infection was to act here only the 5 twins in the OO square could properly be said to come under control of it, because it is the only instance where the performance was the same for both twins. In the cases of the 80 pairs of twins, 1 of which was in some way diseased and the other healthy—that is, those in both rows where 1 twin was an H plant, exclusive of those in HH—it must be regarded that the disease was newly contracted. Before planting, all tubers were cut at the stem end to inspect for vascular discoloration, indicating the presence of a possible disease organism. Cultures were made from diseased tissue. Only 27 tubers, or 6.98 per cent, showed any discoloration. Tabulating then according to the place the tuber occupies in Table I, A, their places are shown in Table I, B.

According to the old conception of the danger of planting diseased seed, the 27 tubers, planting the 54 plants here shown should have given some sign of disease. The 2 which fall without the HH square are healthy plants, for one-half of the tuber would indicate no tendency to disease because of the vascular parasite, but because field conditions acted as in the case of 78 similarly situated plants, as shown in Table I, A.

WISCONSIN RURAL (TABLE I, C–D)

The certified Wisconsin Rural potatoes were treated in the same manner throughout, and were tabulated in the same way: 444 tubers were used, planting 888 hills, and their performance is shown in Table I, C.

The plants in this table are well distributed except in the HD columns. The performance of the twins as representing the strictly inherent tendencies of the tuber seems not to be indicative of any considerable failure because of previous faults. There are 40 pairs in OO, and 30 and 67 pairs in HO and OH, respectively. The factors which placed the 40 pairs in OO did not act on them as parts of 40 tubers, but as 80 individual plants, the same as took place with the 30 and 67 pairs, one-half of which were healthy. This variety is peculiar in showing such a contrast between susceptibility to disease and vigor to survive and grow away from it. The tubers of this lot were planted within 10 feet of the lot represented in Table I, A.

Table I, D, represents the place in which those seed tubers fell which showed discoloration in the vascular system. These 16 tubers (3.60+

per cent) are placed in the table according to the place they occupy in Table I, C, and it is regarded as of no significance that they fall where they do.

EARLY OHIO (TABLE I, E–F)

The Early Ohio seed obtained from the Red River Valley consisted of two lots. These lots were grown separately, but their performances were so nearly alike that they have been combined and presented in Table I, E, as one lot.

This table shows plants falling in the DHDH, DD, AA, and OO squares, a tendency not noted in the previous tables. In the case of the plants falling in DHDH, it would appear that some special weakness had developed in the 22 plants grown from these 11 tubers which placed them there. Inherent weakness, then, can not be predetermined by mere examination of the tubers, because Table I, F, which represents the location of the tubers showing vascular discoloration (9.07 – per cent), placed according to their location in Tables I, E, has none represented in DHDH. These plants outgrew their earliest diseased condition, and finished the season in apparent healthy condition. Table I, E, does not indicate, however, any strong vigor on the part of this lot.

Table I, F, represents the place the 35 tubers of Table I, E, which showed vascular discoloration fell, placing them according to their location in Table I, E.

RURAL NEW YORKER (TABLE I, G.)

One lot of seed of the Rural New Yorker variety was obtained from the Carbondale District of Colorado. It consisted of 320 tubers, free from vascular discoloration, and was regarded as stock of superior quality, selling at an advanced price. Table I, G, illustrates the almost complete failure of this seed through seed-piece infection and rot in the Greeley District.

In this table, where so much disease is represented, the conspicuous absence of plants falling in the HD columns (1 in the even row), and in the DD square, is significant. Root infection did not occur; no vascular discoloration was present in the seed. The great preponderance of plants in the OO square and the O columns, shows clearly that a most serious inherent weakness is present in the seed to withstand infection from the soil. The number of plants that did not eventually become healthy, having previously grown and been diseased, are very few. There is a strong tendency to die or survive (H or DH), for the plants that are H or DH were vigorous at the end of the season. The others either failed, as in O, or gave evidence of a gradually declining health, as in D and A. The lack of plants in the HD columns (1 in the even row) is further evidence that a healthy plant maintains its position.

LATE OHIO DISEASED SEED (TABLE I, H-I)

In the fall of 1915 several fields were visited for the purpose of staking diseased hills of potatoes. The hills selected all showed blackening of the stems and death of the tops, with many cases of rot in the tubers. At harvest the badly decayed tubers were discarded for the reason that they were in no condition to keep through the winter. The tubers showed a large percentage of vascular discoloration, and the remainder were believed to be infected, though not seriously. All came from hills affected by Fusarium-blight. Cultures were made from the stem end of all showing discoloration, and all yielded species of Fusarium. The tubers were cut and planted as twins in adjacent rows and given the same culture as the lots mentioned above. Table I, H, shows the performance of such seed of the variety Late Ohio in the year 1916.

The complete absence of any plants falling in the D columns is the outstanding feature of this table. At best, only the 6 pairs represented in DHDH square could be said to show the results of vascular infection, especially from the occurrence of 5 of them in the DHDH square in Table I, I. Compared with Table I, E, which represents a healthy Early Ohio variety, the advantage in health is with the home-grown seed.

A table representing the places in which the 50 tubers (56.66+ per cent) showing decided vascular discoloration fell, is shown by Table I, I.

PEARL DISEASED SEED (TABLE I, J-K)

One lot of diseased seed consisting of 138 tubers, Pearl variety, acquired in the same manner as the Late Ohios, were cultured from the stem-end, and planted under the same conditions as other lots. Table I, J illustrated the performance of this badly diseased seed stock.

Two things are conspicuous here: The lack of plants in the D columns (one in DO), and the comparatively large number in the O and the HD columns. These are not to be accounted for here altogether because of their vascular discoloration, because Table I, K, which represents where the 53 tubers (38.41 — per cent) fell which showed discoloration, does not account for the majority. Plainly these plants have been weakened by disease, their power of resistance lessened, and their vigor impaired. Examination showed that soil infection acted here to produce the disease, but a comparison of Table I, J, with Table I, A, reveals the great weakness acquired by these plants, which made them so easily attacked. These tubers were extreme cases, being stock that would not ordinarily get into commercial seed. The circumstances surrounding this lot of seed tends to explain the true reason why farmers of the Greeley District prefer to plant newly introduced seed every two years.

Table I, K, represents the places in which that seed fell which showed pronounced vascular discoloration, according to the place they occupy in Table I, J.

PEARL DISEASED SEED (TABLE I, L)

In 1917 one lot of seed, Pearl variety, was secured, each tuber of which showed positive vascular infection by species of Fusarium, as proven by isolation cultures. Conditions were generally more favorable for potato growth early in the 1917 season than they were in the 1916 season, and less favorable late in 1917 than in 1916. Table I, L, shows the performance of this badly diseased stock.

IDAHO RURAL (TABLE I, M)

One lot of seed from Idaho, known as Idaho Rurals, and healthy throughout, were treated in the same manner. No tuber showed disease or infection in the vascular system. The performance of this lot of seed is illustrated in Table I, M.

The similarity between Tables I, L, and I, M, is striking. The presence of plants in the HD columns is attributed to the unfavorable late season in 1917. This is taken to account for the uniform health as represented for the diseased Pearl variety in Table I, L.

There is no reason to suppose that if mere chance had operated so as to have each seed fall where its twin fell, and vice versa, that the result would have been the same in any table. Each seed piece was surrounded by a different set of factors which operated to bring about disease or apparent health. For that reason it is unlikely that the results of a single year can be duplicated, though the average of similar years ought to strike a fair average.

DISEASE RESISTANCE

In the fall of 1915 a field of potatoes was chosen upon which to conduct an experiment in disease resistance. It was one calculated to offer crop failure if one was reasonably possible. One end of the field was white with alkali, the soil was heavy, and drainage was poor. A portion of the field already had potatoes on it, supposedly of the Pearl variety. It presented a very ragged appearance owing to skips, diseased plants, and mixture. Two of the best-looking rows were selected to work upon, and all the diseased and mixed plants were staked. After frost the staked plants were taken out by hand, and the remaining healthy plants were harvested with a machine. In 1916 this seed was planted on an adjoining plot. All the plants came up healthy and with increased vigor. Some plants succumbed to blight during the season, but at least 90 per cent reached harvest. Again in 1916 several rows were inspected, the diseased and mixed plants staked, and the healthy ones harvested as before. These were planted in 1917 in a plot adjoining the one used in 1916. In comparison with other potatoes in the field, the vigor and health of the selected seed was notable. Very few diseased plants were to be found, and skips in the rows were rare. These plants promised

well for another year, when a mistake was made in watering by the farmer, the ground became water-logged, and the entire field was lost through blight in the mature stage.

SOIL CONDITIONS AND IRRIGATION

Soil conditions materially assist the plant or the fungus. If the ground is well moistened and loose when the seed is planted, a strong vigorous start may be obtained by the plant which will carry it well beyond the immediate reach of the fungus. It has been the common practice to withhold irrigation until the new tubers begin to set. If the plant can endure withholding artificial watering until the new tubers set, it is well to delay, but to postpone it until the plant is suffering acutely, brings it to a condition from which it never wholly recovers. The fungus will make headway in a drouthy plant. After irrigation water has been supplied, it is expedient to cultivate deeply, because irrigation water packs the soil tightly. Too great an application of water on heavy soil may leave the soil puddled, in which condition it must remain for several days before cultivation is possible. If this is accompanied by a rising soil temperature, the ill effects are increased. Occasionally a heavy rain will puddle the soil late in the season preceding harvest. This may occur on ground irrigated too late. In such an event it is common for the plants to blight generally and die. The damage now is not in the death of the foliage or the death of the plant, but in the rot which will attack the new tubers. This is a black-rot which may enter by way of the stolons, a common method, or through wound or lenticel. When such tubers have begun to rot, they are a total loss. If the rot has not been detected in the field, it may occur later in the bin, causing a worse trouble. All the tubers of a plant may not be attacked, however, and in such a case control consists in getting them out of the ground without delay. Early varieties in which the new tubers have time to come to full ripeness are more susceptible than late varieties.

At this time correct irrigation practice is unknown. No rule can be formulated, because each piece of ground requires different treatment. During some years it is expedient to "irrigate up," meaning to water the field immediately after planting. If the soil is too dry, irrigation is necessary for germination and will carry the plant for the maximum length of time before rewatering. Harm results if the water applied in addition to the soil moisture present creates an excess. Irrigation of a plowed field in which nothing has been planted is impractical, owing to the absence of row ditches, and the fact that a certain time must elapse before anything can be planted. Cultivation should be given after each irrigation, so long as it can be done without damaging the plants below ground. Sandy soils need less cultivation than heavy soils. The best

judge of the soil on any farm is the farmer who has worked with it. Each parcel of land has its own pecularities, and advice on the handling of land should be specific. The very best conditions obtainable for the potato should prevail throughout the season, and so long as the farmer can control the environmental conditions no trouble is likely to result.

CONTROL

Control of Fusarium-blight has not been attained. Different methods have been employed, three of which offer reasonable hope of success.

First, selection of plants whose progeny will offer resistance to the invading organisms. For this purpose, experiments are being carried out with standard accepted varieties known to be suited to the locality. It is possible to select for resistance and have it gradually evidenced in the performance of the plants, as in the field experiment noted above. That is satisfactory so long as some overwhelming circumstance does not intervene and wipe out the work of years. At best, resistance is but a relative thing.

Second, control by seed treatment. The attempt has been made, not to kill something that may be on the seed as in the orthodox seed treatment, but to coat the cut seed with a preservative or fungicide which would remain vital throughout the season, preventing infection. Could this be done, it would offer an easy solution to the problem. Experiments were carried out in 1917 to test out the effect of different solutions. None of them gave satisfaction. Several lots of potatoes were treated and planted on May 18, 1918, with several different mixtures and compounds, all of which for some reason or other were suspected of having some possible preservation value. The seed used was the Rural variety, and was cut in the usual way. The method of application depended upon the nature of the fungicide, and this is noted under "Remarks" in Table II. One lot was treated with a spore suspension of *F. oxysporum* for comparison. On June 19, 1918, a similar experiment was made with a few lots. The results of these experiments are given in Table II, and are the data taken from counting 600 plants.

TABLE II.—*Effect of various seed treatment on germination of Irish potatoes*

PLANTED MAY 18; COUNTED JUNE 15

Treatment.	Percentage of germination.	Remarks.
Nicotine sulphate	75	Dipped. Solution 1 to 8,000.
Bordeau mixture	68	Dipped. Formula 5–5–50.
Charcoal	90	Dusted. Seed remained unusually firm.
Hypochlorous acid [a]	0	Dusted. Seed killed by treatment and rotted by several organisms.
F. oxysporum	35	Sprayed. Strong spore suspension.
Iron sulphate	40	Dusted. Some seed killed.
Lithum carbonate	0	Dusted. Thoroughly and quickly rotted.
Mercuric chlorid	60	Dipped. Solution, 1 to 1,000.
Mustard oil	70	Sprayed.
Controls	85	Dipped in water.
Whole seed	97	Dipped in 3 per cent solution of copper sulphate.

PLANTED JUNE 19; COUNTED JULY 15

Charcoal	82	Dusted.
F. oxysporum	0	Sprayed.
Onion juice	55	Dipped. Expressed juice of onions.
Control, cut	88	Dipped in water. Average field performance.
Whole seed	99	No treatment.

[a] SMITH, J. L. et al. ANTISEPTIC ACTION OF HYPOCHLOROUS ACID AND ITS APPLICATION TO WOUND TREATMENT. *In* Brit. Med. Jour., 1915, no. 2847, p. 129–136. July 24, 1915.)

In the first planting the charcoal treatment gave better germination than the controls, but fell behind in the second planting. None of the others were worth the trouble of treatment. The whole seed gave much better stands and of more healthy vigorous plants.

Third, in applying the best-known cultural practice to the propagation of the potato. For this no rules can be given. Each farmer should judge the condition of his land, its moisture content, tilth, and apparent needs. Rotation with grain and legumes is advisable, allowing the land to be cropped with alfalfa as many years as possible before potatoes are planted. Methods of irrigating and cultivation during the growing season should be investigated at the time for the field in question. Plate 41, A, shows a field planted with good seed, but owing to the dryness of the soil at planting time infection set in, and the fungus destroyed from 60 to 80 per cent of the seed, with the resulting poor stand.

GENERAL DISCUSSION

Infection of potatoes by *F. oxysporum* from the soil through the seed piece has never been recorded before, so far as is known. That it is of widespread general importance on alkali soils is believed, from conditions noted in several potato-growing regions of the West. In parts of the

San Luis Valley, where so many unfavorable conditions are at work, owing to subirrigation and a high water table, the large majority of the potatoes show signs of this infection. Other investigators have found vascular infection of the seed to be the cause of much trouble, and the seriousness of that manner of infection elsewhere can not be judged from these experiments. In the Greeley District, where the Fusarium-blight has been so serious for many years, a fortunate change has taken place. This is regarded as being due to the introduction of other crops, potatoes being brought into the crop rotation only once in four years or more. The use of seed beans, sugar beets, grain, and alfalfa in the definite rotation is extending the time between the same crops with corresponding advantage to each. The potato was desirable as a high-priced crop, and still is, and the percentage of loss is less with rotation.

Alkaline soils are a favorable medium for *Fusarium* spp. Pratt (*12*) found them to be abundant in virgin desert soils. The prolific and luxuriant growth of species of Fusarium on alkaline media in pure cultures is an indication of what may be expected in part in alkaline soils where humus is abundant. In disease investigations of this kind it was found desirable to conduct the experiments as much as possible in the field, for the reason that conditions there came about naturally, and the response was immediate and proportionate. Greater care must be taken to note and record every conceivable change of condition. In the gross the changes from day to day are observable and are recorded by suitable instruments; but the changes that occur in the plant are more delicate and rapid than gross observations indicate. Each square foot of soil has its own conditions, not distinguishable from the adjoining square foot perhaps, but of sufficient difference to be felt by the plant.

The plant feels these things and responds. If resistant stock is to be selected, these changes and conditions should be known, and the finer symptoms indicated by the plant must be recognized for the purpose of analysis.

Temperatures of the soil are vital as regards infection. The critical temperature for infection has not been determined and it varies for the manner of infection. Seed-piece infection will occur at a considerably lower temperature than root infection. In the Carbondale District, at a higher altitude and in cooler soil than the Greeley District, only those plants show Fusarium-wilt symptoms which have decayed seed pieces. Usually the seed piece remains sound throughout the season there, and the plants are free from blight. In the Greeley District the soil temperatures are higher, and the seed pieces are generally attacked. Root infection occurs with temperatures higher than the average. As the plants get larger and shade the ground, and the roots penetrate deeper the danger from root infection is lessened.

There has been a belief that less blight occurs when the potatoes follow alfalfa than otherwise, and that the older the alfalfa was the

better would be the potatoes. The current reasons for this are many and varied, but the principal one given is that there is less blight in the soil. This may mean fewer fungus organisms in the soil, but that does not seem to be the case. In several instances potatoes grown from good stock on soil previously in alfalfa for nine years have been observed as badly diseased as the same seed on soil only one year in alfalfa. The organism was present as abundantly as ever, and wherever the condition of poor cultivation or heated soil was present, the disease was manifest. The true value of alfalfa preceding potatoes lies in the fertilizer increment and mechanical improvement added to the soil, and not to any dearth of *Fusarium* spp.

The use of whole seed is suggested, not as a means of controlling the blight, but of avoiding it. By the use of whole seed is meant not culls and other small potatoes, but tubers in good condition, well selected, and preferably of 1½-ounce weight or greater. Whole seed has been many times condemned as yielding quantities of unmarketable small potatoes, and from the horticultural point of view this is a serious fault. Under irrigation, however, the writer believes that whole seed can be made to yield nearly as many marketable tubers as cut seed. The increased stand resulting and the fact that no labor is required in cutting would promise a return commensurate with the initial increased cost of the seed. In one commercial field in 1918 the yield from whole seed was 100 per cent greater than that from cut seed of the same variety. This field is shown in plate 41, B. The cut seed was planted on the left and the whole seed on the right. The photograph was taken at midseason. Sandsten (*13*) believes that whole seed is preferable to cut seed in dry-land farming because it prevents seed-piece rot.

SUMMARY

The disease of potatoes in the field caused by *Fusarium* spp., principally *F. oxysporum*, whereby death of the plant or decay of any part of it is brought about, is to be regarded as different phases of the same disease. For that reason it is desirable to apply a generally applicable name covering all stages. The term "Fusarium-blight" expresses this adequately.

Two methods of infection are recognized: Infection from the soil of roots and root hairs, and infection of the seed piece, whereby the plant becomes diseased. The latter method is regarded as the most serious and responsive to environmental conditions in the Greeley district of Colorado.

Three methods of control are suggested, none of which have yet proved wholly effective. First, selection for disease resistance, a method shown to be effective only to a minor degree. Second, superior cultural conditions for the potato plant, whereby it may always maintain a degree of resistance to pathogenic organisms through activity and

health. Lengthened rotation periods employing other crops followed by alfalfa improve the nutritive and mechanical properties of the soil, while a judicious irrigation practice adapted to the particular field and season involved combined with suitable cultivation should constantly maintain a steady and adequate, but never excessive, supply of moisture and insure suitable aeration. This is the method available to the farmer, so far as he knows what constitutes the best conditions for his land throughout a given season. Third, by the use of whole seed, free from wound or injury, thus preventing seed-piece infection, or at least maintaining the plant free from infection for the maximum length of time. The combination of the two last-named measures probably constitutes the most effective measures for control of Fusarium-blight.

It is believed that more than one species of Fusarium is able to bring about each phase of the blight. *F. oxysporum* in pure culture under suitably controlled and natural conditions has been found to do this.

Three general stages of the Fusarium-blight are recognized. First, the stage in which decay and death of the seed piece and new plant occurs before the new shoot emerges from the ground. Germination may or may not have occurred. Second, the later stage, in which the young plant shows many and diverse symptoms of infection by *Fusarium* spp., often resulting in death. Some of these manifestations are not fatal, and recovery is possible. Third, the mature stage, resulting in death, usually at an advanced state of growth, often with infection and decay of the new tubers.

Different varieties of potatoes show marked variation in their behavior under the same general conditions. There is an inherent weakness in different strains of the Rural variety toward Fusarium-blight, accentuated by the conditions under which the seed was grown. The Pearl variety shows these weaknesses, but to a minor degree, unless brought to a poor condition by previous subjection to disease.

Vascular infection of the seed is not the starting point of disease, but is one of the conditions assisting in bringing about decreased resistance to new infection from the soil.

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PLATE 37

Effect of Fusarium-blight on seed pieces of potato:

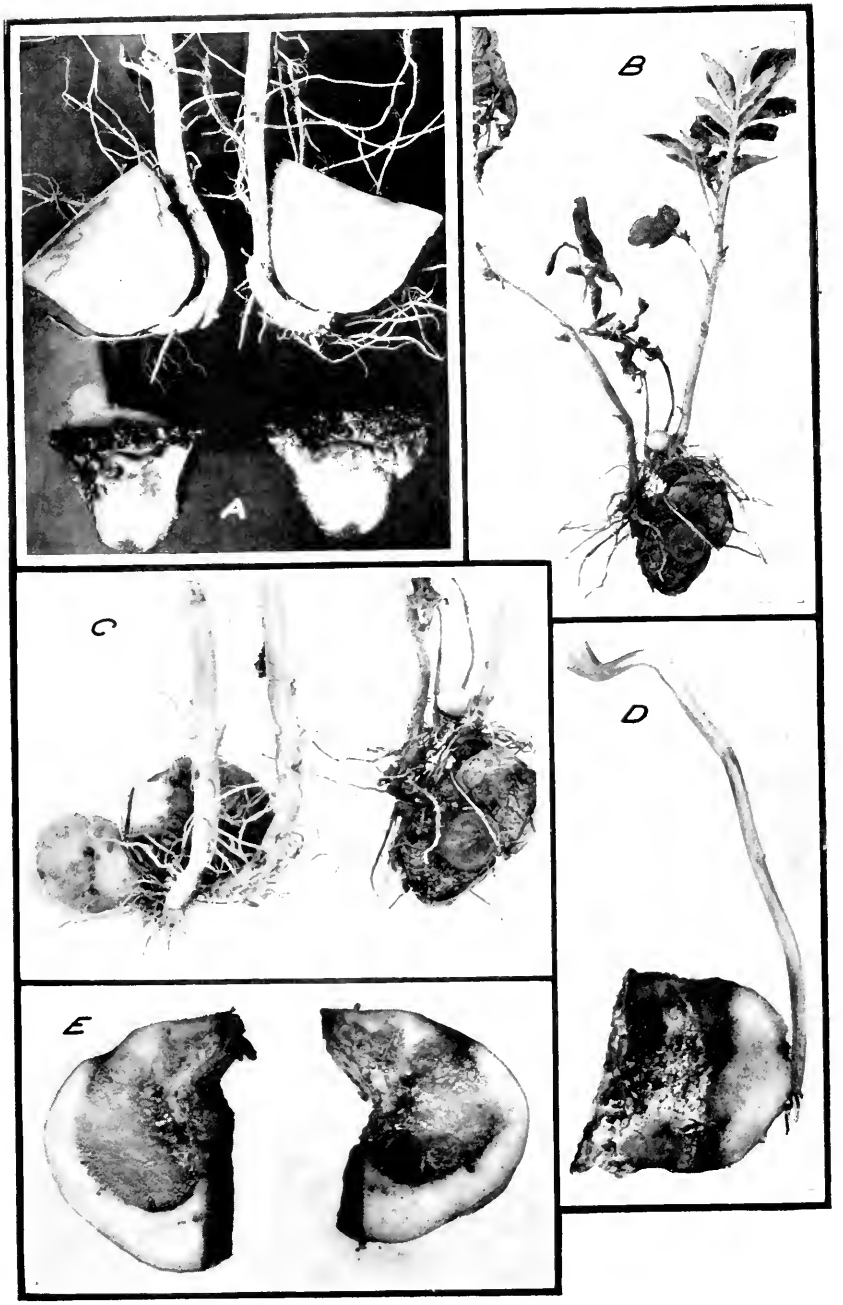
A.—Early Ohio seed pieces: Control above; pieces inoculated with *F. oxysporum* below.

B.—Early Ohio plant. See piece inoculated with *F. oxysporum*.

C.—Early Ohio seed pieces: Control (left) and inoculated (right) seed pieces. The control shows the method used in inoculation.

D.—Seed piece well decayed, resulting from soil infection.

E.—Seed-piece rot in field.


A
B
C
D
E

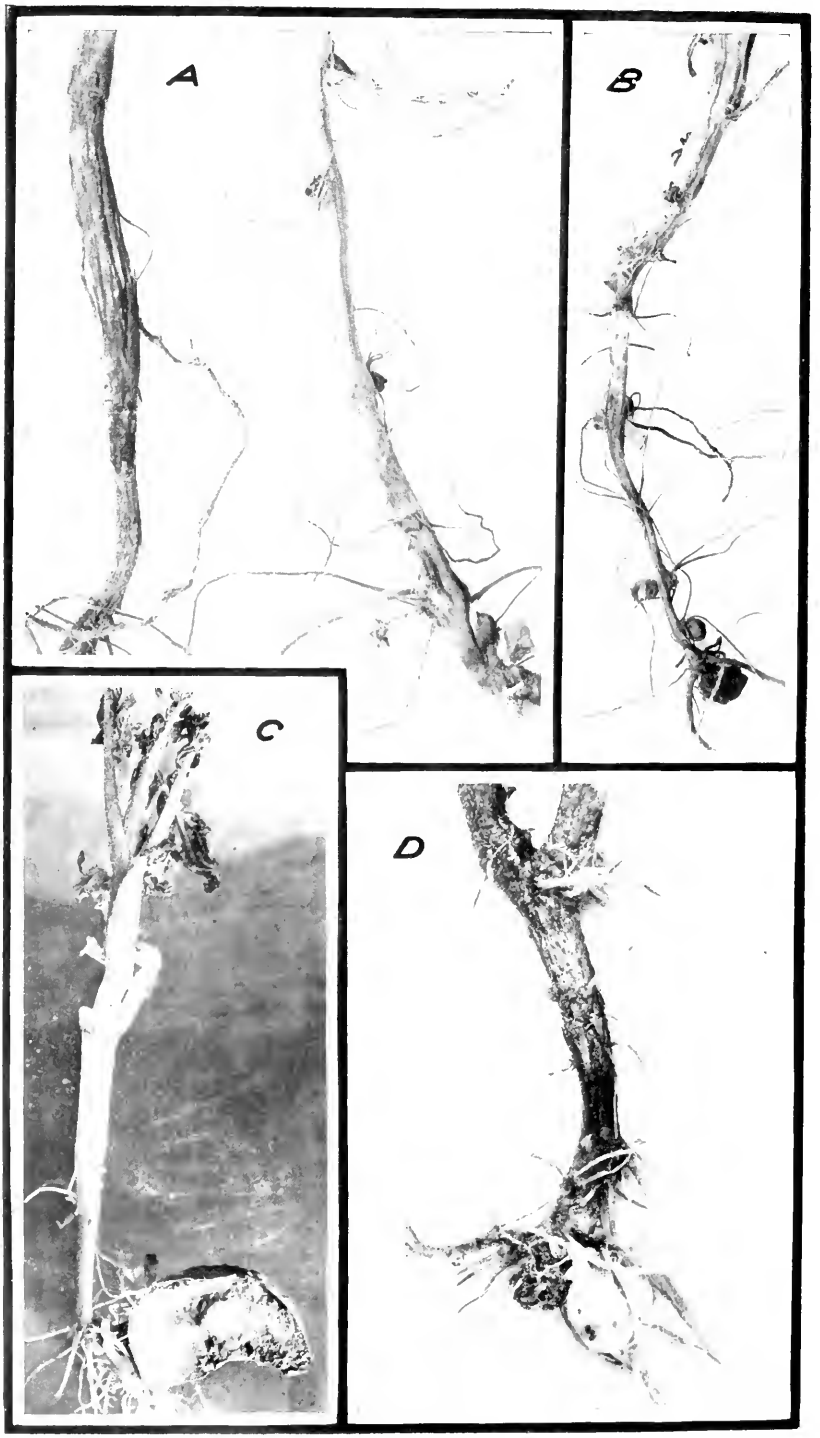

A
B
C
D

PLATE 38

A.—Inoculated and uninoculated stems of same potato plant. Stem at right shattered by *F. oxysporum*.

B.—Potato plant (control), showing method of inoculating with wedge of melilotus stem.

C.—Seed-piece rot in field. The young potato plant has not yet been attacked.

D.—Potato plant naturally infected by *F. oxysporum* in the field.

PLATE 39

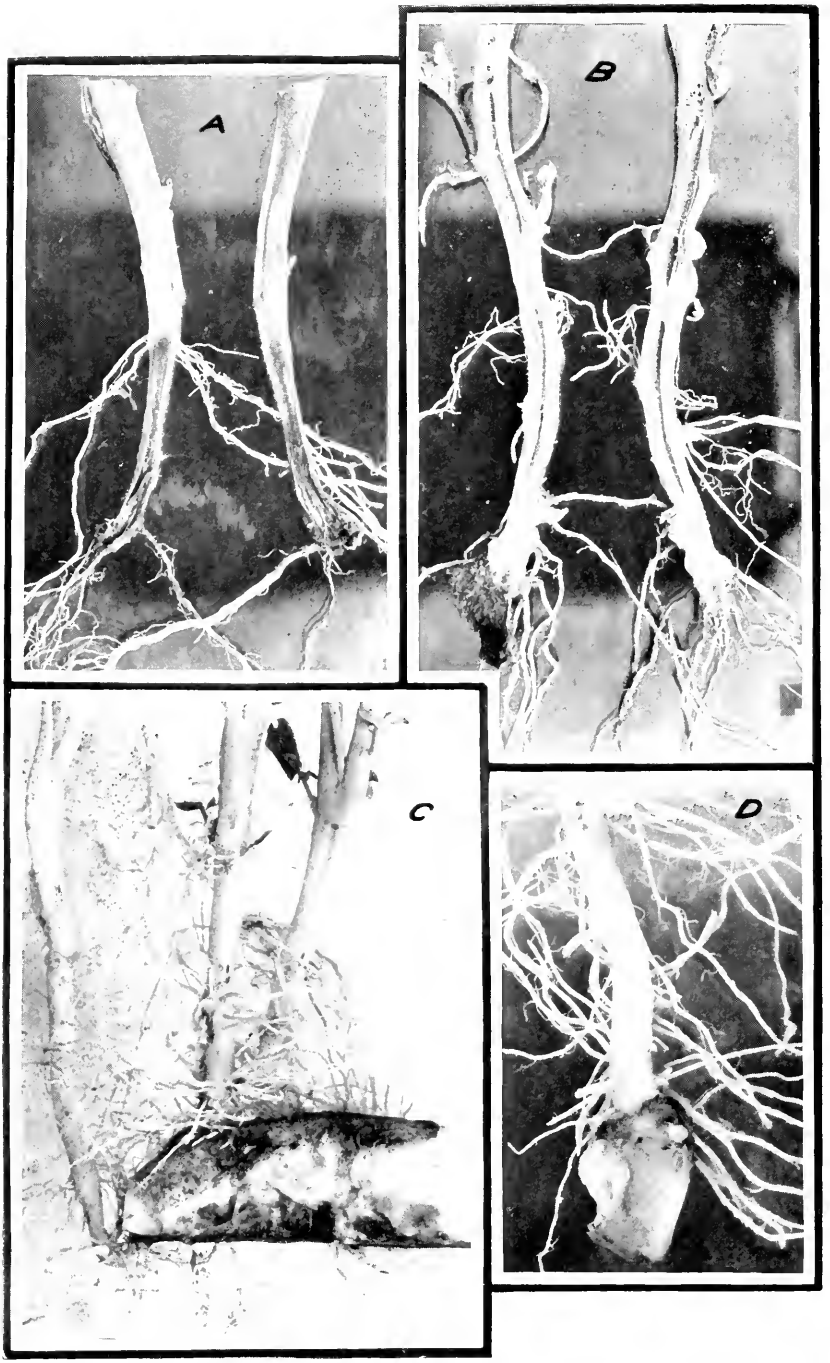
Potato stems showing seed-piece rot:

A.—Stem split to show rotting due to organism entering through seed piece from soil. Note decay of roots from point of attachment outward.

B.—Stem split to show slight discoloration at base where infection from soil-infected seed piece occurred.

C.—Seed piece of potato plant shown in Plate 40, B. The center top leads to the center of decay.

D.—Seed-piece rot in field. The seed piece is well decayed, but plant is unaffected and the roots are healthy.


A
B
C
D


A
B
C
D

PLATE 40

Potato plants affected by Fusarium-blight:

A.—Potato plant late in season with rolled leaves. Fusarium blight.

B.—Top of potato plant consisting of three stems. One leaf on one stem is wilted. See Plate 39, C.

C.—Potato plant of two stems, one at left showing Fusarium blight, or wilt caused by seed-piece infection; one at right healthy.

D.—Plant with rolled leaves gradually dying from Fusarium blight. Severe case late in season.

PLATE 41

A.—A field of potatoes showing the result of unfavorable cultural and soil conditions, by which seed-piece rot destroyed 60 per cent of the stand.

B. A field of potatoes planted with whole seed (rows to right) and cut seed (rows to left) at midseason. The hills planted with whole seed gave a 100 per cent greater yield than those planted with cut seed.


A


B

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PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

All correspondence regarding articles from the Department of Agriculture should be addressed to Karl F. Kellerman, Journal of Agricultural Research, Washington, D. C.

All correspondence regarding articles from State Experiment Stations should be addressed to H. P. Armsby, Institute of Animal Nutrition, State College, Pa.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVI WASHINGTON, D. C., MARCH 24, 1919 No. 12

EFFECT OF CERTAIN GRAIN RATIONS ON THE GROWTH OF THE WHITE LEGHORN CHICK

By G. DAVIS BUCKNER, E. H. NOLLAU, R. H. WILKINS, and JOSEPH H. KASTLE

Department of Chemistry, Kentucky Agricultural Experiment Station

In a former paper[1] from this laboratory evidence was presented to show that the lysin content of the proteins of certain grain mixtures fed to White Leghorn chicks was the limiting factor in their growth. The results of those experiments showed that on a ration consisting of wheat, wheat bran, sunflower seed, hempseed, cracked corn, skim milk, cabbage, and sprouted oats, normal growth was obtained, while on a ration consisting of barley, rice, hominy, oats, gluten flour, butter fat, cabbage, and sprouted oats a condition of arrested growth resulted. The first-mentioned ration was supposed to contain a high percentage of the amino acid, lysin, as compared with the second ration, which was supposed to be low in lysin. These experiments were open to criticism because of the small number of chicks under consideration, the laboratory conditions governing them and the possible inaccuracy in the numbers given for the amino-acid distribution of the grain mixtures fed. In view of this, an experiment was planned which would, as far as possible, eliminate these points of objection.

In 1915 an experiment was conducted by Buckner, Nollau, and Kastle, in which 14 one-day-old White Leghorn chicks were fed a ration consisting of 33 parts of ground soybeans and 67 parts of ground oats, supplemented by 20 per cent of protein-free milk, sprouted oats, shredded cabbage leaves, grit, oyster shell, and a small quantity of sour skim milk. On this ration the chicks failed to thrive and grow and would eventually have died had not the grain ration been changed. It was changed to equal parts of wheat bran, sunflower seed, hempseed, barley, oats, and rice. On this diet a partial recovery was effected, yet the vigor and development of the normal White Leghorn chick of a similar age was not attained. One of us (Kastle) contended that this failure to grow

[1] BUCKNER, G. D., NOLLAU, E. H., and KASTLE, J. H. THE FEEDING OF YOUNG CHICKS ON GRAIN MIXTURES OF HIGH AND LOW LYSIN CONTENT. *In* Amer. Jour. Physiol., v. 39, no. 2, p. 162–171, 1 pl. 1915.

normally was caused by some toxic principle in the soybean, and in order to throw more light on this point the following experiment was planned. It was conducted in an airy, well-lighted basement room of the Kentucky Agricultural Experiment Station.

Two lots of chicks, designated "A" and "B," consisting of 12 and 11 White Leghorn chicks, respectively, were selected entirely at random and fed rations composed of equal parts of soybeans, oats, wheat, ship stuff, sunflower seed, and cracked corn, supplemented by sour skim milk, sprouted oats, shredded cabbage, grit, and oyster shell. The food of lot A was given to them in the usual manner of feeding. In the case of lot B the grains were ground together, well mixed with a small quantity of distilled water, and baked in an electric oven. The temperature of the oven was kept at 420° F., and the mixture was frequently stirred. The baked feed was ground and fed to lot B in the same way as was the uncooked mixture to lot A. Table I gives the weights of the chicks, their increase in weight, and the number surviving each week.

TABLE I.—*Effect of uncooked and cooked rations on the growth of White Leghorn chicks*

LOT A

Date.	Number of weeks.	Number of chicks.	Total weight of chicks.	Average weight of chicks.	Average increase.	Average percentage increase.
1915.			*Gm.*	*Gm.*	*Gm.*	
July 6	.....	11	528	48.0	.....	.....
13	1	11	725	65.9	17.9	37.3
20	2	11	1,004	91.3	25.4	38.5
27	3	11	1,266	115.1	23.8	26.1
Aug. 3	4	10	1,832	183.2	68.1	59.1
10	5	10	2,462	246.2	63.0	34.3
18	6	10	3,290	329.0	82.8	33.6
24	7	10	3,795	379.5	50.5	15.4
31	8	10	4,357	435.7	56.2	14.8
Sept. 7	9	9	4,410	490.0	54.3	12.4

LOT B

1915.						
July 6	.....	12	554	46.2	.....	.....
13	1	12	788	65.7	19.5	42.2
20	2	11	1,014	92.2	26.5	40.3
27	3	8	930	116.2	24.0	26.0
Aug. 3	4	8	1,357	169.7	53.5	46.4
10	5	7	1,655	236.4	66.7	39.3
18	6	7	2,160	308.5	72.1	30.4
24	7	7	2,307	329.5	21.0	[a] 6.8
31	8	4	2,062	515.5	186.0	56.4
Sept. 7	9	4	2,275	566.2	50.7	9.8

[a] Three chickens sick.

This experiment, as shown in Table I, covers a period of nine weeks in which both lots of chicks received identical treatment, except as to the preparation of the food. The growth of lot A was approximately normal as to weight and mortality, but their vigor and general condition was not good. In lot B, which received the cooked grain ration, a decided deleterious effect was shown by the weight and mortality record which can properly be ascribed to the ration. If we regard this ration in the light of the present-day conception of nutrition, it is balanced with reference to the dietary essentials, fat-soluble A being abundantly supplied in the sprouted oats, shredded cabbage leaves, and butter fat contained in the not-too-closely skimmed milk, while sufficient water-soluble B was obtained from the grain mixture before them as a dry mash at all times. The incomplete proteins of the grains were supplemented by the casein of the milk in the wet mash and the proteins in the cabbage and sprouted oats. These factors, with the mineral content amply supplied by grit, oyster shell, and milk, satisfy all conditions in making a complete diet. However, the food hormones were destroyed by heating the ration fed to lot B.

The experiments of McCollum and his coworkers have demonstrated the nutritive limits of seeds. McCollum states[1] in substance that when seeds are fed, supplemented by suitable inorganic salts and sufficient fat-soluble A, the limiting factor with respect to growth is the quality of protein. The data presented in this paper and the experimental work to be described corroborate this statement and throw further light on the following points:

1. That the soybean may enter into the dietary of the White Leghorn chick without having an accumulative deleterious effect.
2. The effect of heat on the food value of certain grain mixtures.
3. That under approximately ideal conditions chicks which had been stunted by dietary measures and had survived by reason of greater vitality may remain in a fairly good state of health over a long period of time.

The plan of the experiments was as follows: Four grain rations were selected so that two would contain grains the proteins of which were supposed to be high in lysin (one to be fed as a mash and one as a grain); the other two grain mixtures were supposed to contain proteins low in lysin and were to be fed in the same way as the first two. After this experiment had been started an effort was made to determine the amino-nitrogen distribution in these mixtures, but, owing to their large carbohydrate content, no satisfactory results have been obtained. The effort to analyze these complex grain mixtures is still in progress, and it is

[1] McCollum, E. V., and Simmonds, N. A biological analysis of pellagra-producing diets. III. The value of some seed proteins for maintenance. *In* Jour. Biol. Chem., v. 32, no. 3, p. 347–368, 12 charts. 1917.

hoped that their amino-acid make-up may be determined at some future time.

In this experiment we selected at random from 600 1-day-old incubator chicks of the White Leghorn breed, three lots, each containing 60 chicks, which were kept under identical conditions except that the diets were different. The conditions governing these three lots of chicks were as follows:

Lot 1 was placed in a large brooder house which opened on a large grass run. The chicks were weighed individually on a torsion balance, sensitive to 0.1 gm. the day after they were hatched and were so weighed each succeeding week. The weekly individual weighings and mortality records were kept, the previous weight records of all chicks which had died being discarded, so that at the end of 28 weeks the weight records represented only those that were living at that time. No particular care was taken of these chicks except to see that they were properly watered, fed, and housed. They were fed a ration consisting of equal parts of finely ground soybeans, wheat, wheat bran, sunflower seed, and hempseed. This was fed as a dry mash and was kept before them at all times; and once a day, at noon, a wet mash of this mixture made with sour skim milk was fed. Once a day a coarsely ground grain mixture, consisting of equal parts of wheat, soybeans, hempseed, and cracked corn, was thrown into the litter in order to make them exercise by scratching for it. Oyster shell, grit, and charcoal were before them at all times, while sprouted oats and shredded cabbage leaves were liberally fed once a day. It may be of interest to note that this ration is in every way identical, with the exception that soybeans were added in an equal quantity, with the ration fed to lot 3 of our previous paper,[1] in which the growth and general physical condition were normal, even though the experiment was conducted under laboratory conditions.

These chicks were closely watched, and any change in their physical condition and habits was noted. When the treading period began, the cockerels and pullets were separated to avoid any loss of weight occasioned by undue exercise.

Lot 2 was kept under the same conditions, only this lot could not be separated into cockerels and pullets, because their external sexual characteristics were depressed to such an extent that they could not be distinguished, and treading did not occur. A dry mash, which was kept before them at all times, consisted of equal parts of finely ground barley, rice, hominy, and oats, to which was added enough gluten flour and butter fat to raise the protein and fat content of this grain mixture up to that of the grain mixture fed as a mash to lot 1. Once a day they received a wet mash made of this grain mixture moistened with protein-free milk. The grain ration fed in the litter consisted of equal parts of

[1] BUCKNER, G. D., NOLLAU, E. H., and KASTLE, J. H. 1915. OP. CIT.

barley, rice, and hominy, to which was added enough pure butter fat and gluten flour to bring the fat and protein content up to the same level as that of the corresponding grain mixture fed to lot 1.[1]

Lot 3 constituted the control for the two other lots of chicks. This lot was kept under conditions identical with those of lots 1 and 2, the cockerels and pullets being separated when treading began. These chicks received a ration known as the standard Cornell ration.[2]

Table II presents the weekly weight and mortality records of these three lots of chicks, covering a period of 28 weeks, in which time there were no unusual weather conditions or epidemics among these chicks, so that these figures represent the degree of nourishment afforded by the rations fed to the separate lots.

TABLE III.—*Effect of various diets on the growth of White Leghorn chicks*

Week.	Lot 1.				Lot 2.		Lot 3 (control).			
	Average weight of—			Number of chicks.	Average weight of cocks and hens.	Number of chicks.	Average weight of—			Number of chicks.
	25 cocks.	17 hens.	Mean.				25 cocks.	17 hens.	Mean.	
	Gm.	*Gm.*	*Gm.*		*Gm.*		*Gm.*	*Gm.*	*Gm.*	
0.....	41.0	39.4	40.2	60	41.8	60	42.1	41.1	41.6	60
1.....	70.4	66.5	68.4	56	52.2	59	72.2	69.2	70.7	55
2.....	100.9	96.8	98.8	55	66.1	54	109.7	95.6	102.6	53
3.....	146.5	138.7	142.6	55	76.3	52	161.4	140.9	151.1	53
4.....	184.1	179.1	181.6	55	89.7	50	231.6	191.7	211.6	53
5.....	256.6	236.5	246.5	55	98.8	46	292.2	248.8	270.5	53
6.....	296.5	276.5	286.5	55	106.9	45	360.9	288.3	324.6	52
7.....	363.4	318.5	340.9	53	124.3	37	442.8	354.4	398.6	52
8.....	420.6	382.5	401.5	52	148.6	35	496.9	395.6	446.2	52
9.....	474.0	423.1	448.5	52	150.1	34	537.6	409.9	473.7	51
10.....	518.2	460.9	489.5	49	162.5	24	630.9	488.7	559.8	48
11.....	588.5	523.7	556.1	48	182.3	22	674.4	506.4	590.4	48
12.....	653.4	582.4	617.9	47	221.6	21	812.9	622.2	717.5	48
13.....	733.4	635.9	684.6	46	244.3	21	902.3	682.4	792.3	48
14.....	795.8	684.2	740.0	45	251.1	21	953.2	695.1	824.1	48
15.....	828.4	740.1	784.2	45	293.4	20	980.4	717.3	848.8	48
16.....	954.9	790.6	872.5	45	325.6	20	1,063.2	780.9	922.1	48
17.....	1,024.5	844.2	934.3	45	361.5	20	1,154.9	841.8	998.3	48
18.....	1,077.8	930.6	1,004.2	45	441.1	20	1,252.7	889.4	1,071.0	48
19.....	1,135.9	964.6	1,050.2	44	474.6	19	1,295.3	922.9	1,109.1	47
20.....	1,184.6	1,010.2	1,097.4	44	526.4	19	1,344.8	956.2	1,150.5	47
21.....	1,231.6	1,057.3	1,144.4	44	543.7	19	1,396.7	992.2	1,194.4	47
22.....	1,214.5	1,055.8	1,135.1	44	565.8	19	1,430.6	1,038.4	1,234.5	47
23.....	1,204.6	1,101.1	1,152.8	44	615.6	19	1,442.6	1,047.6	1,245.1	47
24.....	1,215.5	1,094.3	1,154.9	44	663.8	19	1,462.1	1,066.2	1,264.1	47
25.....	1,206.7	1,079.3	1,143.0	44	734.3	19	1,480.0	1,082.6	1,281.3	45
26.....	1,268.6	1,084.5	1,176.5	44	763.7	19	1,528.5	1,075.4	1,301.9	43
27.....	1,250.4	1,135.3	1,192.8	42	781.9	19	1,560.6	1,090.2	1,325.4	43
28.....	1,294.3	1,182.6	1,238.4	42	809.4	19	1,594.6	1,120.4	1,357.5	42

In studying the growth record of lot 1 it will be seen that the average weights of the pullets closely followed the control throughout the 28 weeks, but that the cockerels were inferior in this respect after the third

[1] At the expiration of these 28 weeks the ration was changed only by the addition of sour skim milk, which brought about profound anatomical and physiological changes. The combs and wattles developed, shortly to be followed by crowing and treading on the part of the cockerels. It is also of interest to note that their growth was greatly accelerated. The exact figures could not be counted on because of errors occasioned by change of student assistants helping in the weighings.

[2] NIXON, Clara. FEEDING YOUNG CHICKENS. N. Y. State Col. Agr. Cornell Reading-Courses, v. 4, no. 88, p. 176. 1915.

week. Regarding their physical condition after the sixth week it was plainly to be seen that lot 1 did not have the same vigorous appearance as lot 3. This was most particularly noticed with regard to the general condition of the feathering which seemed slightly ruffled and unkempt as compared to lot 3. This can be seen only to a degree in the illustrations of these two lots (Pl. 42 A, B). Their general vigor and development did not appear equal to that of lot 3, and these differences became more noticeable as they grew older, until, at the end of the twenty-eighth week, when the experiment was discontinued, it was plainly seen that lot 1 was in no way in the same physiological state as lot 3.

It will also be noted from the data that after the seventeenth week lot 1 did not gain as much as lot 3 over the same period. During these 11 weeks the gain was 234.2 gms. as against 286.5 gms. for lot 3; or, in other words, lot 3 made a gain 18.3 per cent greater than that made by lot 1. It may be of interest to point out that the cockerels of lot 1 showed a greater variation in weight from the normal than the pullets. The results of the mortality record show the same number of deaths at the end of the experiment as in the control.

In lot 2 we see that the rate of growth was retarded to such an extent that at the end of the 28 weeks the average weight of a chick was 809.4 gms. as against 1,238.4 gm. for those receiving the ration containing soybeans and 1,357.5 gms. for the control. The external sexual characteristics were rudimentary, and the feather tracts not properly developed and the fact that they retained the habits of the immature chick during this entire period is of added interest. This is shown in the illustration of this lot (Pl. 42, C) which was taken the same day as those of lots 1 and 3, all having the same focal distance. The ration fed to lot 2 greatly increased the mortality so that only 19 remained at the end of the experiment, the weaklings having died earliest. Therefore we had only those with the greatest initial vitality which had lived for 28 weeks on a diet the biologic value of which was low. From this we would infer that the individual vitality of the animal plays a very important part in determining its ability to grow, and for this reason it is essential that a considerable number of animals should be used in experiments on nutrition. This would seem to follow when we realize that all of the chicks used in these experiments were pure-bred and came from the same parent stock. The chicks of lot 2 seemed to have a good appetite, and while they ate with apparent avidity, yet they always seemed to be in search of something in their feed which they could not find. Regarding their ration, it will be seen that it is satisfactory with respect to every necessary dietary factor except the quality of protein. We are unable at this time to show wherein these proteins are limited, but hope to be able to prove this by experiments which are now in progress.

In conclusion we feel justified in making the following deductions from the results of these experiments:

(1) The proteins of rice, oats, barley, hominy, and gluten flour are inefficient in promoting normal growth in the White Leghorn chick.

(2) The results of these experiments seem to indicate that the proteins in the grains mentioned above have a retarding action on the development of the external sexual characteristics and their functions, which accompanies the arrested growth of the chicks.

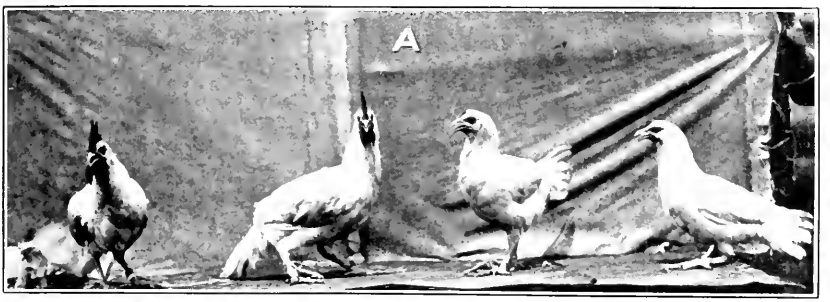
(3) We are unable to account for the apparent weakened vitality of the chicks of lot 1, as shown by their weight record and general condition. However, we do not attribute it to any toxic action of the soybean.

(4) Baking a grain mixture composed of equal parts of soybeans, wheat, wheat bran, sunflower seed, hempseed and cracked corn, moistened with water, materially lowers its efficiency as a food, when all else entering into the diet is sufficient.

(5) The growth and development of any animal is largely dependent on its individual vitality, and for this reason it would seem desirable to use a large number in experiments on nutrition.

PLATE 42

A.—Lot 1 at the age of three months.
B.—Lot 3 at the age of three months.
C.—Lot 2 at the age of three months.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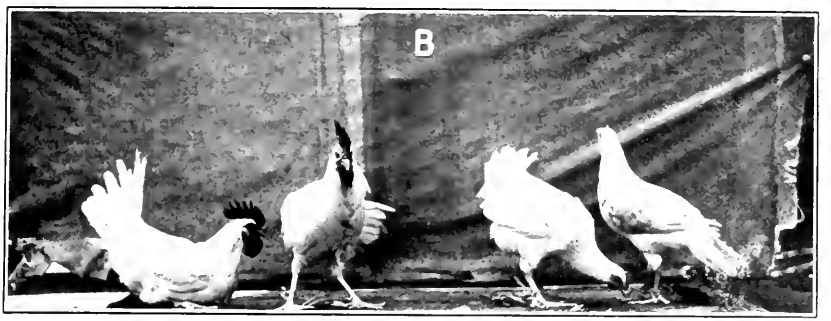
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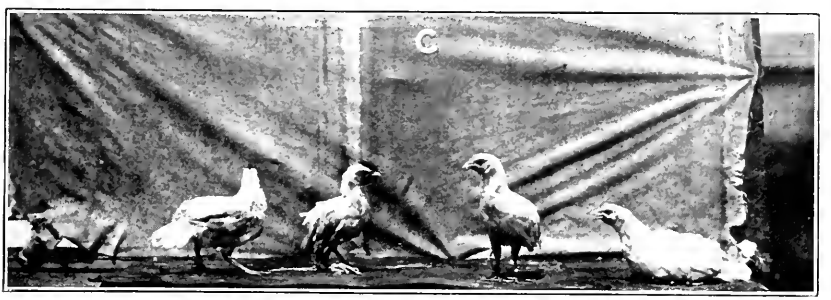
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AMMONIFICATION OF MANURE IN SOIL

By H. J. CONN, *Associate Bacteriologist*, and J. W. BRIGHT, *Assistant Bacteriologist*, *New York State Agricultural Experiment Station*

FOREWORD

A recent series of papers of the New York Agricultural Experiment Station (*8–11*)[1] contained the results of a study of the microscopic flora of the soil. The microorganisms of the soil were classified into a few large groups, some of which were further subdivided, and in a few cases the classification was carried as far as the recognition of species. This preliminary work was considered necessary before studying the different groups with the object of recognizing more of the individual species and learning their functions.

A complete study of all soil microorganisms would be an endless task, and, indeed, rather unprofitable, provided the order of studying the different types were left entirely to chance. To begin a study of this kind, therefore, those organisms should be selected that are presumably important. It is difficult to judge, *a priori*, the importance of any particular microorganism in the soil, but a hint can be obtained by observing which types predominate in natural soil under conditions of considerable importance in practice. The organisms chosen for investigation in the present work were found to multiply in freshly manured soil. In such soil, ammonification and other forms of decomposition are vigorous and there is good reason to believe that the most rapidly multiplying organisms are of practical importance. Upon adding manure to soil several kinds of bacteria have been found to multiply strikingly, but many of them are difficult to recognize and especially difficult to describe so that others may recognize them. It has seemed unwise to make a detailed study of any organism which could not be recognized by other workers; and the work has therefore been limited for the present to two types, both of which have been identified with previously described forms.

The two bacteria investigated belong to the group of non-spore-formers (discussed in an earlier publication (*10*) as one of the three large groups of soil microorganisms) and more specially to that division of this group described as rapid liquefiers (*p. 10, 6–9*). One of them is *Pseudomonas fluorescens* (Flügge) Migula, described on page 6 of that bulletin. The second is described on page 8 of the same bulletin as the "orange liquefying type," and has now been identified with *Bacillus caudatus* Wright. As a single polar flagellum is present, it is renamed "*Pseudomonas caudatus* (Wright)."

[1] Reference is made by number (italic) to "Literature cited," p. 347–350.

Journal of Agricultural Research,
Washington, D. C.
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Vol. XVI, No. 12
Mar. 24, 1919
Key No. N. Y. (Geneva)-5

The present paper is divided into two sections. The first shows the predominance of these two organisms in manured soil and gives the results of an investigation of their function in soil. The second gives a detailed description of the two organisms to aid in their identification by others.

H. J. CONN.

I.—WHAT SOIL ORGANISMS TAKE PART IN THE AMMONIFICATION OF MANURE?

By J. W. Bright

INTRODUCTION

The importance of the ammonification process in the soil has long been recognized, although there has been a tendency on the part of investigators to regard it as secondary in importance to nitrification in soil fertility. Gainey (*19*), however, has recently claimed that the fertility of a soil is limited by processes which precede nitrification—namely, ammonification—rather than by nitrification itself.

The present work has been undertaken for the purpose of determining some of the organisms which actually cause the ammonification of manure in soil under natural conditions; to ascertain the extent to which they can carry on this ammonification; and to compare them with other organisms known to possess the power of ammonifying laboratory media.

A survey of the literature suggests that the active ammonifying organisms in the soil are generally spore formers. This idea seems to be based principally upon the conclusion reached by Marchal (*36*) that the spore-forming *Bacillus mycoides* is one of the most common of the soil organisms and the one that attacks protein most energetically. It should be noted, however, that he worked with a miscellaneous group of organisms and of his eight most important ammonifiers only one, the non-spore-former *B. fluorescens liquefaciens*, is a typical soil organism. J. G. Lipman (*33*) assumed that the spore formers were important ammonifiers, as is evidenced by the fact that he referred to the *B. subtilis* group and the streptothrices as being the most prominent ammonifying organisms numerically important in arable soils. Stephens and Withers (*48*) and C. B. Lipman (*32*) also assumed this when they decided to use *B. subtilis* as the organism with which to do their experimental work on ammonification.

That this idea is still held by some soil bacteriologists is shown by the fact that in a recent investigation by Neller (*39*) (an associate of J. G. Lipman), the spore-forming organisms *B. subtilis*, *B. vulgatus*, *B. mycoides*, and *B. megatherium* are used to represent "some of the more common species of soil organisms" causing ammonification in the soil.

While it is undoubtedly true that a great many spore-forming organisms are capable of very active ammonification in manured soil, yet there is good reason to doubt their activity under natural conditions. Conn (*6*) has already pointed out that the spore formers probably exist in the soil almost entirely as spores rather than as vegetative cells and that their status as active ammonifiers in soil is doubtful. He further shows (*10*) that the non-spore formers not only exist in the soil in great numbers but that one group of them at least have proteolytic powers.

One of this group, *Pseudomonas fluorescens*, is known to be an ammonifier. This, together with the fact that the non-spore formers have been found to be especially abundant in freshly manured soil suggests that they may be among the important soil ammonifiers. The present work was planned to test whether this assumption is correct, and, if so, to obtain as rigid proof as possible of the ammonifying agency of the non-spore-forming organisms.

TECHNIC

The soil used throughout the series of experiments was Dunkirk silty clay loam[1] obtained from a plot on the station grounds. This soil was mixed with fresh horse manure or fresh cow manure, always in the proportion of 20 parts of soil to 1 part of manure.

All samples were plated according to the usual methods, with at least two dilutions. The degree of dilution depended upon the character of the samples to be plated. Four plates were made from each dilution used and the average count of the four plates was taken to represent the count for that dilution. Whenever possible, the count was based upon the dilution averaging between 30 and 150 colonies per plate. In some cases, however, it was necessary to take into account plates which varied from these limits. In a few cases where plates were lost on account of contamination or liquefaction, the count represents an average of three instead of four plates.

The medium used in all the plating was "tap-water gelatin" made by dissolving 200 gm. of "gold-label" gelatin in 1 liter of tap water, adjusting the reaction to about $P_H = 6.8$, with bromthymol blue as the indicator, and clarifying with white of egg.

Nearly all of the plate counts were checked by direct microscopic examination of the soil according to the method described by Conn (*12*). An infusion of the soil to be examined was made by shaking up 1 gm. of the soil in 9.5 cc. of a fixative prepared by dissolving 0.15 gm. of gelatin in 1,000 cc. of hot water. Of this infusion 0.01 cc. was measured out with a capillary pipette and smeared evenly over an area of 1 square centimeter on a glass slide. This smear was then dried and stained with hot rose Bengal for 1 minute.

For all pure culture studies the manured soil was placed in small Erlenmeyer flasks, 150 gm. per flask. These were then plugged with cotton and sterilized in the autoclave at 15 pounds' pressure for two hours. Subsequent platings proved that in this way all organisms and spores were killed. The infusion for inoculating the soil was prepared as follows: A freshly streaked culture of the organism was suspended in sterile water, and the number of organisms per cubic centimeter of this

[1] Described according to the system of the Bureau of Soils of the U. S. Dept. of Agriculture. (MARBUT, Curtis F., BENNETT, Hugh H., LAPHAM, J. E., and LAPHAM, M. H. SOILS OF THE UNITED STATES. U. S. Dept. Agr. Bur. Soils Bul. 96, 791 p., 1913. CARR, M. Earl, LEE, Ora, jr., MAYNADIER, Gustavus B., HALLOCK, D. J., and FROST, V. J. SOIL SURVEY OF ONTARIO COUNTY, NEW YORK. U. S. Dept. Agr. Bur. Soils, Adv. Sheets, Field Oper. 1910, 55 p., 1 fig., 1 map. 1912.)

infusion determined by the above microscopic method. The infusion was diluted to the desired strength and 1 cc. of it introduced into each flask. The flasks were then incubated at room temperature and studied at specified intervals. All flasks were controlled by uninoculated flasks as controls.

The method used for the determination of the ammonia produced was practically that of Potter and Snyder,[1] which is an adaptation of the Folin[2] aeration method. A number of alternating Kjeldahl flasks and absorption cylinders were set up in series so that a continuous current of air could be passed through the system. Twenty-five-gm. samples of the soils to be tested were placed in the Kjeldahl flasks and 200 cc. of *N/50* hydrogen sulphate (H_2SO_4) were put in each absorption cylinder. The flasks and cylinders were so connected that the air from the end flask was driven over into its adjoining cylinder and absorbed in the standard acid. Arranged in this way each Kjeldahl flask and adjacent absorption cylinder with the connecting tubes made one complete unit and any number of these units could be connected in the series.

When the apparatus was set up and all was in readiness for the aeration, 2 gm. of sodium carbonate (Na_2CO_3), and 50 cc. of ammonia-free water were introduced into each Kjeldahl flask. The flasks were then tightly stoppered, and the air was turned on at such a rate that about 6 liters of air per minute passed through the system. The aeration was continued for about two hours and the standard acid in the absorption cylinders titrated against *N/50* sodium hydroxid (NaOH) to determine the amount of ammonia driven off from the soil. Care was taken to have the system absolutely air-tight and all rubber connections dry so that in passing from the flasks to the cylinders none of the ammonia would be absorbed by the water. Absorption in the standard acid was aided by using Folin ammonia tubes to break up the bubbles of air when they entered the absorption cylinders.

The determination of the amount of free ammonia in soil has always been a difficult one. The accuracy of the results obtained is somewhat doubtful, as many of the protein substances present in soil are readily broken up by the reagents used in determining the ammonia present. Consequently the ammonia determinations in this series of experiments can not be regarded as absolutely true determinations of "ammonia production." Still other factors which might tend to destroy the accuracy of the determinations are, first, that the organisms themselves might utilize the ammonia as rapidly as it is produced; and second, that it might escape into the air. The latter is improbable because the ammonia would be more likely to be absorbed by the water present in the soil. Controls of sterilized manured soil were always run at the same time as the inoculated soils, and in this way it was possible to determine whether or not the organisms in the inoculated soil affected the amount of ammonia production in any way.

[1] POTTER, R. S., and SNYDER, R. S. THE DETERMINATION OF AMMONIA IN SOILS. Iowa Agr. Exp. Sta. Research Bul. 17, 19 p., illus. 1914.

[2] FOLIN, Otto. EINE METHODS ZUR BESTIMMUNG DES AMMONIAKS IM HARNE UND ANDEREN THIERISCHEN FLÜSSIGKEITEN. *In* Ztschr. Physiol. Chem., Bd. 37, Heft 2, p. 161–176. 1902.

RELATIVE NUMBERS OF NON-SPORE-FORMING AND SPORE-FORMING BACTERIA IN FRESHLY MANURED SOIL

Work done by Conn (*10, table 3*) on the flora of freshly manured soil, previous to the present series of experiments, offers striking evidence that the non-spore-forming organisms predominate in such soil. During his work the manured soil was plated at intervals extending over a period of six months. On the third day it was found that almost 99 per cent of the entire flora was composed of non-spore-forming organisms. The present work on the flora of freshly manured soil includes experiments designed to verify these earlier results.

The method of procedure in these later experiments was practically the same throughout, except for a few differences in the treatment of samples. Soil was mixed with fresh horse manure or fresh cow manure and, with the exception of the first experiment, the manured soil was then divided into two portions, one of which was placed in an open pot and one in a flask plugged with cotton. In the first experiment the manured soil was kept only in open pots. The moisture content of the pots was controlled somewhat by frequent additions of water to replace that lost by evaporation, but no allowance was made for this in the flasks. Platings were made at frequent intervals at the first of each experiment and at longer intervals as the experiment proceeded. The experiment recorded in Table I was carried on under conditions exactly analogous to those under which Conn did his previous work, and its purpose was the verification of that work. The experiments recorded in Tables II and III were also carried on under similar conditions except that soil mixed with cow manure was used as well as that mixed with horse manure and samples were plated from plugged flasks as well as from open pots.

TABLE I.—*Plate counts of the microorganisms in manured soil in open pots. Experiment I*

[Counts indicate numbers of colonies per gram of soil]

Time since adding manure to soil.	Total count.	Actinomycetes.		Non-spore formers.		Spore formers.	
		Plate count.	Percentage of total flora.	Plate count.	Percentage of total flora.	Plate count.	Percentage of total flora.
Days.							
2	60,000,000	4,000,000	7.5	56,000,000	92.5	None.	0.0
3	80,000,000	6,000,000	7.5	74,000,000	92.5	None.	.0
4	125,000,000	5,000,000	4.0	116,000,000	92.7	4,000,000	3.3
6	235,000,000	6,000,000	2.6	220,500,000	93.5	9,500,000	3.9
9	45,000,000	5,000,000	11.1	38,500,000	85.5	1,500,000	3.4
13	43,000,000	4,000,000	9.3	36,500,000	85.0	2,500,000	5.7
16	35,000,000	12,000,000	34.3	23,000,000	65.7	None.	.0
21	50,000,000	13,000,000	26.0	35,000,000	70.0	2,000,000	4.0
24	55,000,000	12,500,000	22.6	42,500,000	77.4	None.	.0
29	85,000,000	8,500,000	10.0	76,500,000	90.0	None.	.0
38	45,000,000	13,000,000	29.0	32,000,000	71.0	None.	.0
58	95,000,000	8,500,000	8.9	83,500,000	88.0	3,000,000	3.1
94	18,000,000	5,500,000	30.5	11,500,000	63.8	1,000,000	5.7
123	20,000,000	5,000,000	25.0	15,000,000	75.0	None.	.0
Average			16.3		81.6		2.1

TABLE II.—*Plate counts of the microorganisms in manured soil in open pots and closed flasks. Experiment II*

COW MANURE

[Counts indicate number of colonies per gram of soil]

Treatment and time since adding manure to soil.	Total count.	Actinomycetes.		Non-spore formers.		Spore formers.	
		Plate count.	Percentage of total flora.	Plate count.	Percentage of total flora.	Plate count.	Percentage of total flora.
Open pots:							
1 day	164,000,000	9,500,000	5.6	155,000,000	94.4	None.	0.0
2 days	93,000,000	10,500,000	11.2	82,500,000	88.6	Trace.	.2
3 days	98,500,000	10,000,000	12.6	86,000,000	87.2	2,000,000	.2
4 days	127,000,000	8,500,000	7.0	117,000,000	92.2	1,000,000	.8
5 days	55,000,000	7,500,000	14.4	46,000,000	83.8	1,000,000	1.8
7 days	490,000,000	35,000,000	6.8	450,000,000	92.0	5,000,000	1.2
8 days	251,000,000	40,000,000	15.6	208,000,000	83.2	3,000,000	1.2
9 days	52,000,000	7,000,000	12.4	45,000,000	86.7	500,000	.9
11 days	412,000,000	190,000,000	46.1	214,000,000	52.1	7,500,000	1.8
13 days	67,000,000	16,000,000	23.1	51,000,000	76.2	500,000	.7
15 days	395,000,000	160,000,000	40.6	232,000,000	58.7	3,500,000	.7
17 days	245,000,000	50,000,000	22.6	186,000,000	76.0	3,500,000	1.4
Average			18.2		80.9		.9
Closed flasks:							
1 day	27,500,000	3,750,000	18.3	22,500,000	81.7	None.	0.0
2 days	64,000,000	6,000,000	10.0	57,500,000	90.0	None.	.0
3 days	67,000,000	6,000,000	9.0	59,000,000	88.2	1,800,000	2.8
4 days	81,000,000	15,000,000	18.6	64,000,000	79.0	2,000,000	2.4
5 days	101,000,000	15,000,000	14.6	84,000,000	83.1	2,000,000	2.3
7 days	42,000,000	12,500,000	28.5	29,000,000	69.2	1,000,000	2.3
8 days	65,500,000	12,000,000	18.3	53,000,000	81.0	Trace.	.7
9 days	34,500,000	9,000,000	24.3	24,000,000	69.5	1,500,000	6.2
11 days	330,000,000	290,000,000	88.3	38,000,000	11.1	2,000,000	.6
13 days	323,000,000	300,000,000	34.4	21,000,000	65.0	2,000,000	.6
15 days	330,000,000	225,000,000	68.8	99,000,000	30.0	6,000,000	1.2
17 days	350,000,000	190,000,000	54.2	158,000,000	45.2	2,000,000	.6
Average			32.3		66.1		1.6

HORSE MANURE

Open pots:							
1 day	300,000,000	9,500,000	3.4	290,000,000	96.6	None.	0.0
2 days	109,000,000	18,000,000	18.4	87,000,000	79.8	2,000,000	1.8
3 days	157,000,000	12,000,000	7.4	144,000,000	91.7	1,500,000	.9
4 days	907,500,000	22,500,000	2.8	880,000,000	97.0	2,000,000	.2
5 days	775,000,000	56,000,000	7.5	713,000,000	91.6	7,500,000	.9
7 days	625,000,000	65,000,000	10.4	556,000,000	89.0	4,000,000	.6
8 days	67,500,000	6,000,000	8.9	61,000,000	90.4	500,000	.7
9 days	480,000,000	85,000,000	17.7	392,000,000	81.7	3,000,000	.6
11 days	740,000,000	115,000,000	15.6	622,000,000	84.0	3,000,000	.4
13 days	376,000,000	100,000,000	26.6	273,000,000	72.6	3,000,000	.8
15 days	295,000,000	95,000,000	32.2	198,000,000	67.2	2,000,000	.6
17 days	1,705,000,000	700,000,000	41.0	1,000,000,000	58.7	5,000,000	.3
Average			16.1		83.3		.6
Closed flasks:							
1 day	63,000,000	11,000,000	17.5	52,000,000	82.5	None.	.0
2 days	88,000,000	7,000,000	7.8	81,000,000	92.0	Trace.	.2
3 days	82,000,000	8,000,000	9.9	73,000,000	89.2	750,000	.9
4 days	78,000,000	12,000,000	14.6	66,000,000	84.5	750,000	.9
5 days	336,000,000	100,000,000	28.6	234,000,000	70.8	2,000,000	.6
7 days	810,000,000	325,000,000	40.1	481,000,000	59.5	3,500,000	.4
8 days	75,500,000	24,000,000	32.4	50,000,000	66.3	1,000,000	1.3
9 days	58,500,000	30,000,000	51.3	27,000,000	46.2	1,500,000	2.5
11 days	375,000,000	250,000,000	66.6	123,000,000	32.8	2,000,000	.6
13 days	169,000,000	65,000,000	2.8	162,000,000	96.0	2,000,000	1.2
15 days	1,380,000,000	800,000,000	57.9	575,000,000	41.7	5,000,000	.4
17 days	1,045,000,000	800,000,000	76.6	244,000,000	23.3	1,000,000	.1
Average			33.8		65.4		.8

TABLE III.—*Plate counts of the microorganisms in manured soil in open pots and closed flasks. Experiment III*

COW MANURE

[Counts indicate number of colonies per gram of soil]

Treatment and time since adding manure to soil.	Total count.	Actinomycetes.		Non-spore formers.		Spore formers.	
		Plate count.	Percentage of total flora.	Plate count.	Percentage of total flora.	Plate count.	Percentage of total flora.
Open pots:							
1 day	58,000,000	8,000,000	13.8	48,000,000	82.7	2,000,000	3.5
2 days	73,250,000	5,000,000	6.7	68,000,000	93.1	Trace.	.2
3 days	497,500,000	15,000,000	3.0	482,500,000	97.0	None.	.0
4 days	93,000,000	12,000,000	12.9	81,000,000	87.1	None.	.0
5 days	102,500,000	12,500,000	12.2	90,000,000	87.8	None.	.0
7 days	36,250,000	5,000,000	13.8	31,250,000	86.2	None.	.0
8 days	43,000,000	5,000,000	11.7	38,000,000	88.3	None.	.0
9 days	17,500,000	2,500,000	14.3	15,000,000	85.7	None.	.0
10 days	40,000,000	12,000,000	30.0	28,000,000	70.0	None.	.0
12 days	96,000,000	15,000,000	15.7	81,000,000	84.3	None.	.0
15 days	187,000,000	20,000,000	10.7	167,000,000	89.3	None.	.0
18 days	75,000,000	20,000,000	26.7	55,000,000	73.3	None.	.0
21 days	64,000,000	10,000,000	14.4	52,500,000	83.3	1,500,000	2.3
Average			14.3		85.3		.4
Closed flasks:							
1 day	51,000,000	8,000,000	15.7	41,500,000	81.4	1,500,000	2.9
2 days	42,000,000	6,000,000	14.3	35,500,000	84.5	500,000	1.2
3 days	295,000,000	10,000,000	3.4	285,000,000	96.6	None.	.0
4 days	68,000,000	30,000,000	44.1	38,000,000	55.9	None.	.0
5 days	44,000,000	11,000,000	25.5	33,000,000	74.5	None.	.0
7 days	27,000,000	10,000,000	37.6	17,000,000	62.4	None.	.0
8 days	45,500,000	23,000,000	50.6	22,500,000	49.4	None.	.0
9 dyas	31,000,000	20,000,000	64.6	11,000,000	35.4	None.	.0
10 days	36,500,000	17,500,000	48.0	19,000,000	52.0	None.	.0
12 days	49,500,000	20,000,000	40.4	29,500,000	59.6	None.	.0
15 days	53,000,000	25,000,000	47.2	28,000,000	52.8	None.	.0
18 days	125,000,000	65,000,000	52.0	60,000,000	48.0	None.	.0
21 days	46,000,000	25,000,000	54.4	21,000,000	45.6	None.	.0
Average			38.3		61.4		.3

HORSE MANURE

Open pots:							
1 day	110,000,000	12,000,000	10.9	97,000,000	88.1	750,000	1.0
2 days	120,000,000	7,500,000	6.3	111,000,000	92.5	1,500,000	1.2
3 days	195,000,000	10,000,000	5.0	185,000,000	95.0	None.	.0
4 days	150,000,000	15,000,000	10.0	135,000,000	90.0	None.	.0
5 days	122,000,000	12,000,000	9.9	110,000,000	90.1	None.	.0
7 days	95,000,000	15,000,000	5.3	80,000,000	94.7	None.	.0
8 dyas	300,000,000	35,000,000	11.7	265,000,000	88.3	None.	.0
9 days	30,500,000	5,000,000	16.4	25,500,000	83.6	None.	.0
10 days	59,000,000	5,000,000	8.5	54,000,000	91.5	None.	.0
12 days	48,000,000	20,000,000	41.7	28,000,000	58.3	None.	.0
15 days	242,500,000	40,500,000	16.1	202,000,000	83.9	None.	.0
18 days	130,000,000	35,000,000	26.9	95,000,000	73.1	None.	.0
21 days	117,500,000	30,000,000	25.5	87,000,000	74.0	500,000	.5
Average			14.9		84.9		.2
Closed flasks:							
1 day	40,000,000	4,500,000	11.2	34,500,000	86.2	1,000,000	2.5
2 days	182,500,000	8,000,000	4.3	171,500,000	94.0	1,000,000	1.7
3 days	82,500,000	10,000,000	12.1	72,500,000	87.9	None.	.0
4 days	75,000,000	22,500,000	30.0	52,500,000	70.0	None.	.0
5 days	33,000,000	13,000,000	39.4	20,000,000	60.6	None.	.0
7 days	515,000,000	375,000,000	72.9	140,000,000	27.1	None.	.0
8 days	605,000,000	150,000,000	24.8	455,000,000	75.2	None.	.0
9 days	62,500,000	32,000,000	51.2	30,500,000	48.8	None.	.0
10 days	780,000,000	600,000,000	76.9	180,000,000	23.1	None.	.0
12 days	67,000,000	50,000,000	74.6	17,000,000	25.4	None.	.0
15 days	43,500,000	27,500,000	63.2	16,000,000	36.8	None.	.0
18 days	100,000,000	65,000,000	65.0	35,000,000	35.0	None.	.0
21 days	40,000,000	30,000,000	75.0	10,000,000	25.0	None.	.0
Average			46.2		53.5		.3

A survey of the results in Tables I, II, and III shows that the number of non-spore formers in the open pots of manured soil increased rapidly for the first few days (see Table I, column 5). In every instance the highest percentage of this group of organisms was reached within the first week after the addition of the manure and this maximum point was never less than 92.5 per cent, while in some cases it reached 97 per cent. The results in the flasks were much more erratic and, while the percentage of non-spore formers often ran above 90 per cent of the flora, the lines of increase and decrease were not so well marked as they were in the pot experiments. This was undoubtedly due to the fact that conditions of aeration and moisture content were decidedly abnormal. By summarizing the three tables it was found that the non-spore-forming organisms averaged 74.1 per cent of the entire flora in both the pots and flasks; the Actinomycetes 25.1 per cent, and the spore formers only 0.8 per cent.

TABLE IV.—*Results of the isolation of organisms from manured soil*

Source.			Open pots.					Closed flasks.				
Sample No.	Kind of manure.	Time since adding manure.	Total count.	Number of organisms isolated.	Number which grew on agar.[a]	Number of non-spore formers.	Number of spore formers.	Total count.	Number of organisms isolated.	Number which grew on agar.[a]	Number of non-spore formers.	Number of spore formers.
		Days.										
1	Horse.	6	251,000,000	34	32	30	2	317,000,000	.....	.....	.....	.....
2	...do...	10	89,000,000	20	20	20	0	95,000,000	.....	.....	.....	.....
2	...do...	27	73,000,000	25	24	21	3	42,000,000	.....	.....	.....	.....
3	...do...	22	37,000,000	20	20	19	1	78,000,000	17	17	17	0
3	...do...	27	30,000,000	27	27	27	0	15,000,000	5	5	5	0
4	Cow...	9	231,000,000	37	36	35	1	.....	.....	.....	.....	.....
5	...do...	11	.....	.....	.....	.....	.....	136,000,000	41	41	39	2
6	...do...	23	174,000,000	35	33	33	0	143,500,000	35	34	34	0
7	...do...	3	98,500,000	15	14	14	0	67,000,000	14	14	14	0
8	Horse.	8	67,500,000	7	6	6	0	75,500,000	9	9	9	0
9	Cow...	8	251,000,000	8	8	8	0	65,500,000	4	4	4	0
10	Horse.	24	1,705,000,000	13	11	11	0	1,045,000,000	15	15	15	0
11	Cow...	24	245,000,000	11	10	10	0	350,000,000	9	9	8	1
12	Horse.	8	300,000,000	3	3	3	0	605,000,000	5	4	4	0
13	Cow...	8	43,000,000	10	10	10	0	45,500,000	8	8	8	0
			.....	265	254	247	7	.....	162	160	157	3

[a] Plain nutrient agar was used as medium for isolated colonies.

While the data accumulated in the preceding experiments indicated very strongly that the non-spore formers were the predominating organisms in the manured soil, yet the proof was not absolute, because it was based entirely upon the appearance of the colonies upon the plates. Those colonies which possessed the characteristic spreading or filamentous appearance of the typical spore formers were classified accordingly; but some non-spore formers may thus have been inadvertently recorded as spore formers, or some spore formers as non-spore formers. A number of isolations were made, therefore, from the plates poured during the series of experiments described above. All colonies which looked like

spore formers were transferred to agar slants, as were a representative number of colonies of other types. About 97 per cent of these cultures grew and were subsequently examined under the microscope for spore formation.

Table IV, which contains the recorded data from this experiment, shows that of the 254 organisms from the open pots which grew after isolation, only 2.8 per cent were spore formers, and of the 160 organisms from the flasks which grew after isolation only 1.8 per cent were spore formers. And this despite the fact that a special effort was made to include all those colonies whose appearance suggested that they might be spore-forming organisms.

GROWTH OF PSEUDOMONAS FLUORESCENS AND PS. CAUDATUS COMPARED WITH THE GROWTH OF BACILLUS CEREUS IN STERILIZED MANURED SOILS

The organisms selected for the rest of the work were two non-spore formers, *Pseudomonas fluorescens* (Flügge) Migula and *Ps. caudatus* (Wright) Conn, and a spore former, *Bacillus cereus* Frankland.[1] The first two organisms are described in the second section of this article, and were chosen because of the frequency of their occurrence in manured soil. *B. cereus*, according to Conn (*9*) and Laubach and Rice (*27*), is a typical spore former occurring in soil and was selected for the purpose of comparison with these organisms.

SOIL INOCULATED WITH THE THREE ORGANISMS SEPARATELY

In the series of experiments designed to show the relative rates of growth of the three organisms under investigation, manured soil was sterilized in flasks and inoculated with pure cultures in suspensions of carefully determined strength. Samples from each series were plated at similar intervals, and an effort was made to make all results comparable. Microscopic counts were made of all the samples of soil inoculated with *B. cereus* in order to determine the number of vegetative cells actually present in the soil. As *Ps. fluorescens* and *Ps. caudatus*, on the other hand, grow well on plates, form no spores, and show no tendency to clump, a microscopic count of them was not so important as the plate count, and since they are so small as to be easily overlooked under the microscope a microscopic count proved even less accurate than the plate count. The results as set forth in Table VI, Experiment I, show that *Ps. caudatus* increased from a 13,300,000 plate count on the day of inoculation to a 1,720,000,000 count seven days later, or an increase of 132 times the original inoculation. The initial plate count of *Ps. fluorescens* was 4,390,000, and on the seventh day the count was 475,000,000, an increase of 110 times the original count. *B. cereus*, on the

[1] As identified by Conn. This organism agrees with the descriptions of Chester (*2*, *p.* 278), and Laurence and Ford (*28*, *p.* 284–287).

other hand, showed a much lower rate of increase and developed from an initial plate count of 1,800,000 (see Table V, Experiment I) to a count of 15,000,000 on the seventh day, an increase of 8.3 times the original inoculation. The microscopic count on the seventh day showed 36,000,000 vegetative cells, an increase of 20 times the initial count. In the four series *Ps. caudatus* showed its greatest increase in Experiment III (Table IV), on the ninth day, when it showed a count 913 times greater than its initial count; *Ps. fluorescens* registered its greatest increase in Experiment IV on the fourteenth day, when it showed a count 530 times higher than the original count; and *B. cereus* made its greatest increase in Experiment IV (Table V) on the sixteenth day, showing a count 29 times higher than the original.

TABLE V.—*Multiplication of B. cereus inoculated into sterile manured soil*

[Count indicates the numbers per gram of soil]

Experiment No. and time since inoculation.	Plate count.	Microscopic count.			
		Groups.		Individuals.	
		Vegetative cells.	Spores.	Vegetative cells.	Spores.
Experiment I:					
0 days				[a] 1,800,000	None.
3 days	3,000,000	5,000,000	19,000,000	9,000,000	23,000,000
5 days	1,750,000	6,000,000	6,000,000	3,000,000	6,000,000
7 days	15,000,000	36,000,000	17,000,000	50,000,000	18,000,000
Experiment II:[b]					
0 days				[a] 1,300,000	None.
5 days	2,600,000				
8 days	4,000,000				
17 days	7,000,000				
23 days	12,000,000				
Experiment III:					
0 days				[a] 116,000	None.
2 days	4,000,000	2,000,000	18,000,000	13,000,000	33,000,000
4 days	5,000,000	2,500,000	11,500,000	10,500,000	16,500,000
6 days	10,500,000	2,500,000	16,000,000	15,500,000	19,000,000
8 days	10,000,000	2,500,000	14,500,000	7,500,000	18,000,000
Experiment IV:					
0 days				[a] 103,000	None.
2 days	8,000,000	2,000,000	5,000,000	11,500,000	30,000,000
4 days	5,000,000	1,500,000	12,000,000	13,500,000	24,500,000
6 days	12,500,000	2,500,000	11,500,000	9,500,000	27,500,000
8 days	14,000,000	1,500,000	9,000,000	4,500,000	27,000,000
11 days	17,000,000	2,000,000	13,000,000	6,000,000	19,000,000
12 days	15,000,000	2,500,000	12,000,000	7,000,000	21,000,000
14 days	21,000,000	2,500,000	15,500,000	6,500,000	26,500,000
16 days	56,000,000	3,000,000	21,000,000	6,500,000	32,000,000

[a] Computed from the number of organisms in the infusion used for inoculation.
[b] No microscopic count made.

SOIL INOCULATED WITH A MIXTURE OF THE THREE ORGANISMS

Table VII gives the results of placing the three organisms in competition one with another by inoculating flasks of sterile manured soil with all of them together. Infusions were made from fresh cultures of each organism and the strength of these infusions determined by the microscopic method. After infusions of the proper strength had been obtained, equal amounts of each were thoroughly mixed and 1 cc. of the mixture

added to the flasks containing 150 gms. of sterile manured soil. These flasks were then incubated at room temperature and plates and smears made from them at regular intervals. In Experiment I, Table VII, the ratio between the numbers of organisms of *Ps. fluorescens*, *Ps. caudatus*,and *B. cereus* was 1 to 1 to 1; in Experiment II the ratio was 1 to 8 to 33; in Experiment III the ratio was 1 to 7 to 33. Although *B. cereus* was as abundant as the other organisms in Experiment I and was much more numerous than they in the later experiments, it failed to appear upon any of the plates poured. The non-spore-forming organisms multiplied very rapidly, and in Experiment II, *Ps. fluorescens* developed from an initial count of 30,000 to a maximum count of 560,000,000 on the third day, an increase of over 18,500 times its count at the time of inoculation. In the same experiment *Ps. caudatus* developed from an initial count of 180,000 to a maximum count of 1,190,000,000 on the seventh day, an increase of 6,600 times its count at the time of inoculation. The microscopic examination of the smears made during this series of experiments showed that the vegetative cells of *B. cereus* rapidly decreased in numbers and in a few days the organism could be identified only in the spore form, while the non-spore formers, especially *Ps. caudatus*, showed a steady increase in numbers for several days.

TABLE VI.—*Multiplication of non-spore formers inoculated into sterile manured soil*

[Count indicates numbers per gram of soil]

Experiment No. and time since inoculation.	*Pseudomonas fluorescens.*			*Pseudomonas caudatus.*		
	Plate count.	Microscopic count.		Plate count.	Microscopic count.	
		Groups.	Individuals.		Groups.	Individuals.
Experiment I:						
0 days	.....	.....	[a] 4,390,000	.....	.....	[a] 13,300,000
3 days	260,000,000	197,000,000	204,000,000	665,000,000	492,000,000	608,000,000
5 days	185,000,000	133,000,000	152,000,000	4,800,000,000	1,340,000,000	1,614,000,000
7 days	475,000,000	259,000,000	325,000,000	1,720,000,000	1,105,000,000	1,254,000,000
Experiment II:[b]						
0 days	.....	.....	[a] 3,300,000	.....	.....	[a] 6,600,000
5 days	145,000,000	.....	.....	2,700,000,000	.....	.....
8 days	160,000,000	.....	.....	1,500,000,000	.....	.....
13 days	200,000,000	.....	.....	No count.	.....	.....
17 days	210,000,000	.....	.....	4,000,000,000	.....	.....
23 days	300,000,000	.....	.....	No count	.....	.....
Experiment III:						
0 days	.....	.....	[a] 1,600,000	.....	.....	[a] 1,600,000
2 days	140,000,000	79,000,0009	88,000,000	1,440,000,000	728,000,000	728,000,000
4 days	260,000,000	173,000,000	188,000,000	1,340,000,000	760,000,000	784,000,000
9 days	315,000,000	236,000,000	247,000,000	1,460,000,000	736,000,000	802,000,000
11 days	350,000,000	310,000,000	328,000,000	1,190,000,000	794,000,000	822,000,000
Experiment IV:[b]						
0 days	.....	.....	[a] 1,000,000	.....	.....	[a] 1,090,000
2 days	102,000,000	.....	.....	114,000,000	.....	.....
4 days	390,000,000	.....	.....	181,000,000	.....	.....
6 days	395,000,000	.....	.....	194,000,000	.....	.....
8 days	470,000,000	.....	.....	220,500,000	.....	.....
10 days	375,000,000	.....	.....	205,000,000	.....	.....
12 days	460,000,000	.....	.....	220,000,000	.....	.....
14 days	530,000,000	.....	.....	269,000,000	.....	.....
15 days	470,000,000	.....	.....	171,500,000	.....	.....

[a] Computed from the number of organisms in the infusion used for inoculation.
[b] No microscopic count made.

TABLE VII.—*Plate counts of the microorganisms in sterile manured soil inoculated with a mixture of one spore former and two non-spore formers*

[Count indicates number of colonies per gram of soil]

Time since inoculation.	Experiment I.			Experiment II.			Experiment III.		
	Pseudomonas caudatus.	*Pseudomonas fluorescens.*	*Bacillus cereus.*	*Pseudomonas caudatus.*	*Pseudomonas fluorescens.*	*Bacillus cereus.*	*Pseudomonas caudatus.*	*Pseudomonas fluorescens.*	*Bacillus cereus.*
Days.									
0	[a] 250,000	[a] 100,000	.....	[a] 180,000	[a] 30,000	[a] 50,000	[a] 1,380,000	[a] 240,000	[a]680,000
1	None.	194,000,000	*Bacillus cereus* did not appear on the plates.	10,000,000	23,000,000	*Bacillus cereus* did not appear on the plates.	600,000	1,300,000	*Bacillus cereus* did not appear on the plates.
2	None.	188,000,000		89,000,000	106,000,000		No test.	No test.	
3	None.	260,000,000		297,000,000	560,000,000		24,000,000	6,900,000	
4	80,000,000	240,000,000		490,000,000	150,000,000		129,000,000	15,000,000	
5	480,000,000	150,000,000		575,000,000	150,000,000		No test.	No test.	
6	390,000,000	220,000,000		460,000,000	180,000,000		No test.	No test.	
7	570,000,000	135,000,000		1,190,000,000	250,000,000		No test.	No test.	
8	710,000,000	100,000,000		1,090,000,000	200,000,000		No test.	No test.	
9	770,000,000	110,000,000		1,020,000,000	130,000,000		No test.	No test.	
10	865,000,000	72,500,000		920,000,000	90,000,000		No test.	No test.	
11	920,000,000	75,000,000		900,000,000	80,000,000		580,000,000	60,000,000	
12	710,000,000	50,000,000		680,000,000	90,000,000		610,000,000	80,000,000	
13	880,000,000	80,000,000		950,000,000	120,000,000		650,000,000	65,000,000	
14	810,000,000	110,000,000		750,000,000	85,000,000		725,000,000	60,000,000	
15	870,000,000	90,000,000		860,000,000	80,000,000		No test.	No test.	
16	820,000,000	80,000,000		750,000,000	80,000,000		No test.	No test.	
17	865,000,000	80,000,000		180,000,000	30,000,000		No test.	No test.	
18	960,000,000	70,000,000		600,000,000	90,000,000		320,000,000	50,000,000	
19	730,000,000	80,000,000		580,000,000	110,000,000		250,000,000	20,000,000	
20	800,000,000	65,000,000		605,000,000	90,000,000		170,000,000	None.	
21	.....	.....		360,000,000	150,000,000		270,000,000	50,000,000	
22	.....	.....		425,000,000	150,000,000		310,000,000	65,000,000	
23	.....	.....		590,000,000	140,000,000		No test.	No test.	
24	.....	.....		735,000,000	100,000,000		300,000,000	70,000,000	
25	.....	.....		730,000,000	100,000,000		No test.	No test.	
26	.....	.....		No test.	No test.		330,000,000	75,000,000	
27	.....	.....		420,000,000	195,000,000		No test.	No test.	
28	.....	.....		550,000,000	80,000,000		No test.	No test.	
29	.....	.....		430,000,000	110,000,000		460,000,000	65,000,000	
30	.....	.....		330,000,000	50,000,000		No test.	No test.	
31	.....	.....		290,000,000	60,000,000		380,000,000	70,000,000	
36	.....	.....		.....	.....		310,000,000	60,000,000	
38	.....	.....		.....	.....		300,000,000	55,000,000	

[a] Computed from the number of organisms in the infusion used for inoculation.

The results as recorded in this series of experiments indicate quite clearly that the non-spore-forming organisms, *Ps. fluorescens* and *Ps. caudatus* rapidly gain the ascendancy over *B. cereus* when placed in competition with it in sterile freshly manured soil. The vegetative cells of *B. cereus* apparently soon sporulate and remain in the resting condition.

RELATIVE NUMBERS OF THE ORGANISMS IN QUESTION IN A SOIL BEFORE AND AFTER ADDING MANURE

Tables VIII, IX, X, and XI record data which show the relative numbers of *Ps. fluorescens*, *Ps. caudatus*, and *B. cereus* in soil in which no ammonification is occurring, and in the same soil after manure has been added and decomposition is occurring rapidly. Table VIII[1] contains the data obtained as the result of analyses of an untreated field

[1] The data given in this table were obtained by Conn in his earlier work. Much of it has already been used in his soil-flora studies (*8-11*).

TABLE VIII.—*Comparison between numbers of Bacillus cereus and the numbers of certain non-spore formers in Plot I, soil untreated*

[Count indicates the number of colonies per gram of soil]

Date.	Total count.	*Bacillus cereus.*	*Pseudomonas fluorescens.*	*Pseudomonas caudatus.*
1912.				
Sept. 23	38,250,000	350,000	[a] None.	60,000
Oct. 25	17,000,000	150,000	150,000	None.
Dec. 3	35,000,000	200,000	None.	None.
17	23,500,000	No count.	No count.	No count.
1913.				
Jan. 15	17,500,000	200,000	100,000	None.
Feb. 5	27,500,000	350,000	None.	None.
14	54,000,000	300,000	300,000	None.
Mar. 11	29,200,000	400,000	None.	None.
Apr. 4	27,000,000	400,000	200,000	None.
July 10	22,600,000	500,000	None.	None.
Nov. 26	15,000,000	350,000	None.	None.
Dec. 15	12,400,000	200,000	Trace.	None.
1914.				
Jan. 16	16,150,000	700,000	None.	None.
30	29,300,000	500,000	Trace.	None.
Feb. 7	26,700,000	400,000	None.	None.
26	38,500,000	600,000	Trace.	None.
Apr. 15	19,400,000	350,000	None.	None.
29	16,100,000	450,000	None.	None.
Aug. 7	[b]	200,000	None.	None.
19	23,400,000	175,000	600,000	None.
1917.				
May 4	12,500,000	300,000	250,000	None.

[a] Dilution so great that no colonies appeared on the plates. [b] Count lost on account of liquefaction.

TABLE IX.—*Comparison between numbers of Bacillus cereus and numbers of certain non-spore formers in soil from Plot I. Soil manured and kept in the laboratory. Series I*

[Counts indicate number of colonies per gram of soil]

Experiment No. and time since adding manure to soil.	Pot.[a]					Flask.[a]				
		Ps. fluorescens.		*Ps. caudatus.*			*Ps. fluorescens.*		*Ps. caudatus.*	
	Total count.	Plate count.	Percentages of total flora.	Plate count.	Percentages of total flora.	Total count.	Plate count.	Percentages of total flora.	Plate count.	Percentages of total flora.
Experiment I:										
7 days	251,000,000	10,000,000	4.0	None.	0.0	317,000,000	None.	0.0	9,000,000	2.8
16 days	105,000,000	6,000,000	5.7	47,000,000	44.8	99,000,000	10,000,000	9.9	50,000,000	50.5
Experiment II:										
3 days	89,000,000	5,000,000	5.7	25,000,000	28.2	142,000,000	10,000,000	7.0	35,000,000	25.0
13 days	177,000,000	7,000,000	4.0	50,000,000	28.3	485,000,000	75,000,000	15.4	35,000,000	7.2
20 days	73,000,000	3,000,000	4.2	10,000,000	13.7	43,000,000	2,000,000	4.7	4,000,000	9.5
Experiment III:										
5 days	233,000,000	17,000,000	7.3	50,000,000	21.4	145,000,000	10,000,000	6.9	20,000,000	13.8
15 days	37,000,000	2,000,000	5.4	5,000,000	13.5	78,000,000	4,000,000	5.1	3,000,000	3.9
20 days	30,000,000	2,000,000	6.6	1,000,000	3.4	22,000,000	1,000,000	4.5	5,000,000	2.2
Experiment IV:										
4 days	231,000,000	4,000,000	1.7	18,000,000	7.8	136,000,000	17,000,000	12.5	35,000,000	25.7
23 days	174,000,000	No count.	.....	No count.	.....	143,500,000	No count.	.....	No count.	.....
Average of all experiments			4.9		17.9			7.3		15.6

[a] *Bacillus cereus* did not develop on the plates.

soil from Plot I made at intervals for a period of three years. It will be noted that during that time the non-spore-forming *Ps. caudatus* appeared only once and then in comparatively small numbers; *Ps. fluorescens* appeared nine times, and only twice constituted more than 1 per cent and never more than 2.5 per cent of the total flora. It is also a significant fact that the spore-forming *B. cereus* was always present and made up from 0.55 to 4.4 per cent of the total flora. Another plating made just previous to the present work showed that the organisms were present as follows: *Ps. fluorescens*, 2 per cent; *Ps. caudatus*, less than 0.1 per cent; and *B. cereus*, 2.4 per cent.

TABLE X.—*Comparison between numbers of Bacillus cereus and numbers of certain non-spore formers in soil from Plot I. Soil manured and kept in the laboratory. Series II*

HORSE MANURE

[Counts indicate the number of colonies per gram of soil]

Time since adding manure to soil.	Open pot.[a]					Closed flask.[a]				
	Total count.	*Ps. fluorescens.*		*Ps. caudatus.*		Total count.	*Ps. fluorescens.*		*Ps. caudatus.*	
		Plate count.	Percentage of total flora.	Plate count.	Percentage of total flora.		Plate count.	Percentage of total flora.	Plate count.	Percentage of total flora.
Days.										
1.....	300,000,000	Trace.	0.1	Trace.	0.1	63,000,000	None.	0.0	None.	0.0
2.....	109,000,000	2,000,000	1.8	None.	.0	88,000,000	1,000,000	1.2	None.	.0
3.....	157,000,000	7,500,000	4.7	None.	.0	82,000,000	1,500,000	1.8	None.	.0
4.....	907,500,000	30,000,000	3.3	None.	.0	78,000,000	2,000,000	2.6	None.	.0
5.....	775,000,000	25,000,000	3.2	Trace.	.1	336,000,000	5,000,000	1.5	None.	.0
7.....	625,000,000	20,000,000	3.2	20,000,000	3.2	810,000,000	2,500,000	.3	None.	.0
8.....	67,500,000	750,000	1.1	1,250,000	1.8	75,500,000	None.	.0	500,000	.6
9.....	480,000,000	12,000,000	2.5	15,000,000	3.1	58,500,000	None.	.0	None.	.0
11.....	740,000,000	62,000,000	8.4	8,000,000	1.1	375,000,000	750,000	.2	750,000	.2
13.....	376,000,000	22,500,000	6.0	7,500,000	2.0	169,000,000	1,000,000	.6	1,000,000	.6
15.....	295,000,000	5,000,000	1.7	4,000,000	1.3	1,380,000,000	2,500,000	.1	5,000,000	.4
17.....	1,705,000,000	20,000,000	1.1	50,000,000	2.9	1,045,000,000	1,000,000	.9	None.	.0
Average.....			3.1		1.3			.8		.15

COW MANURE

1.....	164,000,000	Trace.	0.1	Trace.	.1	27,5000,00	Trace.	0.1	None.	0.0
2.....	93,000,000	Trace.	.2	Trace.	.2	64,000,000	2,000,000	3.1	Trace.	.1
3.....	98,500,000	500,000	.5	None.	.0	67,000,000	1,800,000	2.7	1,000,000	1.5
4.....	127,000,000	3,500,000	2.7	None.	.0	81,000,000	None.	.0	None.	.0
5.....	55,000,000	500,000	.9	None.	.0	101,000,000	None.	.0	None.	.0
7.....	490,000,000	1,000,000	.2	None.	.0	42,000,000	1,500,000	3.6	None.	.0
8.....	251,000,000	1,500,000	.6	None.	.0	65,500,000	Trace.	.2	None.	.0
9.....	52,000,000	1,000,000	1.9	500,000	.9	34,500,000	None.	.0	None.	.0
11.....	412,000,000	2,500,000	.6	7,500,000	1.9	330,000,000	Trace.	.1	None.	.0
13.....	67,000,000	Trace.	.2	None.	.0	323,000,000	Trace.	.1	None.	.0
15.....	395,000,000	5,000,000	1.3	10,000,000	2.6	330,000,000	Trace.	.1	1,000,000	.3
17.....	245,000,000	None.	.0	1,000,000	.2	350,000,000	2,000,000	.7	None.	.0
Average.....			.8		.5			.9		.2

[a] *B. cereus* did not develop on the plates.

Tables IX, X, and XI record the results of platings made from samples of the same soil after being treated with fresh manure. Examination

of these tables show that either *Ps. fluorescens* or *Ps. caudatus*, or both, almost invariably appeared on every sample plated and often constituted as high as 15 or 20 per cent of the entire flora, while *B. cereus* was very seldom observed; in fact, very few spore formers of any type were recognized. It must be borne in mind that these data were obtained from soil in which it was definitely determined that decomposition processes were occurring.

TABLE XI.—*Comparison between numbers of Bacillus cereus and numbers of certain non-spore formers in soil from Plot I. Soil manured and kept in the laboratory. Series III*

HORSE MANURE

[Counts indicate number of colonies per gram of soil]

Time since adding manure to soil.	Open pot.[a]					Closed flask.[a]				
		Ps. fluorescens.		*Ps. caudatus.*			*Ps. fluorescens.*		*Ps. caudatus.*	
	Total count.	Plate count.	Percentage of total flora.	Plate count.	Percentage of total flora.	Total count.	Plate count.	Percentage of total flora.	Plate count.	Percentage of total flora.
Days.										
1	110,000,000	2,000,000	1.8	1,500,000	1.4	40,000,000	750,000	1.8	1,000,000	2.5
2	120,000,000	6,000,000	5.0	5,000,000	4.2	182,500,000	10,000,000	5.5	25,000,000	13.7
3	195,000,000	4,000,000	2.1	35,000,000	18.0	82,500,000	2,000,000	2.4	15,000,000	18.2
4	150,000,000	6,000,000	4.0	13,000,000	8.7	75,000,000	3,000,000	4.0	5,000,000	6.7
5	122,000,000	4,000,000	3.3	8,000,000	6.6	33,000,000	2,000,000	6.1	4,000,000	12.2
7	95,000,000	10,000,000	10.5	5,000,000	5.2	515,000,000	5,000,000	1.0	15,000,000	3.0
8	300,000,000	55,000,000	18.3	40,000,000	13.3	605,000,000	35,000,000	5.8	15,000,000	2.5
9	30,500,000	1,500,000	4.9	1,500,000	4.9	62,500,000	None.	.0	1,000,000	1.6
10	59,000,000	1,500,000	2.5	1,500,000	2.5	780,000,000	Trace.	.1	1,000,000	.1
12	48,000,000	3,000,000	6.3	5,000,000	10.4	67,000,000	1,000,000	1.5	None.	.0
15	242,500,000	25,000,000	10.3	22,500,000	9.3	43,500,000	None.	.0	1,000,000	2.3
18	130,000,000	7,500,000	5.8	7,500,000	5.8	100,000,000	2,000,000	2.0	None.	.0
21	117,500,000	2,000,000	1.9	2,000,000	1.9	40,000,000	None.	.0	None.	.0
Average			5.9		7.1			2.3		4.8

COW MANURE

1	58,000,000	None.	0.0	750,000	1.3	51,000,000	800,000	1.6	2,000,000	3.9
2	73,250,000	7,500,000	10.3	5,000,000	6.8	42,000,000	1,000,000	2.4	2,000,000	4.8
3	497,500,000	10,000,000	2.0	30,000,000	6.1	295,000,000	25,000,000	8.5	12,000,000	4.2
4	93,000,000	2,000,000	2.1	7,500,000	8.1	68,000,000	2,000,000	2.9	3,000,000	4.4
5	102,500,000	3,500,000	3.4	1,000,000	1.0	44,250,000	2,000,000	4.5	3,500,000	7.9
7	36,250,000	1,000,000	2.8	None.	.0	27,250,000	None.	.0	1,000,000	4.0
8	43,000,000	2,000,000	4.6	None.	.0	45,500,000	None.	.0	1,000,000	2.2
9	17,500,000	None.	.0	None.	.0	31,000,000	None.	.0	2,000,000	6.4
10	40,000,000	None.	.0	1,000,000	2.5	36,500,000	500,000	1.4	5,000,000	14.0
12	96,000,000	3,500,000	3.7	4,500,000	4.7	49,500,000	1,000,000	2.0	5,000,000	10.0
15	187,000,000	7,500,000	4.0	4,000,000	2.1	53,000,000	1,500,000	2.8	1,000,000	1.9
18	75,000,000	10,000,000	13.3	2,000,000	2.7	125,000,000	10,000,000	8.0	2,000,000	1.6
21	64,000,000	1,500,000	2.3	5,000,000	7.8	46,000,000	Trace.	.2	None.	.0
Average			3.7		3.3			2.6		5.0

[a] *B. cereus* did not appear on the plates.

AMMONIFICATION BY THE ORGANISMS IN QUESTION IN STERILIZED MANURED SOIL

SOIL INOCULATED WITH THE THREE ORGANISMS SEPARATELY

Table XII contains the data secured when the soil inoculated with pure cultures of each of the three organisms separately was subjected to the ammonification test previously described (p. 317). All of the organisms were found to be ammonifiers, and, so far as the individual organisms are concerned, the data indicates that, per organism, *B. cereus* is the most powerful ammonifier of the three. When the plate count of *B. cereus* showed 17,000,000 colonies per gram of soil on the tenth day after inoculation, the ammonia production was 22 mgm. per 100 gm. of soil (Table XII, Experiment II). On the other hand, a plate count of a flask inoculated with *Ps. fluorescens* showed 375,000,000 colonies per gram of soil on the tenth day and an ammonia production of 20.28 mgm. per 100 gm. of soil (Table XII, Experiment II); and a plate count from a flask inoculated with *Ps. caudatus* showed 220,000,000 colonies per gram of soil on the eighth day and an ammonia production of 17.84 mgm. per 100 gm. of soil (Table XII, Experiment II). This fact does not prove, however, that *B. cereus* is an important ammonifier in unsterilized manured soil. The data already discussed (p. 325) indicate that under natural conditions the organisms of the *B. cereus* group are present in manured soil in very small numbers and that the vegetative cells quickly disappear.

TABLE XII.—*Ammonia produced by B. cereus and the non-spore formers inoculated separately into sterile manured soil*

Experiment No. and time since inoculation.	*B. cereus.*			*Ps. caudatus.*			*Ps. fluorescens.*		
	Total count.	Quantity of ammonia per 100 gm. of soil.		Total count.	Quantity of ammonia per 100 gm. of soil.		Total count.	Quantity of ammonia per 100 gm. of soil.	
		Inoculated flask.	Blank control.		Inoculated flask.	Blank control.		Inoculated flask.	Blank control.
Experiment I:		*Mgm.*	*Mgm.*		*Mgm.*	*Mgm.*		*Mgm.*	*Mgm.*
2 days.....	4,000,000	11.24	4.6	1,400,000,000	5.12	3.4	140,000,000	5.8	1.36
4 days.....	5,000,000	10.92	.68	1,340,000,000	11.56	1.36	260,000,000	16.32	7.76
9 days.....	10,000,000	12.56	1.36	1,460,000,000	20.4	7.76	315,000,000	11.2	2.72
11 days.....	10,500,000	17.68	5.44	1,190,000,000	14.96	2.72	350,000,000	19.04	5.44
Experiment II:									
2 days.....	8,000,000	7.12	3.04	114,000,000	7.48	4.4	102,000,000	12.76	4.24
4 days.....	5,000,000	12.56	5.08	181,000,000	11.88	4.4	390,000,000	15.32	4.16
6 days.....	12,500,000	10.2	3.4	194,000,000	10.36	5.44	395,000,000	20.00	6.44
8 days.....	14,000,000	15.64	2.72	220,000,000	17.84	6.12	470,000,000	20.24	6.64
10 days.....	17,000,000	22.00	6.24	206,000,000	17.48	5.24	375,000,000	20.28	Lost.
12 days.....	15,000,000	18.92	3.72	220,000,000	16.12	4.4	460,000,000	17.80	6.44
14 days.....	21,000,000	21.68	5.08	269,000,000	16.64	4.4	530,000,000	17.32	5.44
16 days.....	56,000,000	17.32	4.24	171,500,000	16.48	5.24	470,000,000	14.28	6.60

SOIL INOCULATED WITH A MIXTURE OF THE THREE ORGANISMS

Table XIII contains the data obtained as the result of inoculating sterilized manured soil with a mixture of the three organisms. In Experiment I all of the organisms were inoculated in approximately equal numbers. It is a noteworthy fact that while *Ps. fluorescens* and *Ps. caudatus* showed rapid development and were always present in large numbers, *B. cereus* never developed upon the plates and showed a very rapid decrease in the number of vegetative cells present in the smears examined under the microscope.

TABLE XIII.—*Ammonia produced by a mixture of B. cereus and the two non-spore formers inoculated into sterile manured soil*

Experiment No. and time since inoculation.	Total count per gram of soil.			Quantity of ammonia per 100 gm. of soil.	
	Ps. fluorescens.	*Ps. caudatus.*	*B. cereus.*	Inoculated flask.	Blank control.
				Mgm.	*Mgm.*
EXPERIMENT I:					
0 days	[a] 2, 573, 000	[a] 2, 406, 000	[a] 2, 380, 000	.....	.....
4 days	240, 000, 000	80, 000, 000	B. cereus did not appear on plates.	19. 52	5. 88
6 days	220, 000, 000	390, 000, 000		19. 56	4. 8
8 days	100, 000, 000	710, 000, 000		20. 4	5. 08
11 days	75, 000, 000	920, 000, 000		14. 96	4. 76
13 days	80, 000, 000	880, 000, 000		21. 12	5. 08
15 days	90, 000, 000	870, 000, 000		17. 52	No test.
17 days	80, 000, 000	865, 000, 000		20. 36	No test.
19 days	80, 000, 000	730, 000, 000		19. 56	No test.
EXPERIMENT II:					
0 days	[a] 80, 000	[a] 640, 000	[a] 2, 600, 000	.....	.....
7 days	250, 000, 000	1, 190, 000, 000	B. cereus did not appear on plates.	15. 96	7. 12
9 days	130, 000, 000	1, 020, 000, 000		18. 2	7. 48
15 days	80, 000, 000	860, 000, 000		21. 16	5. 92
23 days	140, 000, 000	590, 000, 000		21. 12	6. 12
27 days	195, 000, 000	420, 000, 000		19. 52	6. 12
30 days	50, 000, 000	330, 000, 000		17. 36	6. 32
31 days	60, 000, 000	290, 000, 000		15. 96	6. 12

[a] Computed from the number of organisms in the infusion used for inoculation.

Despite the very evident fact that *B. cereus* was not active in the samples tested, the amount of ammonia produced was quite marked. This was a strong indication that the ammonia produced was due to the activity of the only other organisms present, *Ps. fluorescens* and *Ps. caudatus*. In Experiment II the organisms were inoculated in the proportion of 1 individual of *Ps. fluorescens* to 8 of *Ps. caudatus* to 33 of *B. cereus*. Even with such a favorable start as this, *B. cereus* again failed to develop on the plates and showed a rapid decrease in the number of vegetative cells present. The degree of ammonification was marked also in this series.

Throughout the entire series of ammonification experiments a correlation seemed to exist between the time that had elapsed since the

inoculation of the soil and the amount of ammonia produced rather than between the number of organisms present and the ammonia production. The tests were continued until the apex of ammonia production was apparently reached. After this point had been reached, a steady decrease in ammonia production was noted, regardless of the number of organisms present. This was undoubtedly due to the depletion of available organic matter.

DISCUSSION OF RESULTS

The heterogeneous nature of soil, the great variety of organisms present, and the varying moisture content are all factors which make it practically impossible to carry on a study of ammonification in the soil under absolutely natural conditions. In order to control these things and to obtain comparable data, it is necessary to bring the soil into the laboratory for study. This introduces a difficulty, in that conditions governing the activities of organisms in the laboratory are generally at wide variance with conditions in the natural environment of these organisms. The reason for this variance is twofold: First, laboratory media may often be decidedly different from the soil in which the organisms are native; and second, the organisms in pure culture, as they are generally handled in the laboratory for purposes of control, do not behave as they would in competition or in association with the other organisms normally present in the soil. These artificial conditions must be kept in mind in considering the results obtained in the present work, but despite them there was one striking relation which invariably held true: the spore forming *B. cereus* never multiplied in manured soil with any degree of rapidity, while *Ps. fluorescens* and *Ps. caudatus* always did.

The data indicate that in soil where little organic matter is present and the processes of soil decomposition are practically at a standstill the spore former *B. cereus* occurs much more often than do the non-spore formers *Ps. fluorescens* and *Ps. caudatus*. When organic matter in the form of manure has been added to that same soil, however, and the processes of decomposition become active, the character of the flora changes entirely, and *Ps. fluorescens* and *Ps. caudatus* predominate over *B. cereus*. But the proof that these non-spore formers are the important ammonifiers in manured soil is decidedly difficult to secure.

As Conn (7, *p. 254*) has previously pointed out, there are four points which must be established before we can show conclusively that any particular chemical transformation in the soil is due to certain organisms:

(1) The organism must be shown to be present in active form when the chemical transformation under investigation is taking place; (2) it must be shown to occur in larger numbers under such conditions than in the same soil in which the chemical change is not occurring; (3) it must be isolated from the soil and studied in pure culture; (4) the same chemical change must be produced by the organism in experimentally inoculated soil, making the test, if possible, in unsterilized soil. The fourth

requirement, however, can ordinarily be carried out only by inoculating sterilized soil, a procedure which does not give rigid proof, but which is fairly conclusive if carried out in connection with the other three requirements.

The data presented above offer fairly conclusive proof that these conditions have all been fulfilled by the organisms in question. The first step in the proof may be found in Tables IX, X, and XI, where it is shown that one or both of the non-spore-forming organisms are always present in active form in manured soil in which ammonification is occurring. That the second requirement is fulfilled is shown by Tables VIII, IX, X, and XI, in which it may be seen that the non-spore formers *Ps. fluorescens* and *Ps. caudatus* occur in much greater numbers in decomposing manured soil than in the same soil before the manure has been added, and that the spore former *B. cereus* occurs in great abundance in the soil before adding manure, but disappears almost entirely after manuring. The isolation of pure cultures, the third step in the proof, needs no comment, while the fourth is fulfilled, as seen by reference to Table XII, where it is shown that pure cultures of the organisms have the power of ammonifying manure in soil.

On the basis of the data obtained there are therefore no good reasons for believing that the spore-forming organisms play an important rôle in ammonifying manure in soil, and there is very good evidence that the non-spore formers *Ps. fluorescens* and *Ps. caudatus* are of primary importance in ammonification in manured soils.

II.—TAXONOMIC STUDY OF TWO IMPORTANT SOIL AMMONIFIERS

By H. J. Conn

DISTRIBUTION

The preceding section of this paper is concerned with the ammonifying powers of two soil bacteria, *Pseudomonas fluorescens* (Flügge) Migula and *Ps. caudatus* (Wright) Conn. Both of them are believed to be very widely distributed in nature. There is no question as to the wide distribution of *Ps. fluorescens*, because it has been described again and again by previous investigators as occurring in various localities. This, or some other similar organism, has been found most frequently in soil and in water; but has also been reported in air, butter, maple sap, and other substances. It has been observed by the present writer in practically all soils investigated, especially in soil that has been aerated or manured. *Ps. caudatus* is probably equally widely distributed; but the difficulty in recognizing it from published descriptions renders the literature concerning it of doubtful value. No references to similar organisms in soil have been found, but various writers have described yellow or orange liquefying bacteria found in water, some of which are undoubtedly the same as the organism studied here. Water was the source of Wright's *Bacillus caudatus*. The writer has observed it in water and in many soils, especially in freshly manured soil.

To aid in the identification by others of these two organisms studied by Bright, a detailed investigation of their physiology and cultural characteristics has been made, and the characteristics observed have been compared with those described by other writers. The following paper contains a description of these characteristics and a discussion of the probable relationships of these organisms in others.

PSEUDOMONAS FLUORESCENS

Pseudomonas fluorescens (Flügge) Migula (*1900, p. 886*) was first described by Flügge (*16, p. 289*) as "*Bacillus fluorescens liquefaciens.*" The description is rather meager, but the organism is plainly specified as a motile short rod, liquefying gelatin rapidly with the formation of a greenish-yellow fluorescence, producing a brownish growth on potato, and occurring in water and in decomposing material. This description indicates beyond question the group of fluorescent pseudomonads, even if the exact species or variety is uncertain. In the later edition of this book (*Kruse, 26, p. 292*) the organism is described more definitely with the following additional information: Size 0.3 to 0.5 by 1 to 2 μ; no spores; Gram stain negative; optimum temperature 20° to 25° C. Kruse further

makes the statement that if all small deviations were designated as constant characters dozens of species must be established.[1]

The organism is much more fully described by Lehmann and Neumann (*29, p. 272*) under the name of *Bacterium fluorescens* (the adjective "*liquefaciens*" dropped to avoid a trinomial, and placed in the genus Bacterium because these authors placed only spore formers in Bacillus). They state that it is identical with *B. pyocyaneum* in all essential characters.[2] It is described as having polar flagella. *B. pyocyaneum* is described as producing no indol, hydrogen sulphid (H_2S), nor gas from dextrose, but as converting nitrate into nitrogen; from which it is to be assumed that *B. fluorescens* agrees in these characteristics, although nothing definite is said on the subject except in regard to indol and hydrogen sulphid. Migula (*38*) placed this organism in his genus Pseudomonas, created to contain the rods with polar flagella. Migula describes *Ps. fluorescens* at some length, but lays greatest stress on cultural characteristics and adds little of importance to Kruse's description. Migula gives its diameter as about 0.68 μ.

Many other writers have described the same or some similar organism. Many different names have been given to fluorescent bacteria from time to time, Tanner (*49*) having recently stated that 95 different names had been found in a search through the literature. Many of these names are trinomials or worse, such as *Bacillus fluorescens putidus* Flügge (*16*), *B. fluorescens liquefaciens minutissimus* (Unna) Tommasoli (*14*); but others have conformed to approved rules of nomenclature. The greater number of the fluorescent organisms have been found in water, soil, or decaying organic matter; but one of the best known forms, the *pyocyaneus* type—more correctly named "*Ps. aeruginosa* (Schroeter) Migula"—causes blue pus. As mentioned by Kruse (*26*), there is great variation among these organisms, and if each variation be taken as a constant characteristic an almost endless variety of species could be named. This fact naturally raises the question how many of the names found in the literature are valid and how many are really synonyms, having been applied to mere physiological variations of a previously described species. Even the blue-pus organism is thought by some writers to be identical with the saprophytic forms. We have not yet sufficient data to straighten out completely the resulting confusion, but a careful search through the literature throws a little light on the matter. The information accumulated during the present work has made it possible to review this literature more intelligently than could have been done otherwise; and it seems well, therefore, to summarize the writings of others in regard to some of the more important fluorescent organisms.

[1] Wenn man alle kleinen Abweichungen als Konstante Merkmale auffassen wollte, müsste man Dutzende von Arten aufstellen.

[2] Allen wesentlichen Eigenschaften.

BACTERIUM TERMO

The name "*Bacterium termo* (Müller) Ehrb." was given by Ehrenberg to what he considered the *Monas termo* of O. F. Müller (*34*). The same name was used by various writers during the next three or four decades to designate almost any motile rod found abundantly in decaying organic matter. Finally Cohn (*4, p. 196*) described *B. termo* as a green fluorescent organism obtained from decomposing seeds by making a series of transfers into tubes of Cohn's solution.[1] By means of this same technic, a culture has been obtained agreeing fairly well with Cohn's organism, a vigorous denitrifier,[2] differing from all other fluorescent pseudomonads investigated here. Smith (*47, p. 170*), however, used this technic and obtained a green fluorescent organism differing distinctly (in failure to liquefy gelatin and in having but one instead of two flagella) from the one found in this laboratory. It seems doubtful, therefore, whether Cohn's organism actually represents one or several species; and as there is some question as to whether he was justified in his emendation of the species, the name is not used in the present bulletin.

Van Iterson (*23*) described a nonliquefying, fluorescent denitrifier (*B. denitrofluorescens*) which may perhaps be the same as Smith's "*B. termo*," or closely related to it. Other fluorescent, denitrifying bacteria have been described by Severin (*45, 46*) and by Jensen (*24*). It is evident, therefore, that in the group of fluorescent pseudomonads there are certain denitrifiers, one or more of which are especially adapted to growth in Cohn's solution. Severin and Jensen used the designation "*Bacillus pyocyaneus*" or "*Bacterium pyocyaneum*" for their fluorescent denitrifiers, so it is necessary to review the literature relating to the pyocyaneus type of oragnisms.

PSEUDOMONAS AERUGINOSA

Pseudomonas aeruginosa (Schroeter) (*44, p. 157*) Migula (*38, p. 884*), or *Ps. pyocyanea* (Gessard, *Flügge*)[3] Migula (1900, *p. 29*), the blue-pus organism, has long been known, but there has been much confusion as to its name. Many writers have used the specific name "*pyocyaneus*," although others have recognized the priority of *aeruginosus*. Bacillus, Bacterium, and Pseudomonas have all three been used as the generic name, according to the generic definitions adopted by different authors. The name "*aeruginosa*" seems to be correct.[4]

[1] The formula of this solution is: Distilled water, 1,000 cc.; acid potassium phosphate (KH_2PO_4), 5 gm.; magnesium sulphate ($MgSO_4$), 5 gm.; neutral ammonium tartrate [$(NH_4)_2C_4H_4O_6$], 10 gm.; potassium chlorid (KCl), 0.5 gm.

[2] The term "denitrification" in this paper is used strictly to refer to the eliberation of free nitrogen from nitrate, not to the reduction of nitrate to nitrate or ammonia.

[3] Gessard (*20*) is generally quoted as the author of the term "*pyocyaneus*," although he did not employ it in accordance with strict taxonomic usage and apparently referred to an entirely different organism. The first correct use of the name *Bacillus pyocyaneus* for the true blue-pus organism was by Flügge (*16, p. 288*).

[4] The nomenclature of this organism is to be discussed in a paper by R. S. Breed and H. J. Conn, which is now in course of preparation, and will appear shortly in the Journal of Bacteriology.

Gessard (*21*) made a comparative study of this organism and some other fluorescent organisms. He concluded that it produces two pigments: a yellow-green, water-soluble pigment, and a blue-green, chloroform-soluble pigment, which he called "pyocyanin." He claims that it differs from *B. fluorescens liquefaciens* and *B. fluorescens putidus* (the nonliquefying type), as neither produces pyocyanin. Lehmann and Neumann (*29, p. 272*), however, claimed that the two organisms differ only in the intensity of the pigment, and remark concerning *B. pyocyaneum* that according to their conviction, this organism can not be sharply differentiated from *B. fluorescens*.[1] The opposite conclusion was reached two years later by Niederkorn (*40*), who studied a series of fluorescent cultures from various sources and decided that the *fluorescens* type and the *pyocyaneus* type are distinct, although each has numerous subvarieties. He states that the flagella of the *pyocyaneus* type are well defined,[2] but those of the *fluorescens* type are not; that the former takes the Gram stain more definitely than the latter; that the former grows best at 35° C., the latter at room temperature. The contrary opinion is expressed by Růžička (*42, 43*), who mentions these and other differences (except in regard to the Gram stain), but concludes that they are not constant By cultivating the *fluorescens* type at 37° he obtains cultures of the *pyocyaneus* type; by growing the blue-pus organism in water, aerated with sterile air, he obtains cultures of the *fluorescens* type. Later Lehmann and Neumann (*30, p. 411–413*) continue the discussion, referring to the differences between the two types, laying considerable stress on the denitrifying power of the blue-pus organism, but repeating their earlier statement that one type grades imperceptably into the other. They did not find either organism Gram-positive. Finally, Pribram and Pulay (*41*) made a study of the fluorescent group by serological methods and found it apparently to consist of several different species, *B. pyocyaneum* appearing distinct from *B. fluorescens*, although closely related to it.

The ability of the *pyocyaneus* type to convert nitrate into free nitrogen was apparently first mentioned by Lehmann and Neumann (*29*), who do not, however, mention the source from which the culture they studied was obtained. The following year, Weissenberg (*51*), apparently at the suggestion of Lehmann or Neumann, made a further investigation of *pyocyaneus* cultures from various sources, finding them all to be denitrifiers, while observing this ability with no organism of the fluorescens type. The same year Severin (*45*) wrote a paper on denitrifiers obtained from manure, one of which is fluorescent. This fluorescent culture he calls *B. pyocyaneus*, but does not show it to be the cause of blue pus.

One striking fact in this connection is that no one seems to have found a Gram-positive *pyocyaneus* culture which denitrifies or a Gram-negative

[1] Den Organismus scharf gegen *B. fluorescens* abzugrenzen, geht nach unserer Ueberzeugung nicht an.
[2] Wohl ausgeprägte.

one which does not denitrify. Those who report denitrification either state the organism to be Gram-negative (as do Lehmann and Neumann) or else make no statement in regard to the Gram stain. Those who have found it to be Gram-positive have not studied its action on nitrate. This suggests that two different organisms, one Gram-positive and pathogenic, the other Gram-negative, denitrifying, and probably saprophytic. If this be the case, the former is more likely to be distinct from the *fluorescens* type than the latter.

PSEUDOMONAS PUTIDA

Pseudomonas putida (Flügge) Migula (*38, p. 912*). The name "*Bacillus fluorescens putidus*" [1] was given by Flügge (*16*) to the nonliquefying, fluorescent type of organism. Eisenberg (*14*), besides this name, used the name "*B. fluorescens nonliquefaciens*" for what he considers a different organism, and in this is followed by Kruse (*26*) and Migula (*38*), the latter discarding the polynomial and renaming it "*Ps. Eisenbergi.*" Lehmann and Neumann (*30*), however, do not consider it a distinct type and *Ps. putida* is the only nonliquefying species considered to-day to have good standing.

Whether *Ps. putida* and *Ps. fluorescens* are distinct is also a question that is not entirely settled. Lehmann and Neumann do not question that they are distinct. Pribram and Pulay (*41*), as the result of their serological studies, conclude that they are not only distinct but that they stand the farthest apart of any of the fluorescent cultures studied. Edson and Carpenter (*13*), however, consider that there are so many gradations between rapid liquefiers and nonliquefiers that this characteristic can not be used to distinguish between species.

NUMBER OF FLUORESCENT BACTERIA

A summary of the literature, therefore, gives no satisfaction in deciding how many different pseudomonads possess the property of producing fluorescence in culture media. Some writers consider them all the same; others make two or three different species; still others believe there are several species; while, if we consider every name a distinct species, there are a hundred or more. A study of the literature, however, indicates that there are four or five types standing out more or less distinct from each other: (1) *Ps. aeruginosa*, the blue-pus organism, a Gram-positive, rapidly liquefying organism, producing the blue-green pigment pyocyanin in addition to the fluorescent pigment, and possibly reducing nitrate to nitrogen. (2) *Ps. fluorescens*, a Gram-negative, rapidly liquefying saprophyte, showing poor growth or none at 37° C.,

[1] In the third edition of Flügge's book, Kruse (*26*) uses the name *B. fluorescens putridus*, evidently a misprint or mistake in spelling, because Flügge's description of the organism by the term "*stinkende*" shows plainly that "*putidus*" was the word he meant to use. Migula in renaming the organism follows Kruse's spelling, calling it *Ps. putrida* (Flügge). Other writers, however, such as J. Eisenberg (*14*), Lehmann and Neumann (*30*), and Chester (*2*) have used the spelling "*putidus*."

and unable to convert nitrate to free nitrogen. (3) A Gram-negative, rapidly liquefying denitrifier, such as described by Lehmann and Neumann as *Bacterium pyocyaneum*. Whether these authors worked with the true blue-pus organism or not, there seems to be an organism of this description that is different from the true *Ps. aeruginosa*. Several such cultures have been isolated in this laboratory, all of which fail to show pyocyanin even when grown in nitrite broth (the method described by Eisenberg (*15, p. 470*) as showing the production of this pigment to advantage) and extracted with chloroform. These cultures have differed among themselves and may represent several varieties. Undoubtedly the *B. pyocyaneus* of Severin (*45, 46*), Jensen (*24*), and others, who studied denitrifiers from manure and soil, was an organism (or organisms) of this type rather than of the true *pyocyaneus* type. (4) A nonliquefying denitrifier described by Van Iterson (*23*) as *B. denitrofluorescens*, which is probably distinct from the above and from the following, although it has not been studied here. (5) *Ps. putida*, a nonliquefying organism unable to denitrify. Although some writers seem to think liquefaction an unsatisfactory basis for the separation of these species, there seems no chance for reasonable doubt that an organism unable to liquefy after six months is different from the very rapid liquefiers studied in the present work. The difficulty in making this distinction may perhaps be due to the failure to distinguish between true liquefaction by the living cells and slow digestion of the gelatin by enzyms liberated from the cells after death.

Further investigation is necessary before it can be decided whether these five types represent different varieties of the same species, five separate species, or even five different type species about which distinct groups of species (perhaps genera) should be gathered.

CHARACTERISTICS OF TYPICAL FLUORESCENT ORGANISMS

MORPHOLOGY.—Small, short rods, not much over 0.5 μ, or perhaps somewhat smaller; no spores; a few flagella in a tuft at one pole; Gram-negative. (A few fluorescent spore formers have been described, and Edson and Carpenter (*13*) mention a weakly fluorescent peritrichic rod; but these are apparently unrelated organisms. The *pyocyaneus* type has been described as Gram-positive.)

CULTURAL CHARACTERISTICS.—Growth on agar smooth, soft to slimy; on potato smooth, brownish, medium discolored. Nearly all other cultural characteristics vary.

Greenish fluorescence is the most striking characteristic of the entire group, but it is not a constant characteristic. It is produced in some media and not in others. Gessard (*21, 22*), Lepiere (*31*), and Jordan (*25*) have studied the ability of these organisms to cause fluorescence with rather discordant results. They differ considerably in their conclusions as to the composition of the medium necessary for the produc-

tion of this pigment. The reason for this discrepancy may be in part, as suggested by Jordan, because of difficulty in obtaining absolutely pure chemicals; but it is undoubtedly also due to the varying behavior of different varieties. It has been observed in the course of the present work that two different strains may behave exactly the opposite, so far as concerns their ability to produce fluorescence in one or the other of some two media investigated. Any one strain, moreover, may vary considerably at different times in its ability to produce fluorescence. One particular strain has been studied in this laboratory which was thought to be *Ps. fluorescens* when first obtained from soil, although not showing fluorescence; but after having been cultivated for several generations on a beef-extract-peptone agar containing 0.1 per cent of nitrate, it gradually became fluorescent, and at the time of writing is one of the most strongly fluorescent cultures in this laboratory. (Another substrain of this organism, kept growing meanwhile on the same agar without nitrate, has developed no fluorescence.) A similar phenomenon was observed by Severin (*45*) upon cultivating a denitrifying strain in nitrate broth.

The strain used in Bright's experiments as reported in the first section of this paper was always found to cause decided fluorescence on all ordinary media.

RELATION TO OXYGEN.—Apparently all of the group are strictly aerobic. This is certainly true of all that have been studied here.

LIQUEFACTION OF GELATIN.—Typical *Ps. fluorescens* is a very vigorous liquefier. Slow liquefiers are common, as shown by Edson and Carpenter (*13*), although but few have been found in the present work. Non-liquefiers have been observed occasionally in the soils investigated here.

The gelatin colonies of fluorescent organisms vary according to the rapidity of liquefaction. Typical *Ps. fluorescens* produces a rapidly liquefying colony with entire edges that liquefies the entire plate in a few days. The strain studied in this work produced a colony of this type, also characterized by its clear structureless center; fluorescence was sometimes present, sometimes absent.

AMMONIFICATION.—Ammonia is produced from proteid by all the fluorescent organisms so far as they have been studied. Blanchitière (*1*) has made a careful study of the ammonification of asparagin by a fluorescent liquefier, agreeing well with the strain used in the present work; and has found that it easily converts the amid nitrogen of this compound into ammonia, but the aspartic nitrogen less readily.

ACTION ON SUGARS AND GLYCERIN.—Apparently no fluorescent organism has been recorded as producing gas from sugars or glycerin. Nearly all writers have found acid to be produced from dextrose, but in regard to other sugars the results are conflicting. The reason for this in part is that the technic generally used is bound to give meaningless results. Thus, Tanner (*49*) and Edson and Carpenter (*13*) both determine acid

production by titration, the latter writers titrating hot, a procedure which Clark (*3*) has shown to be illogical. Tanner finds acid production only in dextrose, while Edson and Carpenter find it common with the other sugars, undoubtedly because the H-ion concentration is increased by heating the culture previous to titrating.

Blanchetière (*1*) avoids this error by using litmus agar. He finds acid produced from dextrose and levulose, but not from the disaccharids; but as levulose is a difficult sugar to purify, and as Blanchetière says nothing about the source of his sample, he leaves some doubt in the reader's mind as to whether it was actually free from dextrose. He distinctly states that lack of acid reaction in this medium does not mean failure to produce acid, but simply that not enough acid is produced to neutralize the ammonia formed from the peptone. This shows Blanchetière realizes another source of error, but feels unable to overcome it. Plainly, with these two sources of error, the data in the literature as to acid production by fluorescent organism are not reliable.

In the present work Blanchetière's technic has been modified by using bromcresol purple in place of litmus as an indicator. The result has been in practically every case to find acid produced from dextrose and sucrose, but not from lactose and glycerin. The strain studied in Bright's experiments, above reported, showed these reactions very constantly. A synthetic medium containing ammonium tartrate as its only source of nitrogen [1] was then used in an attempt to overcome the error resulting from the presence of peptone in ordinary agar, and somewhat different results were obtained. Even with this method there was no agreement in the results obtained with the different fluorescent organisms. The strain used by Bright showed acidity from dextrose and sucrose, the latter reaction disappearing after the first day; while another strain agreeing with it in every respect showed strong and persistent acidity in sucrose as well as dextrose. The conclusion was reached that *Ps. fluorescens* produces acid from both dextrose and sucrose, but that with the latter sugar the acid production is likely to be obscured by other activities tending to lower the reaction of the medium.

REDUCTION OF NITRATE.—The literature is full of conflicting data in regard to the action of fluorescent bacteria on nitrate. There are several different possibilities: (*1*) Reduction to nitrite; (2) reduction to nitrite, then to ammonia; (3) reduction to ammonia without appreciable accumulation of nitrite; (4) reduction to free nitrogen—namely, denitrification; (5) assimilation of the nitrogen of the nitrate, with or without previous reduction. It has not proved possible to devise a simple test to distinguish with certainty between these five different possibilities, hence, the confusion.

[1] The formula of this medium was: Distilled water, 1,000 cc.; agar, 15 gm.; calcium chlorid ($CaCl_2$), 0.5 gm; potassium phosphate (K_2HPO_4), 0.5 gm.; ammonium tartrate [$(NH_4)_2C_4H_4O_6$], 10 gm.; with 10 gm.; of the sugar (or glycerin) under investigation.

Conversion into free nitrogen is the easiest to determine. It has already been seen that fluorescent denitrifiers have been described in the past. Here, they prove to be common enough in soil to be obtained frequently from ordinary soil culture plates. Neither Edson and Carpenter (*13*) nor Tanner (*49*) found any among the various cultures they studied; but they both used nitrate broth containing only 0.1 per cent of peptone, in which appreciable gas production has never been observed here. Most vigorous gas production has been observed in broth or agar containing 1 per cent of peptone. Typical *Ps. fluorescens*, however, has never been found to convert nitrate into nitrogen.

Conversion into ammonia is ordinarily impossible to demonstrate by any simple test, because ammonia can be produced from any nitrogenous substance, and some organic nitrogen is ordinarily present in media. Conversion into nitrite is easy to demonstrate, provided the organism investigated does not convert the nitrite into ammonia or assimilate it as fast as produced. *Ps. fluorescens* is generally considered to produce nitrite, but Franzen and Löhmann (*18*) studied two strains of what were presumably *Ps. fluorescens* without observing any action at all on the nitrate. Certain strains of fluorescent liquefiers have been studied here which produce no appreciable amount of nitrite in media containing peptone or ammonium chlorid, but produce considerable nitrite in an agar containing no nitrogen except potassium nitrate.[1] One strain has been found which does not produce nitrite (nor ammonia) even on the latter medium. This suggests that some strains of *Ps. fluorescens* lack the ability to produce nitrate, wholly or in part, and never attack the nitrate in the presence of more readily available nitrogen. This may explain Franzen and Löhmann's findings (*18*).

The question naturally arises whether those organisms that produce no nitrate in ordinary nitrate media constitute a different species from typical *Ps. fluorescens*. So far as tested, these differences between the strains have proved constant. Nevertheless, the different strains agree in all other particulars investigated, and the data at hand are not considered to warrant the establishment of separate species. As typical *Ps. fluorescens* is generally considered to produce nitrite in nitrate broth the strain selected for Bright's work in the preceding section was one showing considerable nitrite on all the nitrate media investigated.

DIASTATIC ACTION ON STARCH.—This test was made by the method of Allen,[2] streaking the cultures over a plate of agar containing soluble starch, and flooding with iodin after seven days. In general no digestion of the starch was observed, although some of the cultures seemed to show a very narrow zone around the growth where the starch had disappeared.

[1] The formula of this medium was: Distilled water 1,000 cc., agar 15 gm., calcium chlorid ($CaCl_2$) 0.5 gm., potassium phosphate (K_2HPO_4) 0.5 gm., potassium nitrate (KNO_3) 1 gm., dextrose or sucrose 10 gm.

[2] ALLEN, Paul W. A SIMPLE METHOD FOR THE CLASSIFICATION OF BACTERIA AS TO DIASTASE PRODUCTION. *In* Jour. Bact., v. 3, no. 1, p. 15–17, illus. 1918.

ACTION ON MILK.—Digestion without previous coagulation.

PRODUCTION OF INDOL.—Statements in the literature are discordant. A number of different strains of *Ps. fluorescens* have been tested here for indol production, a feeble or moderate reaction having been obtained. The test is not considered to have much significance.

BRIEF SUMMARY OF CHARACTERISTICS OF TYPICAL PSEUDOMONAS FLUORESCENS

In the following summary the characteristics written within parentheses apply to typical cultures only (including the strain studied by Bright); the other characteristics apply not only to typical cultures but to all the cultures studied of the *fluorescens* type—(that is, type 2, p. 337).

MORPHOLOGY: Short, lophotrichic, Gram-negative rods about 0.6 μ in diameter. No spores.

GROWTH ON AGAR: Soft, smooth, with greenish fluorescence if conditions are favorable.

GELATIN COLONIES: (Large), liquefied (center structureless), edges entire.

RELATION TO OXYGEN: Strictly aerobic.

AMMONIA produced from organic nitrogenous matter.

ACID PRODUCTION from dextrose and sucrose, but not from lactose or glycerin.

NITRATES reduced to nitrite (in peptone media containing no nitrogen except the nitrate).

DIASTATIC ACTION ON STARCH: Weak or none.

MILK digested without coagulation.

PSEUDOMONAS CAUDATUS

Pseudomonas caudatus (Wright), (*53, p. 444*) Conn has been recognized by the writer for a number of years and has been mentioned in previous publications (*5, 7–11*), but not named. It is now believed to be identical with *Bacillus caudatus* Wright. Earlier surveys of water bacteria by Frankland (*17*), Tils (*50*), and Zimmerman (*54*) contain descriptions of orange or yellow liquefying bacteria, but they are either meagerly described or else show marked differences from the organism studied here. The identification with Wright's organism is based primarily upon a color plate showing the gelatin colony and upon his description of the morphology. He describes the morphology of the organism as follows (*53, p. 444*):

> A rather small, slender, nonmotile bacillus, with conical ends, occurring often in pairs and in longer forms, sometimes threadlike, which may show irregular segmentation; no spore formation observed.

His illustration of the colony agrees well with the present organism as to structure and agrees as nearly with the shade of orange observed as could be expected in a colored plate 20 years old. Although Wright does not give the size of the organism in exact figures and calls it immotile, there is little question as to its identity.

MORPHOLOGY.—Ordinarily the organism is a very slender rod, so small that its diameter is difficult to measure with the ordinary microscope.

It is about 0.2 μ in diameter. Its length is ordinarily about 2 μ, but, as stated by Wright, longer forms occur. These rods stain solid with the ordinary bacterial stains, such as fuchsin or methylene blue; but with the more delicate dye, rose Bengal, they appear to be made up of tiny granules. Cultures a few days old are sometimes made up wholly of these granules, each about 0.2 μ in diameter. Such a preparation looks like a very tiny micrococcus. Cultures of this sort have proved to be alive upon transfer to fresh media, but whether the granules are capable of growth or whether the multiplication is carried on by a few stray rods present in too small numbers to be observed under the microscope is still an unanswered question. This suggests very strongly Löhnis and Smith's idea (*35*) as to life cycles among bacteria, but as yet it has not proved possible to find whether that is the true explanation of this case. The granules may be degenerate forms, a possibility suggested by the rapidity with which cultures die, or the organism may be actually a coccus that has a tendency to produce short chains or filaments in young cultures.

The majority of the cultures show no motility, although occasionally one is observed that is distinctly motile. This undoubtedly explains why Wright (*53*) called the organism immotile. An idea of the difficulty in studying motility can be obtained from the trouble encountered in demonstrating flagella on the strain used in Bright's work. This strain was kept under observation for a few months without observing any motility, when at last, quite unexpectedly, a distinctly motile culture of it was obtained. On the same day two other strains, previously showing no motility, were found to be motile. No apparent reason could be found for this sudden development of motility, which persisted through at least three or four generations. Meanwhile flagella preparations were made from the strain used by Bright in the work reported above, and one or two organisms were observed with a single flagellum each. This flagellum is rather long in comparison to the length of the rod. This finding agrees with previous studies of this organism made by the writer. Three strains in all have been successfully stained, and about 10 different organisms have been observed with a single polar flagellum each. Preparations were always too poor to allow photomicrographs, but there seems to be sufficient evidence to establish the presence of one polar flagellum. For this reason Wright's name, "*Bacillus caudatus*," is changed to *Pseudomonas caudatus*.

CHROMOGENESIS.—Next to its morphology, pigment production is the most striking characteristic of *Ps. caudatus*. The pigment grades from yellow to orange. On potato and gelatin it is generally distinctly orange, while on beef-extract peptone agar it is more of a yellow. Its color on the latter medium is practically the same as that which is typical of the orange pyogenic cocci, designated cadmium-orange by Winslow and Winslow (*52*) in their book on the Coccaceae. The typical color, indeed, is exactly the shade of cadmium-orange which the Winslows

found most common among the orange cocci. One strain has been found which was typical in color upon isolation from soil, but which lost its chromogenesis upon cultivation, not regaining it even after cultivating for a while in sterilized soil. This strain retained its typical morphology and differed from the other cultures at first in no other respect except that it was unable to digest soluble starch. Later it was found to have lost its power of producing nitrite upon nitrate-peptone media. No data are at hand to show whether or not it digested starch before it lost its pigment-producing power. The change in the color of this culture can hardly have been due to an impurity, because three separate substrains of this one strain all lost their pigment-producing power at exactly the same time. This shows that chromogenesis, striking as it is in typical cultures, is not an absolutely constant characteristic.

PHYSIOLOGY.—Perhaps the most striking physiological peculiarity of the organism is the difficulty of cultivating it under laboratory conditions. The only way found to keep it vigorous is by transfers every few days onto agar that has been freshly melted and solidified so as to have considerable water of condensation on its surface. This fact is unfortunate, for it makes it practically impossible to keep stock cultures of the organism for purposes of comparison with cultures of other investigators.

RELATION TO OXYGEN.—The organism is very strictly aerobic. In fact, it grows poorly in liquid media, even in an open test tube.

LIQUEFACTION OF GELATIN.—All cultures liquefy gelatin. The rapidity of liquefaction varies, although in general it is quite rapid.

Gelatin colonies usually liquefy to a diameter of about 1 cm. in four days. Liquefaction is most rapid on the plates made directly from soil, old cultures liquefying more slowly. The colonies have typically a radiate structure, although the typical structure is observed only immediately after isolation from soil. Edges of colonies are entire.

AMMONIA PRODUCTION.—As shown by Bright in the preceding paper, *Ps. caudatus* is a vigorous ammonifier.

ACTION ON SUGARS AND GLYCERIN.—In the early work with this organism (*Conn, 5, 10*) tests for acid production were made in sugar broth as recommended in the report of the committee of water analysis of the American Public Health Association.[1] Very irregular results were obtained and in mentioning this type (*5, p. 103*) question marks were placed over those figures in the group number referring to the dextrose, sucrose, and glycerin, although at that time no evidence at all of acid production in lactose had been obtained. Later (*10, p. 8*) it was thought that this irregularity must be due to poor growth in liquid media, so the recent tests have been made in sugar agar containing some indicator. The most satisfactory indicator has proved to be bromcresol purple. Using standard agar in this work, the writer divided the strains studied

[1] American Public Health Association. Standards methods for the examination of water and sewage, ed. 2, p. 127–128. New York, 1912.

into two groups, one[1] producing no acid and the other (containing the majority of the strains) producing acid from dextrose and sucrose but not from lactose or glycerin. There proved to be some irregularity upon repetition of the test, but not a great deal. It was then felt that the difference between these two groups of strains might be that one produced more alkalinity from the peptone than the other and that its acid production was thus obscured. The test was therefore repeated a few times on a tartrate agar[2] in which *Ps. caudatus* was found to cause no change in reaction unless some sugar were present. With this medium more consistency was observed upon repetition of the test, but the difference between the two groups was still sharp. The acid group acidified lactose in this medium as well as dextrose and sucrose. It is therefore concluded that typical *Ps. caudatus* produces acid from dextrose, sucrose, and lactose, but not from glycerin, its acid production from lactose being too weak to neutralize the alkalinity produced from the peptone if growing in ordinary media. The nonacid strains, with the exception of the nonchromogenic one, died while under cultivation in the laboratory; so it is felt that their failure to produce acid may have been the first evidence of loss of vigor. Hence, they are not considered to be distinct from the typical acid formers. The strain used by Bright in the experiments reported above was a vigorous acid producer.

NITRATE REDUCTION.—Irregular results were obtained with this test also. Ordinary nitrate broth proved so unsatisfactory that tests were made on agar slants as described for *Ps. fluorescens* (p. 341). On beef-extract peptone agar, the acid group of strains, above mentioned, showed a strong nitrite reaction; the nonacid group. with the exception of the nonchromogenic strain, showed no nitrite; the nonchromogenic strain when first tested was distinctly nitrite-positive, but after a few months all three substrains of this organism were found to have lost their nitrite-producing power. To investigate this matter further, the synthetic sucrose-nitrite agar[3] already used for *Ps. fluorescens* was employed. On this agar an occasional culture was found to produce nitrite that had showed no nitrite reaction on the nitrate-peptone agar, and ammonia was observed in almost all cases. Growth was very poor, however, with the nonacid group of strains. Inasmuch as there was no possible source of ammonia in this medium except the nitrate, the conclusion was drawn that *Ps. caudatus* reduces nitrate to nitrite and ammonia, but that some cultures convert the nitrite into ammonia so rapidly that a nitrite test is generally negative. The presence or absence of the nitrite test depends upon the relative rate of these two processes, which is probably associated with the vigor of the culture. Hence, the failure of the nitrite test is no proof that any particular culture is not *Ps. caudatus*.

[1] One of the strains in this group was the one that had lost its pigment-producing power.

[2] Formula given in footnote, p. 340.

[3] Formula given in footnote, p. 341. Sucrose (not dextrose) was used in this formula because of the presence of ammoniacal impurities in the dextrose on hand.

DIASTATIC ACTION ON STARCH.—This test was made by the method of Allen.[1] All the cultures of *Ps. caudatus* studied, except the nonchromogenic strain, gave a strong reaction, but the nonchromogenic strain showed no digestion of the starch. These results were the same upon frequent repetition of the test.

ACTION ON MILK.—No change in appearance or reaction.

PRODUCTION OF INDOL.—The results of this test have generally been negative, although a few cultures have shown a weak reaction. It is not impossible that they would all produce indol if tested under conditions favorable to the growth of this organism; but the test has always been made in liquid media (Dunham's solution), and as yet no effort has been made to improve the technic.

BRIEF SUMMARY OF CHARACTERISTICS OF TYPICAL PSEUDOMONAS CAUDATUS

In the following summary the characteristics written within parentheses apply to typical cultures only (including the strain studied by Bright); the other characteristics apply to all the strains studied:

MORPHOLOGY: Long, slender, granular, Gram-negative rods, about 0.2 μ in diameter, with a single polar flagellum. No spores. Old cultures often appear like cocci, 0.2 to 0.4 μ in diameter.

GROWTH ON AGAR: Soft, smooth (cadmium orange).

GELATIN COLONIES: Small (to medium sized)—i. e., up to 1 cm. in diameter (orange, structure radiate), edge entire.

RELATION TO OXYGEN: Strictly aerobic.

AMMONIA produced from organic nitrogenous matter.

ACID PRODUCTION: (from dextrose, sucrose, and lactose) but not from glycerin.

NITRATES reduced to nitrate and ammonia (with accumulation of nitrite).

DIASTITIC ACTION ON STARCH: (strong).

MILK unchanged.

SUMMARY

(1) The statement recently made by one of the authors that non-spore-forming bacteria are most active in manured soil has been verified. This is contrary to the generally accepted idea that spore-forming bacteria are the important ammonifiers in soil.

(2) Of these non-spore-forming organisms that are especially active in manured soil, two of the most easily recognized are *Pseudomonas fluorescens* (Flügge) Migula and *Ps. caudatus* (Wright) Conn. They have therefore been selected for special study.

(3) Pure cultures of *Ps. fluorescens* and *Ps. caudatus* multiply much more rapidly in sterilized manured soil than do pure cultures of *Bacillus cereus* Frankland (selected as a typical spore former).

(4) When sterilized manured soil is inoculated with a mixture of these three organisms in pure culture, the two non-spore formers immediately gain the ascendency, *B. cereus* occurring in too small numbers for detection by the ordinary methods of study.

[1] ALLEN, PAUL W. Op. cit., 1918.

(5) In field soil to which there has been no addition of organic matter for several years, *Ps. fluorescens* and *Ps. caudatus* were rarely found, while *B. cereus* was a common organism.

(6) When this same soil was mixed with manure and potted, *Ps. fluorescens* and *Ps. caudatus* immediately multiplied rapidly, while but small numbers of *B. cereus* spores and no active forms of *B. cereus* could be found.

(7) All three of these organisms are vigorous ammonifiers when tested in pure culture.

(8) The activity of the non-spore formers and the absence of activity of the spore formers in unsterilized manured soil leads to the conclusion that *Ps. fluorescens* and *Ps. caudatus* are important ammonifiers of manure in soil, while there is no evidence that *B. cereus* takes part in this process.

(9) Detailed descriptions are given of the two ammonifying organisms studied.

(10) The culture of *Ps. fluorescens* studied has been compared with other fluorescent bacteria isolated from soil, and a review of the literature relating to fluorescent bacteria has been made. It has not proved possible to fix definite limits for this species.

(11) *Ps. caudatus* is the name now assigned to the organism previously denoted by one of the writers as the "orange-liquefying type." It is apparently identical with the organism described by Wright in 1895 (*53*), and seems to be quite common in soil and water.

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Vol. XVI MARCH 31, 1919 No. 13

JOURNAL OF AGRICULTURAL RESEARCH

CONTENTS AND INDEX OF VOLUME XVI

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN
AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON, D. C.

WASHINGTON : GOVERNMENT PRINTING OFFICE : 1919

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