FACTORS INFLUENCING THE PATHOGENICITY OF HELMINTHOSPORIUM SATIVUM

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE
SCHOOL OF THE UNIVERSITY OF MINNESOTA

BY

LOUISE DOSDALL, M.A.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

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By Louise Dosdall[1]

INTRODUCTION AND HISTORICAL REVIEW

In 1910 Pammel, King and Bakke (9) described a new *Helminthosporium* disease of barley which they called "late blight." The causal organism was named *Helminthosporium sativum* n. sp. Pammel, et al., had observed the disease in Iowa in 1907 and 1908. In 1909 it was very serious. In the same year, they report that it was also found in South Dakota, Minnesota, and Saskatchewan. These authors describe the disease as follows: "Brown spots of irregular outline occur upon the leaves causing them to turn brown. The leaves are easily broken up, and in some cases completely destroyed. The disease also occurs upon the glumes, spikelets and seed. The straw at harvest is dull brown, and instead of standing erect becomes a tangled mass. The date of ripening of the grain corresponds with the time of full development of the late blight." They observed that there was considerable difference in varietal susceptibility, the degree of infection ranging from 0 to 100 per cent. Late blight was considered the most serious disease of barley in Iowa.

In 1913 A. G. Johnson (7) differentiated clearly the three *Helminthosporium* diseases of barley in Wisconsin, and he designated the one caused by *H. sativum* P.K.B., the "American blotch disease."

Louise Stakman (11), in 1920, showed that a *Helminthosporium* similar to the organism described as *H. sativum* by Pammel, King and Bakke, but isolated from various parts of diseased wheat and rye plants, was capable of causing a serious seedling blight of these hosts, and could also attack the older parts of the plants, namely, the leaves, nodes, culms, roots, glumes, and grains. In addition to wheat and rye, successful infections were obtained on barley and a number of grasses. In the spring and early summer of 1919, serious attacks of seedling blight caused by *Helminthosporium* occurred in practically all the wheat-growing regions of Minnesota.

F. L. Stevens (12), also in 1920, reported that a species of *Helminthosporium* was constantly associated with foot rot disease of wheat in Madison County, Ill. Inoculations with the organism gave positive results. He concluded that *Helminthosporium* was the cause of the disease.

[1] The writer wishes to express her appreciation to Dr. E. C. Stakman, under whom the work was done, for advice and criticism, and to Mr. M. N. Levine for his helpful criticism in the presentation of the biometrical studies.

In January, 1922, Hamblin (5) reported a *Helminthosporium* foot-rot disease of wheat in New South Wales, Australia. The disease symptoms are very similar to those of the true take-all caused by *Ophiobolus graminis* Saccardo, but there are distinguishing characters. Hamblin's description of the foot-rot in Australia corresponds very closely with that of Mrs. Stakman and of F. L. Stevens. His description of the poorly developed root system with an abnormal development of root hairs close to the culm, giving the dead or dying root a "fuzzy" appearance, and the frequent growth of secondary roots above the first node of the affected straws, applies equally well to conditions observed in Minnesota during 1921. In Hamblin's opinion, the *Helminthosporium* disease was responsible for far more damage in 1921 in Australia than was the better known take-all.

In recent years, a foot-rot disease of cereals, particularly wheat, rye, and barley, has been destructive in certain localities in Minnesota. This was especially true on certain peat lands in Anoka and St. Louis counties and on some of the sandy soils in Anoka, Nicollet, and Mahnomen counties. A *Helminthosporium* of the *sativum* type has been consistently isolated from the diseased plants. This organism is very widely distributed throughout the cereal growing region. The severity of its attack apparently must be greatly influenced by ecological conditions. In order to obtain more detailed and accurate information concerning these conditions, a study of the physiology of the fungus, to the extent of its possible correlation with the pathogenicity under given conditions, was undertaken.

PROBLEM

In this study attention was directed primarily to the root- and foot-rots caused by *H sativum*. Little attention was given to secondary infections on leaves and heads. The soil environment was, therefore, of chief concern. In analyzing the factors which might influence the development of a disease of this type, temperature, moisture, and acidity would affect the growth of both the pathogene and the host, and possibly also the reaction between the two. The vigor of the host conceivably might greatly influence the development of a disease caused by a facultative parasite, such as *H. sativum*. The type of soil in which they grew and the available nutriment might, therefore, change the balance between host and pathogene. It is difficult to separate and analyze the individual factors, because certain combinations introduce various complexities which are difficult to interpret.

The following phases of the problem were investigated especially:

1. Relation of temperature to the growth of the fungus, to spore germination, to infection, and to the development of the disease.

2. Relation of hydrogen-ion concentration and temperature to spore germination.
3. Development of the disease in various types of soil.
4. Influence of soil moisture on the development of the disease.
5. Influence of soil fertilization on the development of the disease.
6. Comparison of several root-rotting organisms.
7. Morphological variation in the fungus with regard to its specific identity.

METHODS

SOURCE OF PATHOGENE

During the spring and summer of 1920, tissue cultures were made from lesions caused by *Helminthosporium* on cereals and grasses. Twenty-two strains (isolations from various parts of different hosts or from different localities) of the *sativum* type were obtained from the roots, stems, nodes, leaves, and kernels of barley; from the roots, stems, leaves, and kernels of wheat; and from leaf spots of various grasses. Material was obtained from Anoka, Clay, Mahnomen, Nicollet, Ramsey, and St. Louis counties in Minnesota; from Tennessee, and from Spruce Grove and Edmonton, Alberta.

Seven of these strains were selected for preliminary inoculation experiments. As a virulent root-rotting organism was desired, only soil inoculations were made. Four-inch pots filled with soil were treated with live steam for two hours on each of three successive days. Six pots of such soil were inoculated with each of the various strains of *Helminthosporum*. For this purpose, spores were scraped from the surface of potato dextrose agar cultures and mixed with water. The suspension of spores was poured over the soil, and the pots were incubated for several days. Three pots which had been inoculated with each strain were then sowed with Marquis wheat and three with Manchuria barley (Minn. 105). Some infection was obtained in each case, on both the leaves and the roots. (The check plants were slightly infected, as the seed had not been treated.) The plants inoculated with strain 82a, however, were decidedly more heavily attacked than the others. This was especially true of the barley plants. A *Helminthosporium* of the *sativum* type was re-isolated from lesions on both the barley and the wheat. A single spore culture was then made from the original 82a culture, and all subsequent work was done with this single spore strain.

Culture 82a was originally isolated from the darkened base of badly stunted barley plants sent to the laboratory from the peat plots on the Fens experimental field, St. Louis County, Minn., in the summer

of 1920. A similar *Helminthosporium* was isolated from the nodes, sheaths, and blades of the same plants.

In addition to *H. sativum, Alternaria* was frequently obtained from blackened kernels and nodes; a pink *Fusarium* was sometimes found on the base of the stem and roots; and *Helminthosporium teres* Sacc. was occasionally isolated from the leaves and stems.

SELECTION OF HOST VARIETIES

In all experiments, the effect of the fungus on wheat and barley only was studied. In most cases where barley was tested, both Manchuria (Minn. 105) and Lion (Selection) were used. Manchuria is the barley most commonly grown in Minnesota. It is somewhat resistant to *H. sativum,* as shown by the work of Pammel, King and Bakke (9), of Hayes and Stakman (6), and of Christensen (3). For this reason it was used in the breeding work of Hayes and Stakman. It was crossed with the smooth-awned Lion, which is very susceptible to *Helminthosporium,* in an attempt to obtain a high yielding, smooth-awned, resistant variety.

Marquis (Minn. 1239) was used in most of the experiments with wheat. In some cases Haynes Bluestem (Minn. 169) also was used.

CHECK PLANTS

Since it is difficult to obtain seed entirely free from *Helminthosporium,* especially in susceptible varieties, it was necessary to treat the seed in order to reduce infection in the check plants to a minimum. Silver nitrate was found to be the most useful disinfectant because the seed coats of both barley and wheat are impermeable to it (10), and the seed can be soaked for a long time in the solution without being injured. It also is more effective, especially against *Helminthosporium,* than mercuric bichloride. For experimental purposes, the method of seed treatment followed was essentially that recommended by Schroeder (10). The seed was dipped in 50 per cent alcohol to remove the air from the surface, soaked over night in N/100 silver nitrate solution, dipped in a dilute sodium chloride solution to precipitate as insoluble silver chloride the silver nitrate remaining on the surface of the seed, washed thoroly in running tap water, and dried. Such treatment reduced the germination of Lion barley from 90 per cent to 78 per cent, and of Marquis wheat from 99 per cent to 97 per cent.

SPECIFIC IDENTITY OF THE PATHOGENE

Three species of *Helminthosporium* are known to occur on barley in the United States. These are readily distinguished on the host by the symptoms. *H. gramineum* Rabh. causes the systemic stripe disease characterized by long, narrow, yellowish to brownish spots on the leaves and sheaths. Many spots often coalesce to form parallel striations which run more or less the entire length of the blade and often down the sheath. Eventually the leaves may be reduced to shreds. *H. teres*. Sacc. and *H. sativum* P.K.B. both cause local lesions which are characterized by peculiar blotches on the leaves. *H. teres* causes the European blotch or net blotch disease. The spots are yellowish brown in color, irregular in shape, and are scattered on the leaves. When held to the light, a characteristic net work is apparent. *H. sativum* causes the spot blotch disease characterized by irregular reddish brown spots on the leaves. The spots are usually longer than they are broad, and, when abundant, may tend to form stripes.

These three species also may be distinguished readily by their growth on potato dextrose agar. *H. gramineum* grows slowly, forms a fluffy, aerial mycelium which does not sporulate (at least not readily), and usually gives the medium a reddish or purplish tinge. *H. teres* also grows rather slowly. The mycelium grows very close to the surface of the agar. The color of the reverse side of the colony is greenish black. Grayish white tufts of mycelium are formed irregularly on the surface of the colony. Cylindrical, thin-walled spores are formed, but usually they are not abundant. In contrast to both these species, *H. sativum* grows very readily and sporulates abundantly, forming a flat, black or greenish black colony on agar. The abundance of conidia gives the surface a powdery appearance. Organisms similar to the one isolated from typical barley spot blotch have been isolated hundreds of times by workers in this laboratory from various parts of barley, wheat, and rye plants, and from numerous grasses.

Pammel, King and Bakke (9) described the spores as cylindric in shape, straight or curved, slender, widest at the middle, from 105 to 130 microns in length by 15 to 20 microns in width, pale greenish gray to dark brown in color, with 7 to 14 cells. Later workers have found much shorter spores, altho observations on shape agree fairly well. Johnson (7) states that the spores are narrowly spindle-shaped, usually more or less curved. Mrs. Stakman (11) describes the spores of the organism with which she worked as either straight or curved, dark blue-green to brown in color, averaging 41 by 20 microns in size, and containing from 3 to 8 septa. Two types were isolated from diseased wheat: one a fuscous type measuring 35 by 22 microns and containing from 3 to 4 septa; the other straw-colored to fuscous,

measuring 60 by 20 microns, and containing from 4 to 7 septa. Both of the latter are described as elliptical in shape.

Stevens (12) makes the following statement regarding the form causing the foot-rot of wheat: "The spores, observed as grown on autoclaved wheat leaves or stems in humid air, are from 24 to 122 microns long, the majority of them falling within the limits 80 to 90 microns, with septa or pseudo-septa varying from 0 to 13, usually 5 to 10. The spores are usually typically thickest in the region about midway between the base and the middle point of the spore, approaching a narrow or broadly elliptical shape, tapering somewhat toward each end. They possess an outer dark wall that is thin and extremely fragile and an inner, colorless, thick wall that is frequently soft and gelatinous . . . The spores usually, perhaps always, germinate either from one or both ends, not laterally, and are functionally only one-celled."

After making a large number of isolations from *Helminthosporium* lesions on barley, wheat, and rye, great variations were found in the size of the spores of the various cultures, altho they resembled each other more or less in shape and color. In order to find out just what variations might be expected in one strain, as a guide to the interpretation of the species, a single spore was again isolated from culture 82a and a biometric study was made of the spores produced under various conditions.

The single spore was planted on a potato dextrose agar slant and incubated at 24° C. for ten days. Transfers were then made to potato dextrose agar and to ripe autoclaved barley heads. Agar cultures were incubated at the following temperatures: 5°, 14°, 18°, 24°, 28°, 32°, and 36° C. The cultures grown at 5° and 36° did not produce spores. The barley head cultures were incubated at 24° C. Fresh barley leaves were taken from the greenhouse, placed in moist chambers, inoculated with spores of the same culture, and incubated at 24°. The length of time required for the cultures to sporulate at the different temperatures varied considerably; those at 24°, 28°, and 32° were ready for measurement in 16 days, while those at 14° required 37 days.

TABLE I

VARIATIONS AND CONSTANTS FOR LENGTH OF SPORES OF *Helminthosporium sativum* OBTAINED FROM MEASURING POPULATIONS OF DIFFERENT SIZE

Conditions under which spores were produced		Distribution in classes (microns)												No. of spores measured	Mode	Mean	Standard deviation	Coefficient of variability
Temperature, degrees C.	Medium	10	20	30	40	50	60	70	80	90	100	110	120					
32	Potato dextrose			3	7	14	32	29	14	1				100	60	62.30 ±0.85	12.56 ±0.60	20.16 ±1.18
32	Potato dextrose	1	13	8	34	52	92	77	22	1				300	60	57.44 ±0.57	14.69 ±0.40	25.57 ±0.75
32	Potato dextrose	1	16	15	48	73	149	136	57	4	1			500	60	59.66 ±0.45	14.81 ±0.31	24.82 ±0.56
32	Potato dextrose	3	29	25	94	191	281	268	103	6				1000	60	59.07 ±0.31	14.34 ±0.22	24.27 ±0.39

Spores from these various sources were then measured for length. In all cases, measurements were made with a Bausch and Lomb microscope, using the 4 mm. objective and an eyepiece micrometer calibrated so that one space was equal to 3.4 microns. It was observed that at an extreme temperature, such as 32° C., there was a great deal of variation in length and a large number of measurements would be required to obtain a normal curve. Data were therefore recorded for the measurements of 100, 300, 500, and 1000 spores. From these data the mean was calculated for each group and the differences were compared in relation to the probable errors, according to the methods given by Babcock and Clausen (2). These data are summarized in Tables I and II. For 100 spores the mean was found to be 62.30 ± 0.85 microns; for 300 spores 57.44 ± 0.57 microns; for 500 spores 59.66 ± 0.45 microns; and for 1000 spores 59.07 ± 0.31 microns. Their accuracy can be seen by comparing these results with their probable errors. In Table II the comparison of the means for 100 and 300 spores with the means of each of the other three groups, shows that the difference between any two is from 3 to 5 times the probable errors of the difference. This borders on the verge of a significant difference, so that 100 or perhaps even 300 spores are scarcely enough to use as a basis for drawing conclusions. When the mean for 500 spores is compared with that for 1000, the ratio is 1:1. The results obtained by measuring 1000 spores are only very slightly more accurate than those obtained by measuring 500 spores. The difference is certainly not great enough to necessitate the measurement of the second 500 spores.

TABLE II

SUMMARY OF COMPARISONS BETWEEN MEANS AND COEFFICIENTS OF VARIABILITY FOR LENGTH OF SPORES OF *Helminthosporium sativum* OBTAINED FROM MEASURING POPULATIONS OF DIFFERENT SIZE (FROM DATA SUMMARIZED IN TABLE I)

Conditions compared	Means		Coefficients of variability	
	Difference	Difference divided by the probable error of the difference	Difference	Difference divided by the probable error of the difference
No. of spores				
100 and 300	4.86 ± 1.02	5	5.41 ± 1.73	3
100 and 500	2.64 ± 0.96	3	4.66 ± 1.31	4
100 and 1000	3.23 ± 0.91	4	4.11 ± 1.24	3
300 and 500	2.22 ± 0.72	3	0.75 ± 0.93	1
300 and 1000	1.63 ± 0.65	3	1.30 ± 0.85	2
500 and 1000	0.59 ± 0.55	1	0.55 ± 0.68	1

These comparisons are perhaps brought out more clearly by the curves in Figure 1, in which the data obtained from measuring the different lots of spores have been plotted after grouping the measurements into 10 micron classes. The lowest curve, representing 100 spores, very clearly does not give a true index of the lower extreme of the total population. This explains why the mean obtained from 100 spores is too high. The three succeeding curves show that, as the number of individuals increases, the curve gradually approaches

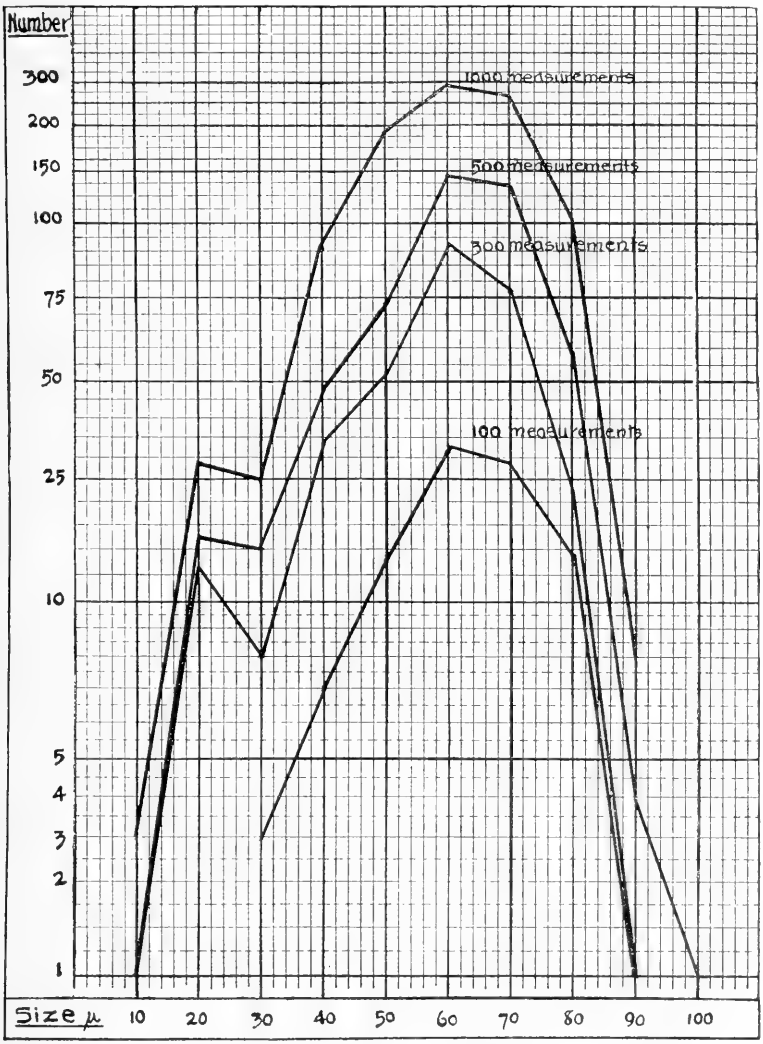

Fig. 1. Types of Curves Obtained from Measuring the Length of 100, 300, 500, and 1000 Spores of *Helminthosporium sativum* Produced on Potato Dextrose Agar at 32° C.

a normal one. In general contour the 300-spore curve and the 500-spore curve approach the 1000-spore curve, altho the first is somewhat more irregular. The slight rise at the lower extreme indicates that the short spores tend to group themselves about a mode of their own. It is possible that improvement in the method of sampling might increase the accuracy of the results obtained from a smaller population. In the present study about 100 spores were measured from one mount. The spores were distributed as evenly as possible in the drop of water and each spore was measured in passing systematically over the slide from the upper left to the lower right hand corner. An attempt was made to make the mount so that two or three spores would come into the field at once. For all other conditions, 500 spores were measured.

Results obtained in the study of the morphology of spores developed on potato dextrose agar at various temperatures are interesting. Table III shows very little difference in the means of spores developed at 18° and 24°. From the comparisons in Table IV it is seen that these differences are insignificant. If, however, we examine the coefficients of variability, we find that there is a significant difference in the amount of relative variation in the length of spores. This fact is very clearly brought out in the curves in Figure 2. The degree of variation is not increased by a temperature 4 degrees lower (14° C.), but the mean length of the spores is slightly increased. This may be due to the fact that at a lower temperature the black outer wall on the spores and mycelium is laid down much more slowly, so that the spores have a longer time in which to form. This is further substantiated by the fact that at 32° the spores are very much shorter. The amount of relative variation is practically the same as at the lower extreme. These differences in length of spores produced at various temperatures are graphically represented by the curves in Figure 2.

The most striking difference in spore morphology was obtained by comparing the spores produced on different media. As the fresh leaf and the autoclaved head cultures were incubated at 24° C, we may compare these results with those obtained from the agar culture at 24° C. Comparing first the spores from the head and from the agar, we find that the former are slightly longer. The amount of relative variation in the two is practically the same. On the fresh leaves, however, the spores are very much longer and decidedly more uniform. These differences are illustrated in the curves in Figure 3.

TABLE III

VARIATIONS AND CONSTANTS FOR LENGTH OF SPORES OF *Helminthosporium sativum* PRODUCED UNDER DIFFERENT CONDITIONS

Conditions under which spores were produced		Distribution in classes (microns)												No. of spores measured	Extremes	Mode	Mean	Standard deviation	Coefficient of variability
Temperature, degrees C.	Medium	10	20	30	40	50	60	70	80	90	100	110	120						
14	Potato dextrose		4	25	36	48	85	138	99	38	10	15	2	500	24.5–119.0	70	67.32 ±0.55	18.41 ±0.39	27.30 ±0.62
18	Potato dextrose	1	21	22	22	38	93	147	131	22	2	1		500	14.0–105.0	70	65.02 ±0.52	17.23 ±0.37	26.50 ±0.60
24	Potato dextrose	1	4	8	17	56	107	217	88	8				500	14.0 87.5	70	65.75 ±0.37	12.28 ±0.26	18.68 ±0.41
28	Potato dextrose		11	14	48	127	197	98	5					500	17.5– 80.5	60	55.98 ±0.35	11.47 ±0.24	20.49 ±0.46
32	Potato dextrose	1	16	15	48	73	149	136	57	4	1			500	14.0– 98.0	60	59.66 ±0.45	14.81 ±0.31	24.82 ±0.56
24	Sterile barley head	1	5	7	14	33	100	203	122	13	2			500	14.0– 98.0	70	67.74 ±0.38	12.63 ±0.27	18.64 ±0.40
24	Fresh barley leaves					3	8	89	117	180	39	3	1	500	49.0 115.5	90	83.14 ±0.29	9.77 ±0.21	11.15 ±0.24

TABLE IV

SUMMARY OF COMPARISONS BETWEEN MEANS AND COEFFICIENTS OF VARIABILITY FOR LENGTH OF SPORES OF *Helminthosporium sativum* PRODUCED UNDER DIFFERENT CONDITIONS FROM DATA SUMMARIZED IN TABLE III.

Conditions compared	Means		Coefficients of variability	
	Difference	Difference divided by the probable error of the difference	Difference	Difference divided by the probable error of the difference
14° C. and 18° C.	2.30±0.75	3	0.80±0.86	1
14° C. and 24° C.	1.57±0.66	2	8.62±0.74	12
14° C. and 28° C.	11.34±0.65	17	6.81±0.77	9
14° C. and 32° C.	7.66±0.71	11	2.48±0.83	3
18° C. and 24° C.	0.73±0.64	1	7.82±0.73	11
18° C. and 28° C.	9.04±0.63	14	6.01±0.76	8
18° C. and 32° C.	5.36±0.68	8	1.68±0.82	2
24° C. and 28° C.	9.77±0.51	19	1.81±0.62	3
24° C. and 32° C.	6.09±0.58	11	6.14±0.69	9
28° C. and 32° C	6.32±0.56	11	7.41±0.73	10
Head and leaves	15.40±0.47	33	7.49±0.47	16
Head and agar 24° C.	1.99±0.53	4	0.04±0.57	0
Leaves and agar 24° C.	17.39±0.47	37	7.53±0.48	16
Leaves and agar 28° C.	27.16±0.45	60	9.34±0.52	18

These facts show that in a single spore strain of a *Helminthosporium* of this type, marked variations may be found in the length of spores developed under various conditions. Differences in spore measurements by various authors are therefore to be expected, and very fine specific or varietal differences can not be drawn on the basis of spore size unless a large number of carefully controlled comparative studies have been made. Seemingly, the original spore measurements given by Pammel, King and Bakke are rather large (105 to 130 microns). Stevens has come nearest to approaching this length with a maximum of 122 microns. The same author states that the majority of his spores fall within 80 to 90 microns. By examining Table III it will be seen that the majority of the spores developed upon fresh barley leaves in a moist atmosphere fall within the 80 and 90 classes, or within 75.0 to 94.9 microns. Out of 4000 spores measured, only 43 were longer than 100 microns. On the fresh leaves the longest spore measured was only 115.5 microns. However, the optimum conditions for maximum and minimum spore length have not

necessarily been obtained in these studies. On the basis of spore shape and similarity with the organism obtained from typical spot blotch lesions and the ability to produce spot blotch symptoms on barley, the organism undoubtedly should be included in the species *Helminthosporium sativum* P.K.B.

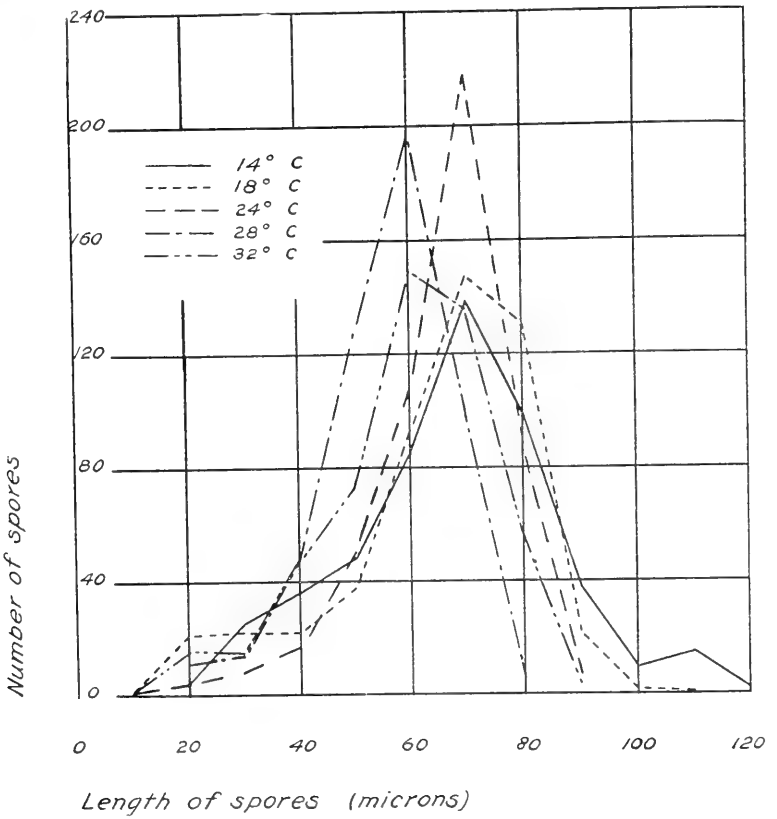

Fig. 2. Length of Spores of *Helminthosporium sativum* Produced on Potato Dextrose Agar at Various Temperatures

The shape of the spores was found to be more or less the same under various conditions. At 24° and 28°, the spores tended to be fat, spindle-shaped to broadly elliptical, sometimes slightly curved. At 32° the thickening in the middle was less evident, and they tended to be more uniform in diameter. The small spores were globose to ovate. At 14° the longest spores were mostly narrowly cylindrical. There was a marked tendency for the thickened portion to occur nearer the base than the apex, giving the spore the shape of a slender flask.

Throughout the culture work, bizarre forms frequently appeared, especially forked spores which were sometimes almost stellate. Seven or eight such single spores were isolated and planted on agar slants. In each case normal spores were produced and the bizarre type occurred so rarely that it was quite impossible to isolate another single spore of the same shape from the progeny.

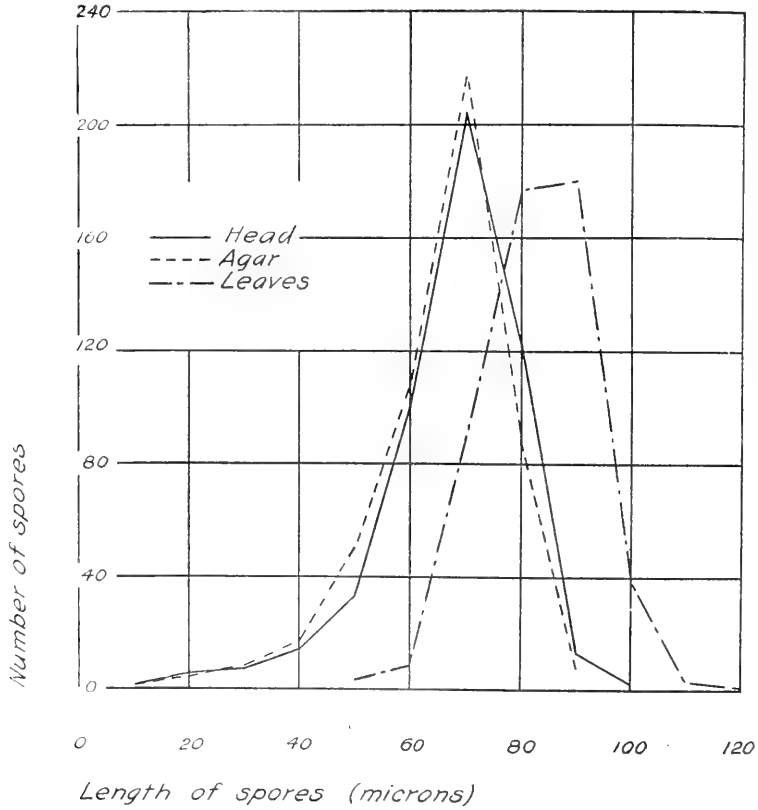

Fig. 3. Length of Spores of *Helminthosporium sativum* Produced on Different Substrata at 24° C.

The solid line represents spores produced on autoclaved ripe barley heads; the line with short dashes, spores produced on potato dextrose agar; the line with alternate long and short dashes, spores produced on fresh barley leaves in a moist chamber.

TEMPERATURE RELATIONS

GROWTH OF FUNGUS ON POTATO DEXTROSE AGAR

In determining the temperature relations of *H. sativum*, the first problem studied was the growth of the fungus in pure culture. In all, four series were run to determine the range of growth, the same general method being used in each. Thirty cubic centimeters of potato dextrose agar were poured into petri dishes 10 centimeters in diameter.

The plates were inoculated in the center and incubated at the various temperatures. Each series was run in triplicate. The diameter of the colony was taken as the index of growth. In some cases it was impossible to control the temperature within several degrees, so that one series can not be checked quantitatively against the other. Different lots of potato dextrose also were used in the different series. The results of two series are given in Table V. In each case, the size of the colony represents the average of three plates.

TABLE V

EFFECT OF TEMPERATURE ON GROWTH OF *Helminthosporium sativum* on Potato Dextrose Agar

First Series (Feb. 28–March 3, 1921)		Second Series (Dec. 15–22, 1921)	
Temperature, degrees C.	Diameter of colony (after 9 days)	Temperature, degrees C.	Diameter of colony (after 7 days)
	mm.		mm.
0– 2	4	3– 6	9
6– 8	14	12–13	18
13–15	28	15–18	30
17–22	42	20–23	77
21–24	51	27–28	89
30–32	36	31–33	35
34–35	13	35–39	4
40–42	0	.....	..

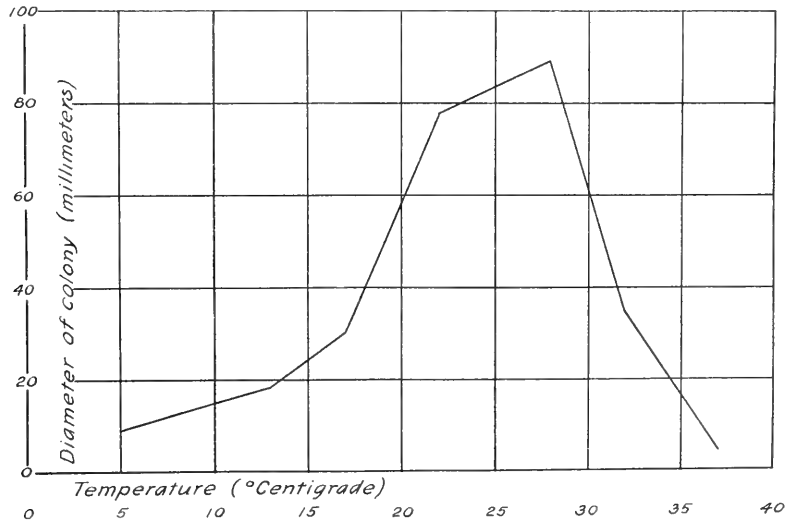

Fig. 4. Growth of *Helminthosporium sativum* on Potato Dextrose Agar in Petri Dishes The curve represents the diameter of the colonies at the end of seven days.

Data obtained in the second series are shown graphically in the curves of Figures 4 and 5. Figure 4 shows the relative growth of the fungus at the various temperatures after seven days. Figure 5

shows the daily rate of growth at each of the temperatures tested. Plate I shows the final appearance of the colonies in the first series, Plate II in the second.

From these results we may conclude that the minimum temperature for the growth of *Helminthosporium sativum* lies near 0-2° C., the maximum temperature between 35° and 39° C. and the optimum between 24° and 28° C.

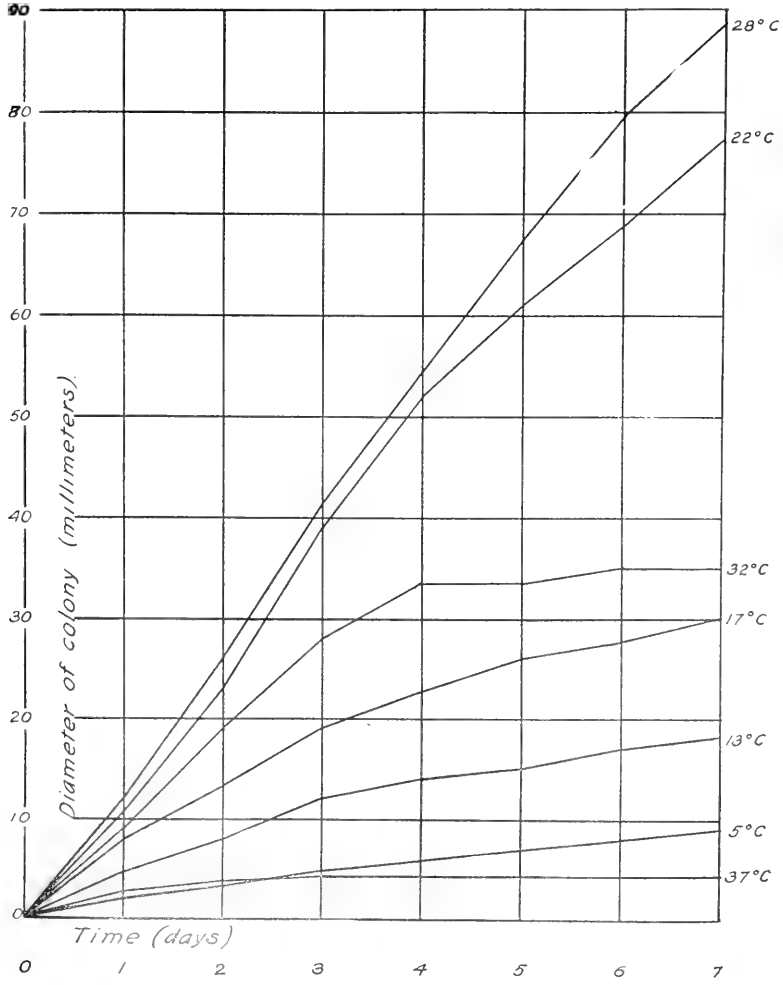

Fig. 5. Daily Rate of Growth of *Helminthosporium sativum* on Potato Dextrose Agar in Petri Dishes

SPORE GERMINATION

In the first series of studies to determine the effect of temperature on spore germination, hanging drop cultures were made on the covers of petri dishes, using distilled water and Czapek's solution, minus the sugar, as media. The spores were taken from a six-days-old bean agar culture. Germination counts were made after 48 hours. The results are given in Table VI.

TABLE VI

SPORE GERMINATION OF *H. sativum* IN DISTILLED WATER AND IN CZAPEK'S SOLUTION AT VARIOUS TEMPERATURES

Temperature, degrees C.	Distilled water PH 6.7					Czapek's solution—sugar PH 6.0				
	1st drop	2nd drop	3rd drop	4th drop	Av.	1st drop	2nd drop	3rd drop	4th drop	Av.
	%	%	%	%	%	%	%	%	%	%
1	1—	1—	1—	1—	1—	20	10	15	10	14
6	10	8	20		13	30	50	40	50	42
14	10	20			15	55	50	62	40	52
18	40	40	30		37	66		50	60	59
24	68	41	44		58	85	86	75	75	80
30	45	65	48	41	50	77	70	59	80	72
34	50	60			55	36	50	25	42	38
42	0	0	0	0	0	0	0	0	0	0

With the exception of the results obtained at 34° C., a higher percentage of germination was obtained in Czapek's solution than in distilled water. In both cases the optimum occurred at 24°. At a temperature of 1° C. 14 per cent of the spores germinated in Czapek's solution, while less than 1 per cent germinated in distilled water. At 42° no germination occurred in either case, while at 34° the germination in distilled water was practically the same as at 30°, but in Czapek's solution a marked inhibition occurred at the higher temperature.

After trying various methods for germinating spores, including hanging drops in petri dishes, in van Tieghem cells, films on slides in moist chambers and in moist atmosphere, the most satisfactory method proved to be floating the spores on the surface of a thin layer of a liquid medium in Syracuse watch glasses. In such cultures the spores can be counted directly on the surface under the low power of the microscope.

In several series, through the different temperature ranges, consistently high percentages of germination were obtained at the extreme temperatures when water which had been redistilled over glass was

used. Fluctuations occurred in the different cups at any one temperature. The results given in Table VII are typical. The percentages given in the table represent the average of several counts.

TABLE VII

SPORE GERMINATION OF *H. sativum* IN REDISTILLED WATER AT VARIOUS TEMPERATURES

Temperature, degrees C.	Average percentage of germination	
	Spores floating on surface of water	Spores lying on bottom of cup
5.5– 6.5	87	76
11.5–12.0	93	89
17.0–19.0	73	57
21.0–24.0	62	61
28.0–29.5	70	66
30.5–32.0	83	46
34.0–35.0	65	63
38.0–39.0		65

In a second series, using hanging drops in petri dishes, 67 per cent of the spores germinated at 6°, 54 per cent at 12°, 79 per cent at 18°, 91 per cent at 22°, 72 per cent at 28°, 91 per cent at 29-30°, 80 per cent at 32°, 82 per cent at 35°, and 87 per cent at 39°.

In a third and fourth series in watch crystals, the spores were not counted but the germination was indicated as poor, moderate, and good. After 24 hours incubation, in these series, the germination was poor at 6°, moderate to good at 12°, 18°, and 22°, and good at the higher temperatures. By count, 89 per cent of the spores germinated at 39°. At the end of 48 hours the germination was good at 6°.

From these results with redistilled water it is difficult to detect any quantitative effect of temperature on the number of spores which germinate. Even at 1° C. a small number of spores will germinate. This, however, is probably very near the lower limit. At the lower temperatures, 1°, 6°, and 12°, pieces of mycelium in the cultures always germinated much more readily and sent out longer tubes than did the spores. At 40-42° no germination occurred in the first series for which the results are given. In later series, however, high germination sometimes occurred at 38-39°. Comparing these results with the data presented in Table V, we find 35-39° to be the maximum temperature for the growth of the mycelium on potato dextrose. At temperatures as high as 35° and 39° the germ tubes appeared very quickly, but were always short and did not increase much in length after two or three days. On the other hand, at 22°, 28°, and 32°,

the tubes formed such a mat of mycelium by the end of 24 hours that it was often difficult to determine the percentage of germination. At the lower temperatures a longer time was required for the germ tubes to appear, and they increased in length very slowly.

In redistilled water, therefore, spores of *H. sativum* germinate about equally well at temperatures from 6° to 39° C. No very definite optimum temperature for germination is apparent. The character of the germ tubes and the length of time in which they appear, however, would indicate that an optimum temperature lies between 22° C. and 32° C. From these results it would seem that for the above-ground parts of the host, temperature is not a limiting factor in infection so far as spore germination is concerned.

From the data in Table VII it will be seen that in most cases the percentage of germination of the submerged spores is only slightly less than that of those on the surface. In all cases, however, the germ tubes produced under water were very short and abnormally branched in comparison with the long straight tubes produced on the surface.

The germ tube first appears as a hyaline tip at the apex of the spore. It is difficult to determine whether the tube breaks through the wall or emerges through a pore. After the tube has increased in size, the delicate exospore is split, sometimes for a third of the length of the spore. A second tube soon appears at the base of the spore, just to one side of the scar where the spore was attached to the sporophore. The connection between the two tubes is continuous through the spore, showing the false nature of the septation in the endospore. The endospore is frequently drawn away from the exospore and forms a constricted tube through the latter. Two germ tubes are not always formed from each spore. In one lot of spores germinated in redistilled water at 22° C., it was found that 52 per cent of the spores possessed germ tubes at both ends and 39 per cent at only one end. Nine per cent of the spores did not germinate. Very rarely lateral tubes are found. One spore was observed with a lateral germ tube from each of five adjacent cells at one end of a ten-celled spore. In a few other cases, one or two lateral tubes were observed, usually arising from central cells. Fusions between germ tubes are very common.

EFFECT OF HYDROGEN-ION CONCENTRATION AND TEMPERATURE ON SPORE GERMINATION

As so many spores always germinated in redistilled water at the various temperatures which permitted germination at all, the effect of hydrogen-ion concentration on germination was studied. Culture solutions, based on Clark and Lub's (4) titration curve for ortho-phosphoric acid, were made by adding varying quantities of n/5 KOH to 50 cc.

n/5 H_3PO_4 to give a series of hydrogen-ion concentrations ranging from pH 2.4 to pH 12. The pH value of the solutions was determined colorimetrically, except for the highest three alkaline solutions for which the theoretical value according to the curve is given. Spores from a seven-weeks-old barley head culture were dusted over the surface of the solutions in Syracuse watch glasses. The percentage of germination was determined after 18 hours and after 36 hours. Similar series were run in triplicate at 19°, 24°, and 32° C. The results are given in Table VIII.

TABLE VIII

SPORE GERMINATION OF *H. sativum* IN H_3PO_4—KOH SOLUTIONS OF VARIOUS HYDROGEN-ION CONCENTRATIONS AT DIFFERENT TEMPERATURES

pH	Hours	Germination											
		19° C.				24° C.				32° C.			
		1	2	3	Av.	1	2	3	Av.	1	2	3	Av.
		%	%	%	%	%	%	%	%	%	%	%	%
2.4	18	0	0	0	0	0	0	0	0	0	0	0	0
	36	3	4	1	3	0	0	0	0	0	2	0	1
3.4	18	2	0	4	2	5	6	0	4	8	10	10	9
	36	2	4	7	4	4	0	2	2	10	13	7	10
4.4	18	15	17	14	15	40	36	28	35	44	44	28	39
	36	22	19	14	18	41	33	16	30	31	53	27	28
5.2	18	27	28	25	27	55	38	46	46	40	42	54	44
	36	28	33	25	29	52	39	52	48	44	41	52	44
6.4	18	31	25	40	33	50	59	57	55	47	76	52	44
	36	32	33	34	33	56	64	62	61	41	66	55	54
7.0	18	40	42	60	47	71	71	79	74	86	76	72	76
	36	36	45	53	45	68	65	84	72	65	74	60	66
7.4	18	30	39	68	46	81	75	72	76	78	80	84	81
	36	34	60	67	54	85	83	73	80	72	83	87	81
7.8	18	55	35	36	42	83	78	80	80	88	90	94	91
	36	30	34	35	33	83	90	90	88	97	94	92	94
8.0	18	35	24	29	29	72	62	68	67	72	65	74	70
	36	35	22	38	32	76	68	82	75	75	79	78	77
8.2	18	21	18	18	19	60	43	50	51	70	80	90	80
	36	19	24	19	21	72	61	65	66	75	85	84	81
9.2	18	25	40	34	33	62	58	63	61	87	82	87	84
	36	36	47	35	39	85	70	60	72	89	90	95	91
11.4*	18	22	22	20	21	33	40	32	35	84	83	82	83
	36	40	35	36	37	73	74	82	76	86	93	90	89
11.8*	18	12	18	12	14	0	0	8	3	40	26	39	35
	36	26	34	37	32	10	0	20	10	30	69	48	39
12.0*	18	0	0	1	0	0	0	0	0	0	1	0	0
	36	2	14	1	6	0	22	0	7	9	35	0	15

* Theoretical value according to Clark and Lub's titration curve for ortho-phosphoric acid.

After an incubation of 18 hours at 19° C. no germination was obtained at a hydrogen-ion concentration of pH 2.4. Very slight germination occurred at pH 3.4; while at pH 4.4 the germination showed a marked increase, rising steadily until a hydrogen-ion concentration of pH 7 was reached. From this point a gradual decrease occurred, reaching the lowest point at pH 8.2. At pH 9.2 there was a second rise followed by a gradual falling off, until at pH 12 no germination occurred. After incubating for 18 hours longer there was scarcely any change in the amount of germination on the acid side. There was a slight increase on the alkaline side.

At the higher temperatures the results were very much the same except that the percentage of germination was increased and the point of maximum germination was shifted slightly to the alkaline side. At both 24° and 32° the optimum germination occurred on the alkaline side of neutrality at a hydrogen-ion concentration of pH 7.8. A much greater increase in germination occurred at the higher temperatures on the alkaline side than on the acid. The average germination after 18 hours incubation at the different temperatures is represented by the curves in Figure 6.

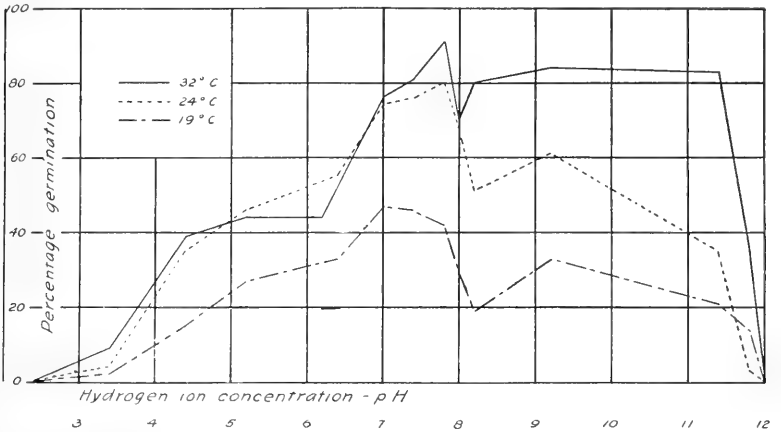

Fig. 6. Percentage Germination of Spores of *Helminthosporium sativum* in Phosphoric Acid—Potassium Hydroxide Solutions of Various Hydrogen-Ion Concentrations

Webb (14) germinated spores of *Aspergillus niger, Penicillium cyclopium, Fusarium sp., Botrytis cinerea,* and *Lenzites saepiaria* in n/5 mannite solutions in which the hydrogen-ion concentrations were adjusted by the use of H_3 PO_4 and NaOH according to Clark and Lub's titration curve for ortho-phosphoric acid. The results obtained with *Fusarium sp.* are the only ones comparable with those obtained with *H. sativum* in the wideness of the range of hydrogen-ion con-

centration which permits spore germination. It may be pointed out that both *Fusarium* and *Helminthosporium* are chiefly soil organisms. Among the organisms that Webb studied, only *Fusarium* responded favorably to an alkaline medium. Maximum germination occurred at hydrogen-ion concentrations of pH 2.8 and pH 7.4. From pH 6.2 a steady increase in germination occurred with the increase in hydrogen-ion concentration up to pH 2.8. From the same point, a steady increase in germination also occurred with the decrease in hydrogen-ion concentration and practically the same maximum was reached at a concentation of pH 7.4. Examining the data of *H. sativum* again, there is a steady decrease in germination from the neutral point with the increase in hydrogen ions up to a concentration of pH 2.4, where no germination occurred during 18 hours and only very slight germination during 36 hours. However, the usual bimodal curve is obtained, but, in this case, both maxima occur on the alkaline side at hydrogen-ion concentrations of pH 7.8 and pH 9.2. With *H. sativum,* germination occurred chiefly in the alkaline solutions.

A series of spore germination tests was also made in Czapek's solution minus the sugar, with various hydrogen-ion concentrations ranging from pH 2.6 to pH 9.8. The results are represented by the curve in Figure 7. In this case also the bimodal curve was obtained. The first maximum, however, occurred on the acid side of neutrality at a hydrogen-ion concentration of pH 6. The second was on the alkaline side at a concentration of pH 8.

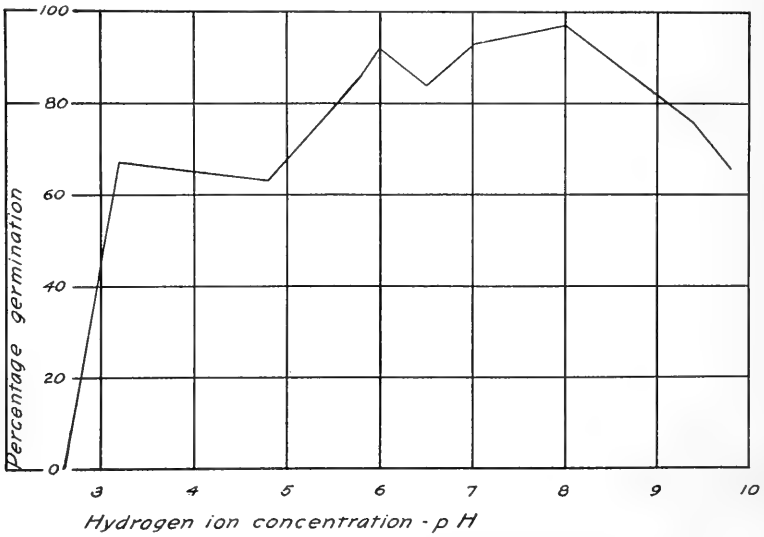

Fig. 7. Percentage Germination of Spores of *Helminthosporium sativum* in Czapek's Solution Minus the Sugar at Various Hydrogen-Ion Concentrations

While the germination of *H. sativum* spores in these solutions is not necessarily the same as in a soil solution, certain general relationships may be pointed out. The spores will germinate through a wide range of hydrogen-ion concentration. Optimum germination occurs near the neutral point or on the alkaline side. The spores will tolerate high degrees of alkalinity. Germination studies in solutions more nearly approximating soil solutions are still desirable from the standpoint of a closer analysis of the development of the disease.

INFECTION

Marquis wheat and Lion barley were grown under sterile conditions in test tubes containing white sand. When the seedlings were about an inch high, the coleoptile was inoculated with a suspension of spores and incubated at various temperatures. At 22°, 25°, and 30° C. characteristic minute brown lesions were visible after 18 hours. At the end of five days no infection had occurred at 6° on the barley; very light infection was evident on the wheat. Light infection also occurred on both wheat and barley at 14° and 34°, and on wheat at 30°. Moderate to heavy infection occurred on both hosts at 22° and 25°, and also on the barley at 30°. In these cases, the typical basal browning characteristic of the seedling blight occurred. This was as far as it was possible to follow the disease under these conditions. The results indicate that infection will take place to some extent through a rather wide range of temperature from 6° to 34° C. but that for the severe development of the disease the range is narrower, probably 22° to 30°. To some extent moisture, as well as temperature, was the limiting factor at the extremes.

F. L. Stevens (13) reports that, "In an adaptation of the rag-doll seed tester, which allows the use of seedlings under aseptic conditions and variations of moisture and temperature as desired, inoculation by spores of *Helminthosporium* upon the uninjured sheath was followed within 24 hours by entrance of the mycelium into the host cells, and within 48 hours by a browned, diseased spot visible to the naked eye. Subsequently, when conditions favored, the mycelium invaded the innermost leaves and caused general rotting and death. When inoculated upon the roots, there was general invasion of the cortex with very slight discoloration." Stevens does not report under what conditions of temperature and moisture the disease developed best.

An attempt was made to arrive at the temperature relations governing leaf infection by inoculating fresh excised leaves with spores of *H. sativum,* placing them in moist chambers and incubating them at various temperatures. After incubating for 72 hours at 6° C., both

inoculated and uninoculated check leaves were dark green, turgid, and normal in appearance. No signs of infection were apparent. Microscopic examination showed that many spores had germinated but so far as could be detected from free hand sections, the germ tubes had not penetrated. After the same incubation period, at 12° C., very small blue-green water-soaked areas were visible at the points of inoculation. The remainder of the leaf tissue and the uninoculated check leaves were still green and normal in appearance. These water-soaked areas were not yet visible at the end of 48 hours' incubation. At 18° C., by the end of the third day, there were green water-soaked areas on which conidiophores were beginning to appear on the inoculated leaves. The tissue of the leaves was still firm and the cells were turgid. While the infected areas retained a dark blue-green color, the rest of the leaf was yellow. The uninoculated check leaves were light green to yellow in color. After 72 hours' incubation at 23°, 27°, and 30° C., there were large dark green blotches of infected tissue covered by a velvety mass of conidiophores. The leaf tissue was beginning to soften and the check leaves and non-infected areas were yellow. In the infected areas the cells were beginning to disintegrate, but the chloroplasts were still green. At 34° C. small water-soaked areas, 3 or 4 mm. in diameter, were apparent after 36 hours' incubation. At this time the border was beginning to turn brown. By the third day, there were small, brown, definite leaf spots, similar to the normal lesions produced on leaves in the greenhouse and in the field. The remainder of the leaf tissue and the check leaves were yellow.

Under the conditions just described, there was always an abundance of moisture, so that the difference in reaction must have been due to the influence of temperature on host and fungus. During the first 36 hours the results were probably more or less comparable to results obtained in growing leaves attached to the plant; during the second 36 hours, at some temperatures at least, the relationship was probably saprophytic. The most that can be claimed for results obtained in this way is that they are only indicative of what may happen on growing plants.

The results obtained from these experiments would indicate that at temperatures of from 18° to 30° C., penetration into the leaf will take place about equally well in the presence of sufficient moisture. Below 24° the spots increase in size more slowly, above 24° more rapidly. At 12° a much longer incubation period is necessary for the development of water-soaked areas than at higher temperatures. At 6° no visible infection was obtained. At a temperature as high as 34°, on the other hand, the development of the spots and the browning of

the host tissue occurred so rapidly that further development of the fungus was checked.

While no control experiments were made with soil or leaf infection on growing plants, results obtained in the greenhouse agreed in general with those obtained on the temperature relations of the fungus. When the average temperature was between 75° and 85° F., much better infection was obtained than when the average was lower. Better results were obtained on an inner bench over the steam pipes than on an outer bench next the outside wall on the west end of the house where it was always cool, and vigorous plants developed in spite of heavy soil inoculation.

These results also agree with those reported by McKinney (8). He says, "Controlled soil temperature experiments, conducted in the 'Wisconsin temperature tanks,' and field experiments show that seedling infection in both spring and winter wheat and in spring barley is greatest at relatively high temperatures. The optimum temperature apparently lies between 26° and 28° C. This is very near the optimum rate of growth of *H. sativum* in pure culture."

INFLUENCE OF TYPE OF SOIL

The statement has already been made that particularly severe infections of *Helminthosporium* foot- and root-rots were observed during the summer of 1920 on sandy soils and on peat soils in certain localities in Minnesota. Consequently one of the first tests undertaken was a study of the development of the disease in different types of inoculated soil in order to gain, if possible, an insight into the individual factors which might be influencing the situation.

A heavy loam, a sandy loam, a sand, and a peat soil were selected for use. The heavy loam was a black dirt used without modification; the sandy loam was obtained by mixing two parts of the heavy loam with one part of quartz sand; and the sandy soil by mixing one part of the heavy loam with two parts of coarse sand. All this soil was passed through a 5-millimeter mesh screen before being packed into the pots. The peat was a high-lime peat obtained from Anoka County through the Division of Soils, and fertilized according to directions with acid phosphate and potassium chloride to secure maximum yield from this particular type of soil (1).

Small pots of steam-sterilized soil were planted with Marquis wheat and Lion barley. After the seeds were planted, the soil was watered several times with a heavy suspension of *Helminthosporium* spores.

When the plants became crowded in the small pots, they were transplanted to larger pots containing sterilized soil which had been inoculated in the same way. In these pots, the plants were grown to maturity.

There was no very serious seedling blight in any of the pots. The coleoptiles of most of the plants were darkened, and lesions were formed on the first leaves. The seedlings in the inoculated soils were not noticably smaller than those in the uninoculated, sterilized, check soils. When about six weeks old the height of the plants was measured in order to determine the effect of the disease on growth. The results are given in Table IX. In each case the table gives the average height of 30 to 40 plants.

Any differences in height of the plants in the different soils in either the uninoculated or inoculated series may be considered due to the influence of the soil in which they grew. As will be seen from Table IX, the differences between the plants in the different types of soil in the inoculated series, altho small, agree fairly well with similar differences in the uninoculated series. The differences between check plants and inoculated plants in the same type of soil may be considered to be the result of the disease. A comparison of the differences between plants in inoculated and uninoculated soils of the different types will give an index of the influence of the soil type on the development of the disease.

TABLE IX

AVERAGE HEIGHT OF WHEAT AND BARLEY PLANTS GROWN IN INOCULATED AND UNINOCULATED SOILS OF VARIOUS TYPES

Type of soil	Marquis wheat		Lion barley	
	Inoculated	Check	Inoculated	Check
	cm.	cm.	cm.	cm.
Heavy loam	22.8	30.2	24.5	27.6
Sandy loam	21.0	32.3	29.0	31.1
Sand	23.0*	33.6*	25.3	28.3
Peat	29.4*	41.7*	29.4	33.6

* Plants were measured two days later than those in the heavy loam and in the sandy loam, and so can not be compared with these.

Judging by the height of the plants at this stage, the barley developed about equally well in the heavy loam and in the sand, the difference in the average height being 8 millimeters in the inoculated series and 7 millimeters in the check series. The increases over this amount were about equal in the sandy loam and in the peat, the advantage being slightly in favor of the latter. The difference between the height of plants in inoculated and uninoculated soils was practically the

same in the heavy loam and in the sand. This would indicate that these two types of soil had practically an equal influence on the development of the disease.

The difference was less pronounced in the sandy loam, showing that here the disease had least influence on the size of the plants. The greatest difference in height was in the peat soil, indicating that here the disease had most influence on the growth of the plant. From these results it is apparent that root-rot of barley produced the greatest effect on the host in the peat, a less marked effect in the sand and heavy loam, and the least effect in the sandy loam. On the whole, the differences were very small. The further development of the disease on the barley plants was not followed.

The conclusions to be drawn from the height of the wheat plants at this stage must be derived from comparisons between the differences in the height of diseased and check plants in the same type of soil. It is obvious that the effect of the disease is much more marked on the wheat than on the barley. The least effect of the disease on the growth of the plants was obtained in the heavy loam. There was practically an equal increase in effect in the other three types of soil.

The wheat was then transplanted to larger pots of inoculated soil. In each case, the most severely diseased plants were transferred. After transplanting, the check plants grew much faster than the diseased plants, and headed several days earlier. Plate III shows the comparative vigor and size of the plants in the different types of soil at maturity. Final observations were made on the Marquis wheat just before the heads began to turn yellow. The plants were removed from the soil, carefully washed, and examined for foot- and root-rot.

In the heavy loam soil, both diseased and check plants averaged 3.5 culms per plant. While the severity of infection, measured by the degree of browning at the base of the plant, was moderate, there was very little difference in the extent of the root systems. The check plants headed four days earlier than the diseased plants and were considerably more vigorous. A slight browning occurred at the base of most of the mature check plants which resembled slightly a light infection by *Helminthosporium*. The lesions, however, were less definite and no organism was obtained from tissue cultures. *H. sativum* was isolated from the base of diseased plants.

In the sandy loam, the average number of culms on each diseased plant was 3, on each check plant 2.5. The basal infection was moderate. There was little difference in the root systems.

In sand the average number of culms on each diseased plant was 3, on each check plant 2. The infection at the base of the diseased plants

ranged from moderate to heavy minus. The root systems of the diseased plants were considerably less extensive, brown lesions were numerous, and the roots were very easily broken. The contrast between diseased and check plants was greatest in this type of soil.

In the peat soil the average number of culms on inoculated plants was 2.7, on uninoculated 2.6. Basal infection was light to moderate. There was very little difference in general appearance of the plants grown in inoculated soil and in uninoculated soil. The best plants in both series were obtained in the peat soil.

Under the conditions studied, the root-rot inhibited the growth of Lion barley most, during the first six weeks, in the peat soil. The effect of the disease was less evident in the heavy loam and the sand, and least evident in the sandy loam. During the same period, the growth of Marquis wheat was least inhibited in the heavy loam. The effect of the disease on the growth of the plants was markedly increased, and to practically the same extent, in the other three types of soil. By the time of maturity, however, the disease had developed much more severely in the sand, as evidenced by the smaller size of the plants, their decreased vigor, the amount of basal browning, and the breaking down of the root system. The effect of the disease was almost as severe in the heavy loam. In both the sandy loam and the peat there was only a very slight difference between the plants grown in inoculated and uninoculated soil.

In analyzing the factors involved in these various soils, it may be pointed out that in the loam soils, in addition to the change in physical texture brought about by adding increasing quantities of sand to the original heavy loam, there has been a dilution of the mineral nutrients of the host, a decrease in the water-holding capacity, a decrease in the amount of organic matter in the soil, and an increase in the amount of soil aeration. All these factors may be assumed to have an influence on both the host and the pathogene. On the other hand, in the peat soil, we have a high organic content, a high water-holding capacity, and an optimum of mineral nutrients for the host. The abundant moisture and high organic content of the peat soil should seemingly be conducive to extensive saprophytic growth of *Helminthosporium,* thus greatly increasing the amount of inoculum and the chance for infection of the growing host. This tendency, however, seems to be counterbalanced by the optimum conditions offered for the growth of the host. On the other hand, the greater severity of the disease in sand and heavy loam suggests a possible influence of the soil water. These results led to a further study of the influence of the soil moisture and of soil fertility on the development of the disease.

INFLUENCE OF SOIL MOISTURE

Preliminary series of experiments were carried out in the greenhouse in the following manner in order to determine the effect of soil moisture on the development of *H. sativum* on Lion barley. Light loam soil was sifted through a 5 millimeter screen, packed into jars, and sterilized. The sterilized soil was mixed with a culture of *H. sativum* grown on sterilized oats seed. Five degrees of soil moisture were maintained more or less uniformly by adding definite amounts of water each day. In the fifth series the soil was kept saturated by standing the porous pots in jars of water. In the other four series the soil was in glazed jars and the soil moisture was regulated by adding different amounts of water. Each moisture series was carried out in triplicate, in both inoculated and uninoculated soil. The seed was sterilized with silver nitrate before planting.

Comparative results on the infection above ground at the end of three weeks and below ground at the end of four weeks are summarized in Table X. In this table the infection is designated by fractions; the denominator represents the number of plants in one pot, the numerator the number that were infected. On examining the data, it is seen that, as far as the above ground parts of the plants are concerned, the percentage of infection, as well as the severity, is increased as the amount of soil moisture is increased. Comparatively few infections occurred on the check plants.

The relation of soil moisture to root infection is a little more difficult to see, as here the development of the roots in inoculated and uninoculated soils with the same moisture content must be compared, and then these differences compared for the various series. The roots were most severely rotted in the saturated inoculated soil, and the difference in the extent of the root systems of diseased and check plants was greatest here. The next greatest difference was in the first series, with a soil moisture content averaging 9 per cent, while the least difference was found in the third and fourth series. In these two series the plants grew best of all, in both the inoculated and uninoculated soils. Injury to the roots is brought about by rather limited local lesions which kill the root tips or cut off portions of the roots when the lesions occur back from the tips. Very often the roots are rotted off near the seed.

These results would indicate that plants suffer most from root infection by *H. sativum* in soils containing both maximum and minimum extremes of moisture. When conditions are more nearly favorable for the optimum growth of the plants, the effect of the disease can be overcome, and root systems are developed in inoculated soil almost equal in extent to those in clean soil.

TABLE X

SUMMARY OF COMPARATIVE RESULTS ON THE DEVELOPMENT OF THE *Helminthosporium* FOOT-ROT ON LION BARLEY UNDER VARYING CONDITIONS OF SOIL MOISTURE

Series	Average soil moisture	Above ground infection (Three weeks after planting)		Soil infection (Four weeks after planting)	
		No. of plants	Notes	Foot-rot	Roots
I	9%	3	Brown spots on first leaves.	Moderate	Roots few, long and coarse, few secondary branches. Many brown lesions on the roots, especially near the seed and at the tips, but to some extent on all parts.
		—			
		23			
		8	3 seedlings killed, 1 with leaf spotted, remainder with lesions on the sheaths.		
		—			
		17			
		2	Light infections on first leaves and sheaths.		
		—			
		13			
II	13%	7	Light infection on sheaths and first leaves.	Moderate	Root system slightly better than in I. Roots coarse with few secondary branches. Lesions numerous. Difference between inoculated and check roots not so marked as in I.
		—			
		19			
		12	1 seedling killed, 1 with first leaf destroyed, moderate infections on remaining sheaths.		
		—			
		23			
		22	2 seedlings killed, moderate infections on remaining sheaths.		
		—			
		22			

TABLE X—*Continued*

Series	Average soil moisture	Above ground infection (Three weeks after planting)		Soil infection (Four weeks after planting)	
		No. of plants	Notes	Foot-rot	Roots
III	16%	28 — 28 26 — 26 25 — 25	1 seedling with severe infections on first and second leaves, stunted; remainder moderate. 1 seedling with first leaf killed, 1 with plumule killed. 1 seedling with first leaf killed.	Moderate minus	Tendency toward coarse roots without branches but not to such a marked extent as in I and II. Few but very severe lesions. Very little difference in extent of root systems of diseased and check plants. Best growth of plants in any of the inoculated series.
IV	25%	13 — 21 11 — 18 16 — 28	1 seedling killed, 1 with first and second leaves killed. 1 seedling killed, one with first leaf killed. 1 seedling with first and second leaves killed, 1 with first leaf killed.	Moderate	Roots relatively few and very coarse. Most of them killed at tips.

TABLE X—*Continued*

Series	Average soil moisture	Above ground infection (Three weeks after planting)		Soil infection (Four weeks after planting)	
		No. of plants	Notes	Foot-rot	Roots
		8	1 seedling killed.		
		—			
		8			
		17	12 seedlings killed.	Heavy	On 50% of the plants the roots were completely rotted off. Roots were very few in number and very coarse. Many severe lesions. Marked difference between diseased and check plants in extent of root systems.
V	Mud	—			
		17			
		18	10 seedlings killed.		
		—			
		18			
		2	Small lesions on sheaths.		
		—			
		24			
		0		Light	Root systems almost twice as extensive as in corresponding inoculated series. Main roots much finer with many secondary branches. No lesions.
I	9%	—		2	
Check		18		—	
		0		15	
		—			
		17			
		3	Small lesion on sheaths.		
		—			
		25			
		5	Small lesion on sheaths.	None	Not so many roots as in I check. Roots fine with many branches. No lesions.
II	15%	—			
Check		30			
		2	Small lesion on sheaths.		
		—			
		25			

TABLE X- *Concluded*

Series	Average soil moisture	Above ground infection (Three weeks after planting)		Soil infection (Four weeks after planting)	
		No. of plants	Notes	Foot-rot	Roots
III Check	18%	2	First and second leaves killed.	Light	Many roots, fine and branched. No lesions. Best growth of plants in any of the check series.
		—		2	
		26		—	
		3	Small lesions on sheaths.	15	
		—			
		27			
		3	1 seedling with plumule killed, 2 with small lesions on sheaths.		
		—			
		25			
IV Check	25%	—	Small lesions on sheaths.	None	Roots coarse with few secondary branches. No lesions. Marked difference between extent of root systems in check and diseased plants. Growth of plants good.
		17			
		0			
		—			
		17			
		0			
		—			
		15			
V Check	Mud			None	Comparatively few roots, coarse and with few secondary branches. No lesions. Growth of plants fair.

An attempt was made to check up the moisture relations of the disease under field conditions. In a series of six square-rod plots, three were planted to barley and three to wheat. The north half of the barley plots was planted with Manchuria and the south half with Lion; the north half of the wheat plots was planted with Marquis, the south with Bluestem. A field drill was used for planting. Several days before planting, the soil was inoculated by applying *H. sativum* grown on sterilized wheat seed. One half gallon of the culture was applied to each square rod. After the seed was planted, one of the barley plots and one of the wheat plots was sprinkled for ten minutes each morning and evening, a second of each was sprinkled for five minutes each morning and evening, and to the third no water was added. During the first part of the season, very little infection appeared on any of the plants except Lion barley. The weather was very cold during the first two weeks after seeding and scarcely any infection occurred. There was no seedling blight on the unwatered plot, very little on the moderately watered plot, and only a moderate amount on the heavily watered plot. Infection occurred mainly on the above-ground parts, resulting in leaf spots and lesions on the sheaths. Less than 1 per cent of the plants were killed. After the first two weeks, the amount of infection increased very rapidly on the heavily watered plot, so that at the end of six weeks 100 per cent of the plants were infected, and most of them were severely attacked. On the moderately watered plot about 80 per cent of the plants were infected, the severity of the infection ranging from light to moderate plus. On the unwatered plot, about 50 per cent of the plants were infected, the severity of the infection ranging from light to moderate.

The method of applying the water tended to keep a film of moisture on the lower, shaded leaves, forming almost a moist chamber near the surface of the soil. The sprinkling also offered a good opportunity for the spores to be splashed from the soil onto the leaves. During June, the weather was very hot. As a result of this combination of circumstances, the plants on the heavily watered plot were literally covered with *H. sativum* lesions. In many cases the plants were so badly infected at the base that they rotted off. This was not true to such a marked extent on the moderately watered plot owing, probably, to the fact that the surface of the soil was not kept wet enough to maintain a more or less constant layer of moisture just above the surface of the ground. The Manchuria barley was moderately affected, but the wheats only slightly.

Final data were taken just before the heads ripened. An attempt was made to obtain a quantitative estimate of the percentage of plants infected for the whole plot and an average of the degree of infection on the roots, foot, and node from individual plants. For this purpose, approximately equally large groups of plants were dug from the center, and also from each corner of the plots, two feet from the margins. Ten plants were taken from each group and for these fifty plants, the following data were recorded: the number of culms which headed; the number of tillers which did not head; the degree of infection—indicated as heavy, moderate, or light on the roots, foot, and nodes. Finally, the seed from each plot was weighed after threshing and the yield per acre was calculated from this. These data are summarized in Table XI. In order to obtain a simple mathematical expression for making comparisons, the percentage of heavy infections was multiplied by 10, the moderate by 5, and the light by 1, and the sum was taken as the index of the total infection. In order more easily to make comparisons, these sums were reduced to unity. Finally, these expressions for root and foot infections were totaled to obtain a means of comparing the combined foot- and root-rot with the relative amount of soil moisture and with the yield. For the sake of comparison these were also reduced to unity. In some cases the *Helminthosporium* infections were so complicated by *Fusarium* infections that it is quite impossible to say how much of the damage was due to each organism. This was especially true on the Manchuria barley. In general, the amount of injury was small. Altho the root-rot and the basal infection as measured by the degree of browning was sometimes heavy on a large number of plants, the plants were not noticeably stunted or immature as is often the case in severe cases of foot-rot. On the whole, there was more foot- and root-rot on the barley than on the wheat. The Lion barley alone shows an increase in the amount of foot-rot as the relative amount of soil moisture is increased. The differences are so small, however, that they can not have much significance. There were no indications of a correlation in yield with either the relative amount of soil moisture or the amount of foot- and root-rot. For both varieties of wheat, foot- and root-rot was slightly worse on the driest plot. In no case did the relative amount of soil moisture or infection influence the tillering of the plants.

TABLE XI

SUMMARY OF DATA ON INFECTION OF BARLEY AND WHEAT BY *H. sativum* UNDER VARYING DEGREES OF SOIL MOISTURE UNDER FIELD CONDITIONS

Variety	Soil moisture	Culms Av. no. per plant		Infection													Yield per acre, bu.	Remarks
				Root				Foot				Node				Combined totals (root and foot)†		
		Mature	Immature	% H	% M	% L	Total*	% H	% M	% L	Total*	% H	% M	% L	Total*			
Lion barley	+++	1.8	.9	50	50	0	1.4	8	62	30	7.0	94	6	0	12.1	1.9	13.5	6% infected by Fusarium
Lion barley	++	1.6	.8	50	48	2	1.4	2	38	60	4.5	58	42	0	9.9	1.7	11.7	
Lion barley	+	1.8	.6	32	68	0	1.3	0	46	54	4.7	100	0	0	12.5	1.6	15.8	2% infected by Fusarium
Manchuria barley..	+++	1.4	.4	74	26	0	1.7	0	58	42	5.5	24	58	18	6.9	2.0	22.9	10% infected by Fusarium
Manchuria barley..	++	1.3	.4	81	18	0	1.7	0	34	52	3.7	43	45	12	8.3	1.9	14.6	36% infected by Fusarium
Manchuria barley..	+	1.2	.3	88	12	0	1.8	0	45	55	4.7	61	34	5	9.8	2.0	25.0	26% infected by Fusarium
Marquis wheat	+++	1.2	.8	8	88	4	1.0	0	2	68	1.2	0	22	4	1.4	1.0	11.5‡	
Marquis wheat	++	1.1	.6	34	56	10	1.2	0	0	70	1.2	6	54	0	4.1	1.2	15.0	
Marquis wheat	+	1.0	1.0	40	60	0	1.3	0	20	70	2.8	18	48	0	5.3	1.4	17.2	
Bluestem wheat....	+++	1.1	.9	32	60	8	1.2	0	4	40	1.0	0	16	8	1.1	1.1	9.8‡	
Bluestem wheat....	++	1.0	.7	22	78	0	1.2	0	4	42	1.0	4	16	9	1.6	1.1	10.5	
Bluestem wheat....	+	1.0	.8	24	56	20	1.0	0	4	72	1.5	0	16	0	1.0	1.1	10.7	

* These factors were arrived at in the following manner: the number of heavy infections was multiplied by 10, the moderate by 5, and the light by 1, the products were added and the summations in each column reduced to unity by dividing each by the smallest sum in the column.

† These factors were arrived at by combining the sums for root and foot infection and reducing these to unity.

‡ Stand poor, as many of the seeds were washed out during first few sprinklings.

Many factors that are difficult to control enter into field experiments and complicate the results in such a way as to make them indicative rather than conclusive. In these experiments foot- and root-rot developed slightly more vigorously on Lion barley in the wettest soil and on the two varieties of wheat in the driest soil. This may be only a confirmation of the earlier greenhouse experience that root infection tends to be worse under either extremely dry or extremely wet conditions.

INFLUENCE OF SOIL FERTILITY

The effect of soil fertilization on the development of foot- and root-rot caused by *H. sativum* was studied in field plots on Lion and Manchuria barley, and on Marquis and Bluestem wheat. Potassium and nitrogen in the form of muriate of potash and nitrate of soda were added to square-rod plots at the rate of 600 and 300 pounds of fertilizer to the acre. Treble superphosphate was added at the rate of 200 and 100 pounds. These fertilizers were so applied that there were plots with a heavy and a light application of each alone and in combination with a heavy and light application of each of the others, except that there were no combinations of nitrogen and potassium. In addition to these, complete fertilizer was applied at the rate of 600 and 300 pounds and manure at the rate of 20 tons and 10 tons per acre. Unfertilized plots were left as checks. All the plots were run in duplicate, one series planted with wheat and one with barley. The north half of the wheat plots was planted with Marquis, the south half with Bluestem, the north half of the barley plots with Lion, the south half with Manchuria.

Several days before planting, *H. sativum* grown on sterilized wheat seed was applied on the surface of the soil at the rate of one half gallon of the culture to the square rod. The plots were seeded with a field drill, wheat at the rate of 90 pounds to the acre, and barley at the rate of 86 pounds. This is the normal rate of seeding for this section of the country.

Practically no seedling blight developed on any of the plots. Leaf lesions and foot-rot first appeared on the barley during the second and third weeks, and soon after lesions developed also on the wheat.

There was considerable difference in the vigor and height of plants on the different plots in response to the different fertilizers. During the latter part of the season, there were differences in the amount of lodging on the various plots. Final data on the amount of foot- and root-rot were taken just previous to the ripening of the grain. In order to obtain an approximately quantitative expression for the amount of infection in each plot, 50 plants were selected from each half square rod, 10 from each corner, two feet in from the margins, and 10 from the center of the plot. For each of these plants the following data

were recorded: the number of culms which developed heads; the number of tillers which did not mature; the degree of infection (designated as heavy, moderate, or light) on the roots, the foot, and the nodes. After harvesting, the weight of the straw and of the threshed grain was recorded, and from this the yield per acre was calculated. The infection of Lion barley was slightly more pronounced than on the other hosts. These data for Lion barley are summarized in Table XII.

In order to arrive at a simple factor which would express the total infection for the roots, the foot, and the nodes for a single plot and would also take into account the severity of infection as well as the percentage of plants infected, the number of heavy infections was multiplied by 10, the number of moderate infections by 5, and the number of light infections by 1, and the three products were summed. This was taken as an arbitrary index of the infection. In order to make comparison more simple, these summations were reduced to unity by dividing each by the lowest sum. This is the factor designated as total infection in Table XII. In order to compare the combined effect of root- and foot-rot, the summation for each was added and these sums in turn were reduced to unity.

In order to bring out the relation of infection to fertilizers and yields, the arbitrary indices of infection were grouped into three classes and the yields per acre into three classes, and the fertilizer plots were arranged according to their infection and yield in the various classes as shown in Table XIII. From this summary table it is quite clear that the amount of foot- and root-rot is not correlated with any particular fertilizer.

The disease did not appear in its severest form on any of the plots. Under the conditions of this experiment, there was no evidence of severe stunting of the plants or of excessive tillering.

COMPARISON OF SEVERAL ROOT-ROT CAUSING ORGANISMS

In order to obtain comparative results on the pathogenic effect of different soil organisms on Marquis wheat and Lion barley, a culture of *Helminthosporium* isolated from the foot-rot of wheat in Illinois by F. L. Stevens and a culture of *Fusarium culmorum* (W. Sm.) Saccardo isolated from scabby wheat, were compared with the *Helminthosporium sativum* isolated from barley foot-rot in Minnesota.

TABLE XII

EFFECT OF *H. sativum* ON LION BARLEY IN RELATION TO VARIOUS FERTILIZERS

Fertilizer, lbs. per acre			Culms, Av. no per plant		Infection												Yield	
					Root				Foot				Node					
K	N	P	Mature	Immature	% H	% M	% L	Total*	% H	% M	% L	Total*	% H	% M	% L	Total*	Combined totals (root and foot)†	Grain, bu. per acre	Straw, tons per acre
600	600	200	2.6	1.7	24	25	38	1.4	18	44	38	3.1	24	46	18	1.3	1.3	30.0	1.340
300	300	100	1.2	0.6	24	36	38	1.6	8	48	44	2.6	38	56	6	1.8	1.3	34.6	1.570
	600	200	2.1	0.5	26	72	2	2.2	54	46	0	5.5	42	52	6	1.8	2.2	28.3	1.520
	600	100	1.4	0.6	4	72	20	1.5	6	62	32	2.9	78	22	0	2.3	1.3	32.1	1.490
	600		2.6	0.8	12	56	28	1.5	38	62	0	4.9	80	20	0	2.4	1.8	32.5	1,580
	300	200	2.6	0.8	8	52	40	1.4	24	66	10	4.1	94	6	0	2.6	1.5	24.8	1.345
	300	100	1.3	0.8	16	52	32	1.6	18	50	30	3.3	72	28	0	2.3	1.5	35.8	1.800
	300		2.3	1.1	4	36	60	1.0	10	66	24	3.2	12	88	0	1.5	1.2	28.8	1.430
		200	2.6	0.7	6	34	66	1.1	12	54	32	3.0	64	30	6	2.1	1.2	22.3	1.265
		100	1.0	0.1	14	70	16	1.8	24	40	36	3.4	20	72	8	1.5	1.6	30.6	1.645
600		200	2.7	0.6	8	54	38	1.4	28	54	18	4.1	70	28	2	2.2	1.5	25.0	1.360
600		100	1.0	0.1	20	56	22	1.8	4	36	60	2.0	16	34	50	1.0	1.3	24.8	1.485
600			1.7	1.1	4	84	12	1.7	22	66	12	5.0	34	44	10	1.5	1.7	20.6	1.385
300		200	2.3	0.8	6	56	38	1.4	6	58	36	2.8	68	26	6	2.1	1.2	23.3	1.440
300		100	1.0	0.2	18	56	26	1.7	0	10	90	1.0	20	42	38	1.2	1.0	18.8	1.030
300			1.7	0.9	12	68	20	1.7	14	72	12	3.7	26	66	16	1.5	1.6	21.7	1.160
20 tons manure			2.4	1.0	0	48	48	1.0	26	48	22	3.7	30	60	0	1.6	1.3	30.0	1.365
10 tons manure			1.8	1.0	6	80	14	1.7	70	24	6	5.9	60	14	16	1.8	2.1	27.1	1.470
No fertilizer			1.9	1.2	10	64	26	1.6	26	60	12	4.1	86	8	4	2.4	1.6	19.4	1.135
No fertilizer			1.8	1.1	16	68	16	1.8	26	64	8	4.2	46	54	0	1.9	1.8	19.0	1.580

* These factors were arrived at in the following manner: The number of heavy infections was multiplied by 10, the moderate by 5, and the light by 1, the products were added and the summations in each column reduced to unity by dividing each summation by the smallest sum in the column.

† These factors were arrived at by combining the sums for root and foot infection and reducing these to unity.

These organisms were grown on sterilized wheat seed to obtain a large amount of inoculum. At the end of two months, the cultures were practically masses of mycelium and spores. These masses were passed through a meat grinder and the pulp was thoroly mixed with sterilized soil in which the wheat and barley were then planted. The observations on seedling injury at the end of twenty days are summarized in Table XIV.

TABLE XIII

SUMMARY OF DATA IN TABLE XII

Infection classes

Yield	1.0 to 1.3			1.4 to 1.8			1.9 to 2.2		
	K	N	P	K	N	P	K	N	P
18.0 to 23.9 Bu. per acre	300		100	600					
			200	300					
	300		200	No fertilizer					
				No fertilizer					
24 to 29.9 Bu. per acre	600		100		300	200		600	200
		300		600		200			
							10 tons manure		
30 to 35.9 Bu. per acre	20 tons manure								
	600	600	200		600				
		600	100						
	300	300	100		300	100			

The results on the Lion barley were very sharp. Unfortunately, rats molested some of the pots. In the barley series, however, only the check plants were injured. Three plants were left in each of the three pots. The comparative size of the plants grown in soil inoculated with the various organisms is very well shown by Plate IV.

The figures in Table XIV show that all three of the organisms caused a dwarfing of the barley, the two cultures of *Helminthosporium* to a much greater extent than the *Fusarium*. The Minnesota strain almost completely destroyed the plants. The effect of the three organisms on the root systems is shown by Plate V. The nature of the injury caused by severe infections of *Helminthosporium* is further illustrated by Plate VI, where eight seedlings showing various degrees of infection are shown beside a normal seedling of the same age grown in sterilized soil.

TABLE XIV

SEEDLING INJURY CAUSED BY *Fusarium culmorum*, AND TWO STRAINS OF *Helminthosporium sativum*
OBSERVATIONS MADE 20 DAYS AFTER PLANTING

Organism	Marquis wheat			Lion barley		
	No. plants*	Av. height†	Above-ground infection	No. plants	Av. height	Above-ground infection
Check, sterile soil	25	19.8cm. —— 10	Very small lesions on sheath of 7 seedlings.	9	21.8cm. —— 9	None
Fusarium culmorum	32	18.5 —— 12	Slight browning of sheath on 11 seedlings.	38	14.7 —— 13	4 seedlings killed, less than 2.5 cm. high; 5 seedlings badly dwarfed, first 3 leaves rose at nearly the same place just above soil line; 17 with dark brown sheaths; 12 without discoloration.
Helminthosporium sativum Illinois culture	17	13.7 —— 13	Seedlings killed; 5 seedlings moderately brown at base.	50	9.1 —— 17	7 seedlings with small lesions on leaves; 22 seedlings browned at base, infection varying from slight to severe.
Helminthosporium sativum Culture 82a	28	9.9 —— 17	6 seedlings with leaf lesions; 7 seedlings darkened at base, injury very slight.	35	4.1 —— 13	100% infection; 12 seedlings killed, less than 4 cm. high; 8 seedlings with first leaf killed; 2 seedlings with small lesions on first leaf; all severely browned at base.

* Irregularity in the number of plants is due to the fact that some were destroyed by rats.

† Numerator denotes average height, denominator number of plants measured. Except for the uninoculated Lion barley, all the plants measured in each case were in one pot.

The results obtained with Marquis wheat are not so clear, as all the pots were molested more or less. The amount of injury caused by foot- and root-rot was very much less than on the barley. The Illinois strain of *Helminthosporium* seemed to cause slightly more injury than the Minnesota strain.

This series was started in the greenhouse during warm weather early in October when the temperature in the house was very high. The subsequent development of the disease was most interesting. After three weeks, the plants were thinned so that only three remained in each pot except for those inoculated with *H. sativum,* culture 82a, of which only three plants in each pot survived. These were badly stunted and infected at the time. The pots were kept next to the outer west wall of the greenhouse, where the temperature was always low during winter. The position of the pots was changed periodically so that all the plants would have more or less equal advantages as to sunlight. The plants grew remarkably well, and after a few weeks scarcely any differences could be detected between the different series. The barley stooled excessively and did not head well. The wheat was very good. At heading time, late in April, there was practically no difference between either the wheat or the barley plants grown in the clean soil and in the soil inoculated with the Minnesota strain of *H. sativum* or the *Fusarium culmorum.* The wheat in the soil inoculated with the Illinois strain of *Helminthosporium* was very bushy and developed only one or two heads per pot, while the other wheats developed from four to eight. The Lion barley was also slightly poorer in the soil inoculated with the Illinois strain than in the others. The barley did not head well, however, in any case.

Under the conditions of this experiment, then, the *Helminthosporium* caused more injury to Marquis wheat and Lion barley than the *Fusarium,* both in the seeding and the mature stages. While the Minnesota strain of *Helminthosporium* caused decidedly more seedling blight on the barley, the Illinois strain caused slightly more stunting of the mature plants. The Illinois strain caused more injury to the wheat at both stages.

SUMMARY AND CONCLUSIONS

In recent years a foot- and root-rot of wheat, rye, and barley has been serious in certain localities in Minnesota. A *Helminthosporium* of the *sativum* type has been constantly isolated from the diseased plants. In addition to causing a foot- and root-rot, the same type of organism attacks the leaves and stems and especially the nodes, glumes, and kernels of cereals and a large number of wild grasses. A strain of the organism was isolated from a foot-rot of barley. A pure culture was secured by isolating a single spore. The morphology of the organ-

ism was studied under various conditions with regard to its specific identity. The physiology and pathogenesis were studied with special reference to environmental conditions most favorable to the development of foot- and root-rot.

The organism is capable of causing disease symptoms similar to those described by Pammel, King and Bakke in 1910. Discrepancies are found between the spore measurements of this organism and that described by Pammel, King and Bakke, but since wide variations occurred under different conditions in a single-spore culture of the organism studied, the similarity of disease symptoms is considered sufficient justification for considering the organism to be *Helminthosporium sativum* P. K. B.

Variations in the morphology of the spores were found to occur under different conditions of growth. For spores as variable in length as those of *H. sativum,* it was found necessary to measure 500 spores in order to obtain accurate results.

On potato dextrose agar, significant differences in mean length of the spores occur when the organism is grown at different temperatures. The shortest spores with a mean length of 55.98 ± 0.35 microns were produced at 28° C. The longest spores, with a mean length of 67.32 ± 0.55 microns, were produced at 14° C. The difference between the two means is 14 times the probable error of the difference.

The greatest differences in length were found between spores produced on different substrata. At 24° C. the mean length of the spores produced on potato dextrose was 65.75 ± 0.37 microns, on autoclaved ripe barley heads 67.74 ± 0.38 microns, and on green barley leaves 83.14 ± 0.29 microns. The difference between the means of the spores produced on the agar and on the leaves is 37 times as great as the probable error of the difference.

The temperature relations of the fungus were studied and it was found that the mycelium will grow at from 1° C. to 37° C., the optimum lying near 28°. The spores germinated in redistilled water about equally well at temperatures ranging from 6° to 39°, but the length of the germ tubes indicated that the optimum temperature is between 22° and 32°. Germ tubes penetrated the tissue of both the coleoptile and the leaf at from 12° to 34°, but severe infection occurred through a narrower range, from 22° to 30°, the disease developing faster at the higher temperatures. Above 30°, however, the development of the lesions seemed to be checked, altho they appeared very soon after inoculation. In general, we may say that rather high temperatures are most favorable to the growth of the fungus, to spore germination, to infection, and to the development of the disease.

In phosphoric acid–potassium hydroxide solutions, the spores germinated through a wide range of hydrogen-ion concentrations. A double optimum occurred, both maxima falling on the alkaline side of neutrality at pH 8.2 and pH 9.2. In Czapek's solution minus the sugar, the maximum germination occurred at pH 6 and pH 8. In general, the spores germinate better in alkaline solutions than in acid solutions. The spores will tolerate high degrees of alkalinity.

Leaf infection increases directly with the amount of moisture present. Greenhouse experiments indicate that the effects of root and foot infections are more severe in extremely dry and extremely wet soils than in soils containing an optimum amount of moisture for the growth of the host plant.

During one year's field experimentation, no correlation was found between the fertility of the soil and the development of foot- and root-rot.

The pathogenic effect of *H. sativum* isolated from barley plants in Minnesota was compared with that of a *Helminthosporium* isolated from stunted wheat in Illinois and with *Fusarium culmorum* isolated from scabby wheat. Experiments were made to determine the ability of these organisms to cause root- and foot-rot of Marquis wheat and Lion barley. Under the conditions of the experiment, the *Helminthosporiums* caused more injury than the *Fusarium*. The Minnesota strain of *Helminthosporium* caused the greater amount of seedling injury on the Lion barley, while the Illinois strain caused the greater dwarfing of the mature plants on both wheat and barley.

As a result of these studies, the wide-spread occurrence of *H. sativum* may be explained by the fact that the fungus responds saprophytically to such a wide range of environmental conditions. Neither the effect of temperature nor acidity seems to be a limiting factor in the development of the disease so far as spore germination is concerned. As a parasite, the fungus causes rather limited local infections. The amount of injury is determined largely by the number and size of the lesions. A direct correlation exists between the amount of moisture present and the number of lesions. The severity of the infection is greater at rather high temperatures than at low temperatures. The disease may be expected to develop most severely, therefore, at high temperatures in the presence of sufficient moisture.

Root and foot infections are more severe in certain soils than in others. This is probably largely due to differences in soil moisture and temperature. In general, the disease causes the greatest injury under conditions unfavorable to the growth of the host. Factors, such as soil fertility, which might then be expected to influence the disease, apparently have little effect.

LITERATURE CITED

1. Alway, F. J. Agricultural value and reclamation of Minnesota peat soils. Minn. Agr. Exp. Sta. Bul. 188. 1920. (Out of print.)
2. Babcock, E. B. and Clausen, R. E. Genetics in relation to agriculture. New York, 1918.
3. Christensen, J. J. Studies on the parasitism of *Helminthosporium sativum* P.K.B. Master's Thesis. Minn. Agr. Exp. Sta. Tech. Bul. 11. 1923.
4. Clark, W. M. The determination of hydrogen-ions. Baltimore, 1920.
5. Hamblin, C. O. "Foot-rot" of wheat caused by the fungus *Helminthosporium*. Agr. Gaz. N. S. W. **33**:13-19. 1922.
6. Hayes, H. K. and Stakman, E. C. Resistance of barley to *Helminthosporium sativum* P.K.B. Phytopathology 11:405-411. 1921.
7. Johnson, A. G. *Helminthosporium* diseases of barley in Wisconsin. Phytopath. **3**:75. 1913.
8. McKinney, H. H. The *Helminthosporium* disease of wheat and the influence of soil temperature on seedling infection. Phytopath. 12:28. 1922.
9. Pammel, L. H., King, Charlotte M., and Bakke, A. L. Two barley blights, with comparison of species of *Helminthosporium* on cereals. Iowa State College of Agr. and Mech. Arts. Bul. 116, June, 1910.
10. Schroeder, H. Die Widerstandsfalhigkeit des Weizen-und Gerstenkorns gegen Figte und ihre Bedeutung fuer die Sterilisation. Centralblatt für Bakt. Parasit. und Infekt. 28:492-505. 1910.
11. Stakman, Louise J. A *Helminthosporium* disease of wheat and rye. Minnesota Agr. Exp. Sta. Bul. 191. July, 1920.
12. Stevens, F. L. Foot-rot of wheat. Science N. S. **51**:517-518. 1920.
13. ——— *Helminthosporium* and wheat foot-rot. Phytopathology 11:37. 1921.
14. Webb, Robert W. Studies in the physiology of the fungi. X. Germination of the spores of certain fungi in relation to hydrogen-ion concentration. Annals Missouri Botanical Garden **6**:201-222. 1919.

PLATE I

Helminthosporium sativum P. K. B. grown on potato dextrose agar at the following temperatures:

(1) 0° — 2°C., (2) 6° — 8°C., (3) 13° — 15°C.,
(4) 17° — 22°C., (5) 21° — 24°C., (6) 30° — 32°C.,
(7) 34° — 35°C., (8) 40° — 42°C.

Incubated 9 days.

PLATE II

Helminthosporium sativum P. K. B. grown on potato dextrose agar at the following temperatures:

(1) 3° — 6°C., (2) 12° — 13°C., (3) 15° — 18°C.,
(4) 20° — 23°C., (5) 27° —28° C., (6) 31° — 33°C.

Incubated 7 days.

PLATE III

Marquis wheat showing the effect of *Helminthosporium* root-rot in the following types of soil:

A 1. Heavy loam, soil inoculated with *H. sativum* P.K.B.
A 1C. Heavy loam, uninoculated, sterilized soil
A 2. Sandy loam, soil inoculated with *H. sativum* P.K.B.
A 2C. Sandy loam, uninoculated, sterilized soil
A 3. Sand, soil inoculated with *H. sativum* P.K.B.
A 3C. Sand, uninoculated, sterilized soil.
A 4. Peat, soil inoculated with *H. sativum* P.K.B.
A 4C. Peat, uninoculated, sterilized soil.

PLATE IV

Lion barley plants, 3 weeks old, growing in soil inoculated with the following organisms:

1. *Helminthosporium sativum* P.K.B. Culture 82a, isolated from stunted barley plants in Minnesota
2. *Helminthosporium* isolated from stunted wheat plants in Illinois
3. *Fusarium culmorum* (W. Sm.) Saccardo
4. Uninoculated, sterilized soil

PLATE V

Lion barley plants, 3 weeks old, showing effect of the following soil organisms on development of root systems

1. *Helminthosporium sativum* P.K.B. Culture 82a
2. *Helminthosporium* from stunted wheat plants in Illinois
3. *Fusarium culmorum* (W. Sm.) Saccardo
4. Normal roots grown in uninoculated, sterilized soil

PLATE VI

Lion barley plants, 3½ weeks old, showing effect of root infection by *Helminthosporium sativum* P.K.B. The eight seedlings on the left were grown in inoculated soil, the one on the right in uninoculated, sterilized soil.

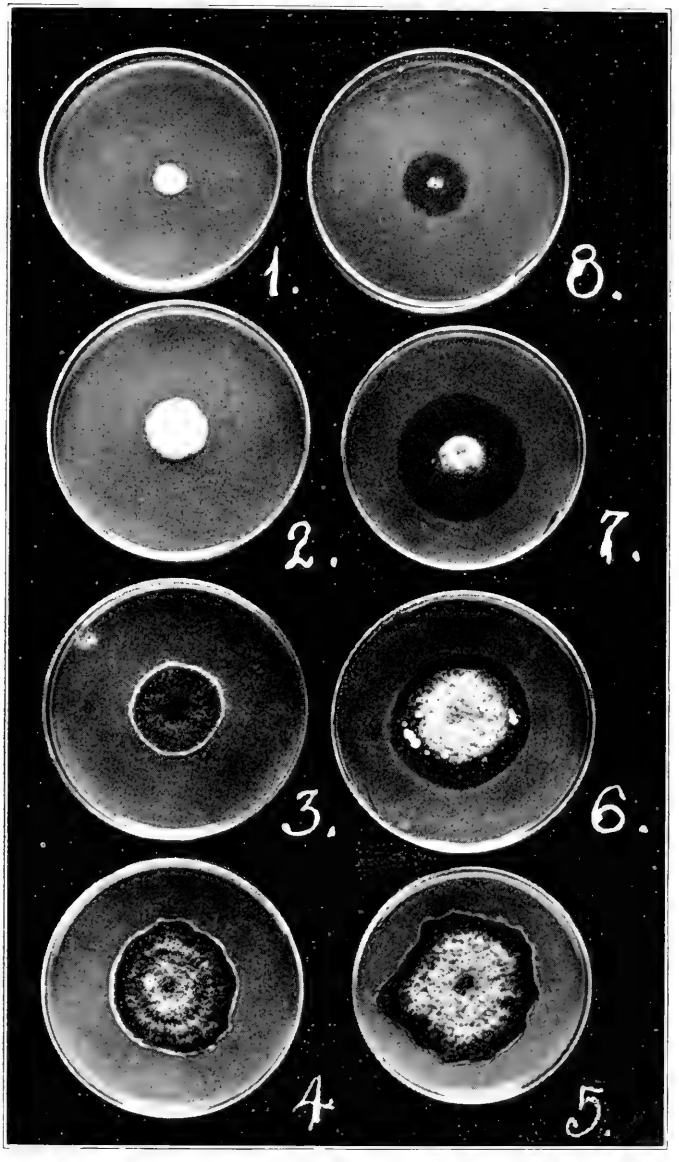

PLATE I

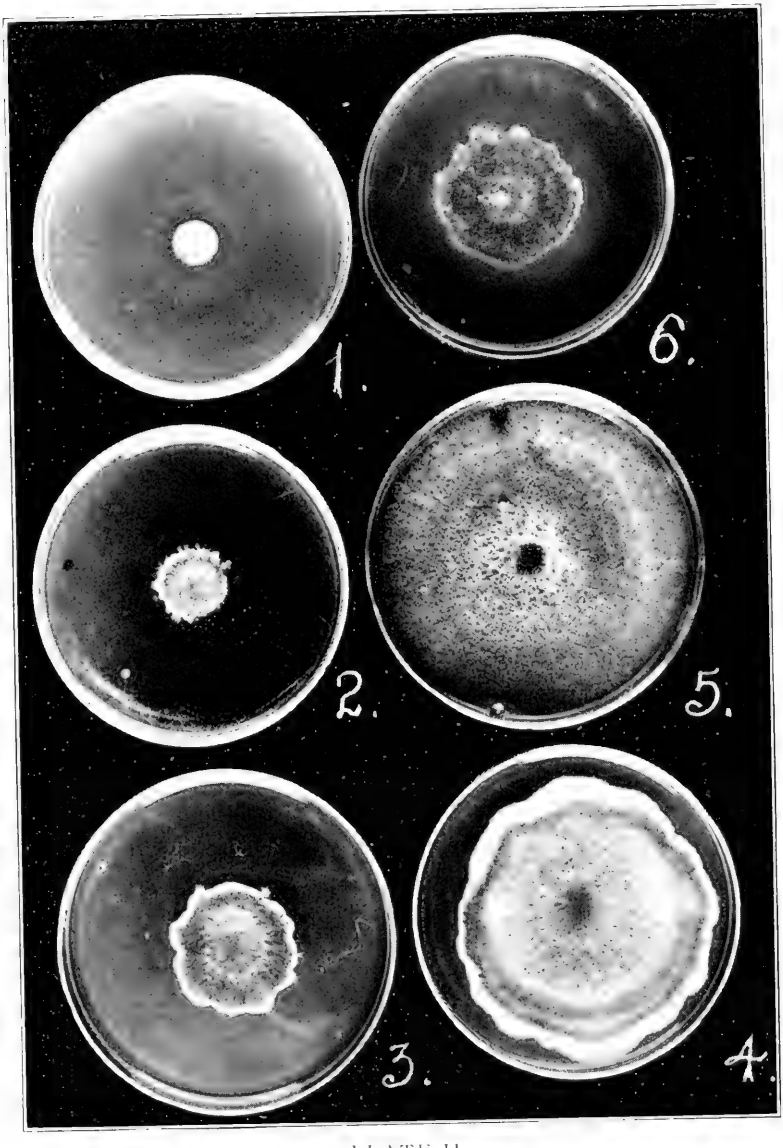

PLATE II

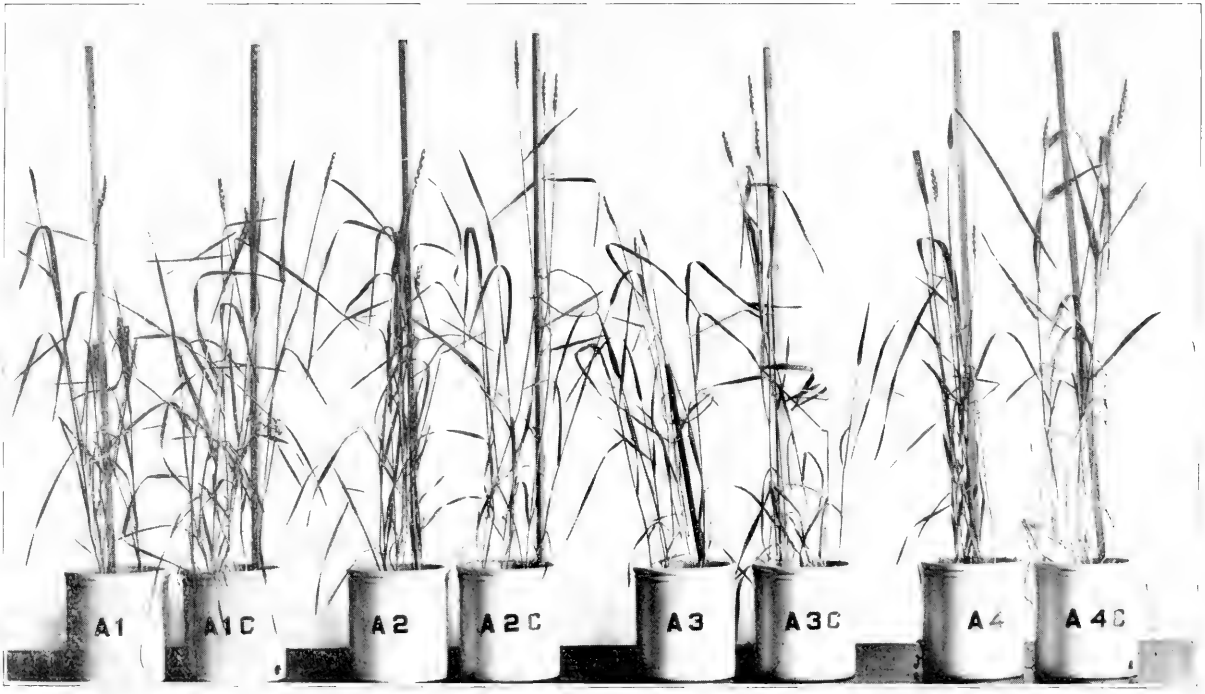

PLATE III

PLATE IV

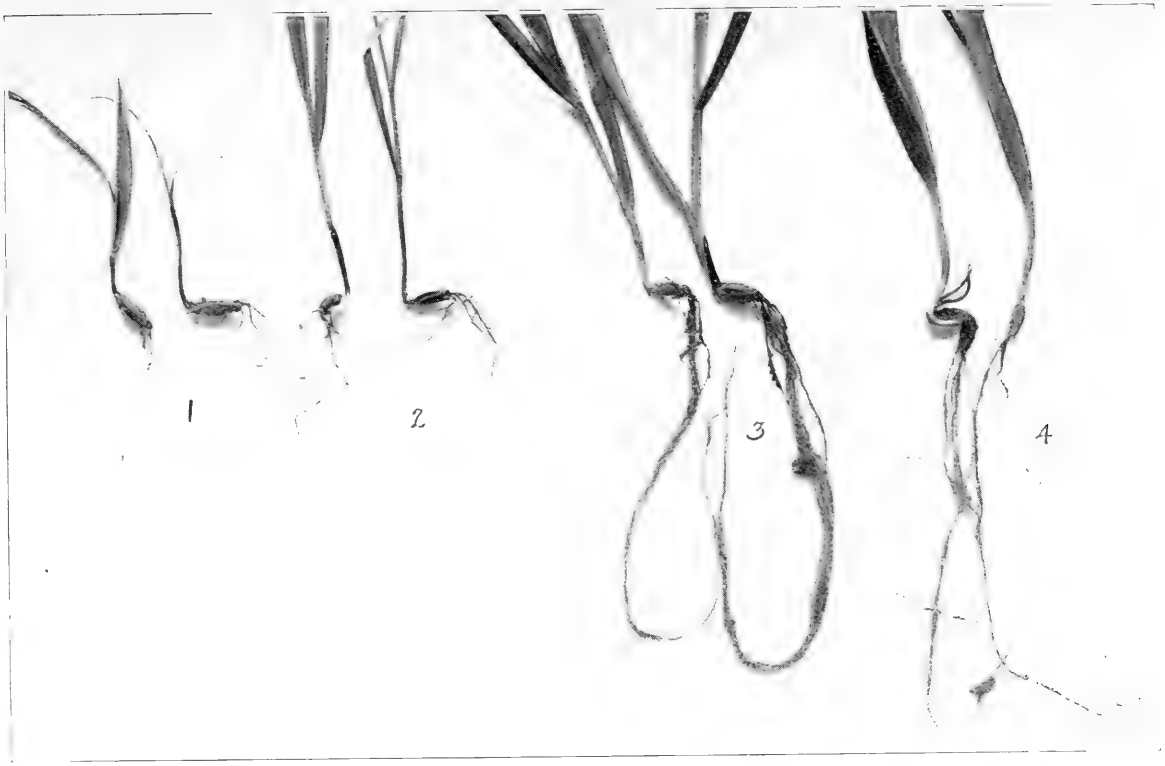

PLATE V

PLATE VI

Louise Dosdall was born in Waco, Texas, December 11, 1893. In 1901 her parents moved to LeSueur, Minnesota. She attended the public schools there, and then finished the grammar schools at St. Paul, Minn. From 1908 to 1912 she attended Humboldt High School, in St. Paul.

In 1912 she entered the College of Science, Literature, and the Arts of the University of Minnesota. As an undergraduate, she majored in botany, specializing particularly in plant ecology. Her minor work was in chemistry and education. In 1916 she received the B.A. degree. During the following year she acted as teaching fellow in botany at Macalester College, St. Paul, and pursued graduate work in plant ecology and plant pathology at the University of Minnesota, receiving the M.A. degree in 1917.

From June, 1917, to July, 1920, she was a half-time assistant in plant pathology in the College of Agriculture, University of Minnesota, devoting the remainder of her time to graduate work in plant pathology and mycology.

Since July 1920 she has been an instructor in the College of Agriculture and Mycologist in the Agricultural Experiment Station of the University of Minnesota.