STUDIES OF THE PATHOGENICITY OF *Helminthosporium sativum* P. K. AND B. ON WHEAT

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Bachelor of Science

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1956

Submitted to the Faculty of the Graduate School
of the Oklahoma State University in partial
fulfillment of the requirements for
the degree of
MASTER OF SCIENCE
May, 1959

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ACKNOWLEDGMENTS

The writer wishes to express his appreciations to Dr. Harry C. Young, Jr. for constructive criticism during the course of this study and his aid in the preparation of the manuscript.

The writer is also indebted to Dr. W. W. Hansen and Dr. J. E. Thomas for reading the final copy.

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INTRODUCTION

The organism Helminthosporium sativum Pammel, King, and Bakke, has been known to cause wheat root and crown rot as well as leaf blotch since 1910. A serious outbreak of the disease, then called "late blight", occured in Iowa in 1909 and brought considerable attention to the disease. In 1922, losses from H. sativum were reported by Hamblin (8), to be as high as 85 to 90 percent of some fields in New South Wales, Australia. During the past 50 years numerous studies have been made of the organism and the disease caused by it, and several investigators have presented observations on the effect of climate upon the development of the organism on its host. They indicate or suggest that the temperature and available moisture greatly influence or even limit the amount of infection which occurs. Other reports have dealt with the competitive ability of H. sativum and suggest that soil type, particularly organic content, is an important factor in disease development. Indeed, the only control measure now recommended for wheat or barley root rot control is rotation with a legume which will increase the organic content of the soil.

In the present study an attempt was made to determine the effect of varieties, depth of planting, soil type, and available soil moisture on disease development. Also, an attempt was made to devise a method for testing the resistance or susceptibility of a large number of varieties in a relatively short period of time in the greenhouse.

LITERATURE REVIEW

Stevens (16), 1919, cites literature referring to root-rot of wheat in France as early as 1849, and from most other parts of the world at later dates.

In 1909, Pammel (10) described a wheat blight and barley browning which caused the affected plants to mature much earlier than healthy plants with a whitish appearance of the head instead of a golden yellow. He found that the disease symptoms appeared about the time the head began to mature. The disease was attributed to Helminthosporium sativum in 1910 (11).

Backwith (1) made a survey of wheat fields in North Dakota which were showing reduction in yield and found the soil infested with species of Helminthosporium, Colletotrichum, and Fusarium, which were not present in large numbers in virgin soil. Spores or mycelium of these fungi were found on the wheat plants grown on the infested soil. In his opinion the wheat was infected with these fungi, and some had caused lesions on the roots. He stated that light, heat, and moisture produced marked differences in total infection.

Bolley (2), in 1913, called attention to the seriousness of root- and foot-rot of wheat, and stated that constant cropping of wheat on the same land caused wheat sickness and wheat sick soil. He maintained that this sickness of the soil was not due to a lack of essential elements or to permanent excrements or poisons detrimental to succeeding crops, but that it was caused by fungi, especially Helminthosporium, Alternaria, Fusarium, and Colletotrichum.

In 1920, Stakman (14) demonstrated by cross inoculation that Helminthosporium sp., apparently identical with Helminthosporium sativum, caused root-rot and seedling blight of wheat and rye as well as spot blotch of barley. She also found that weather conditions were intimately involved in infection, and cited a case of rapid development of the disease during an interval of dry weather preceded by a week of rain.

Stevens (17) reported, in 1920, that a species of Helminthosporium was constantly associated with the root-rot disease of wheat. Inoculation of wheat with the organism gave positive results, and he concluded that Helminthosporium was the cause of the disease. Here again the seriousness of the disease depended upon the environmental conditions of climate and soil.

Later, Stevens (18) conducted inoculation tests upon wheat seedlings under aseptic conditions. He reported from these studies that the mycelium was found to have entered the host cells within 24 hours, and within 48 hours, to produce a browned diseased spot visible without magnification.

McKinney (9), in 1922, studied strains of Helminthosporium and found that those isolated from wheat appeared to be very similar if not identical to those isolated from spot blotch lesions on barley. Cross inoculations made with the wheat and barley strains of the organism on the foliage and the underground portions of the tillers of wheat and barley showed that both produced the typical symptoms on both hosts. He found also, through the use of controlled soil-temperature experiments, that the optimum soil temperature for the development of H. sativum is between 26 and 28 degrees centigrade.

In 1922, Stakman, et. al. (15), through the use of ordinary microscope slides smeared with vaseline and placed in a mechanical spore trap on an airplane, collected Helminthosporium spores as high as 10,000 feet, thus demonstrating the movement of this type of spore by air currents.

Stevens (19), in 1922, presented evidence agreeing with Beckwith (1) that Helminthosporium was the cause of the basal rot of wheat stems. It was found to be the only parasite constantly present, and was repreatedly, by several methods, proved to be capable of causing such rot.

Christensen (3), in 1922, made an extensive study of the pathogenicity of Helminthosporium sativum. He was able to infect 98 species of grass with H. sativum. Wheat, barley, and rye were the most susceptible cereals, while oats were either immune or highly resistant.

Dosdall (6) studied the factors influencing the pathogenicity of H. sativum, in 1923, and found that the optimum temperature for the growth of the organism was 28 degrees centigrade. Leaf infection increased directly with the amount of moisture present, in her studies, and root and foot infection were 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.

Christensen (4), in 1925, described numerous physiologic forms of H. sativum. He studied 37 distinct forms in detail and reported that they differed in many cultural characteristics as well as pathogenicity. Later (5), he stated that the development of the disease in wheat depended upon several factors: The physiologic form present; amount of inoculum produced; effect of enviromental factors on development of the pathogen; varietal susceptibility; and factors predisposing the host. Heavy dews and rains, together with high temperatures, were conductive to foliage and spike infection. He found the disease most severe on peat soil with deep drainage, and that plants seem to be most susceptible to the disease from the milk stage on. He concluded that temperature and moisture affect the development of the disease, but that the virulence of the particular form concerned is often more important than any of the enviromental conditions.

Greaney (7), in 1938, showed that there is a significant varietal difference in the resistance of wheat to H. sativum. He made two types of inoculation tests, soil and seed. The soil was inoculated with finely ground oat hulls overgrown with the fungus. The seed were inoculated by dipping them into a solution of spores and mycelial fragments. The susceptibility or resistance of a variety was determined by the percent of emergence, the infection found on roots and crown 10 days before full maturity, and the yield.

In 1950, Simmonds, et. al. (13) made a study of the occurrence of H. sativum in relation to primary infection in common root-rot of wheat. They were of the opinion that primary infection develops from conidia, found in or on the surface soil or on stubble.

Tinline (20), in 1951, produced the ascogenous stage of H. sativum, Cochliobolus sativus (Ito and Kurib) Drechsler ex Dastur. This was accomplished by growing two sexuality compatable strains of H. sativum together. Apparently the sexual stage is seldom, if ever, found in nature.

MATERIALS AND METHODS

The strain of Helminthosporium sativum P. K. and B. used throughout these studies was isolated from an infected wheat seedling. In culture it has been grown on potato dextrose agar and potato dextrose broth. Isolation was accomplished by incubating the infected wheat seedlings in a moist chamber at 28 degrees centigrade until spores were produced. The spores were then picked off singly and placed upon petri-dishes of potato dextrose agar and incubated at 28 degrees centigrade for growth.

Inoculum for the experiments was prepared by allowing the organism to grow for 15 days on 60 ml of potato dextrose broth in a 250 ml Erlenmeyer Flask at 28 degrees centigrade, after which the mycelial mat was placed in a blender with 50 ml of water and macerated for 2 minutes. An additional 250 ml of water was then added.

The first experiment consisted of the following factors: two contrasting soil types; two varieties of wheat; two depths of planting; and three soil moisture levels. The soils were taken from two locations in Oklahoma. One, a loamy sand soil, came from near Dover and the other, a clay loam soil, cam from near Tonkawa. These soils are characterized in Table I. The soils were placed in one gallon glazed crocks with the drain hole plugged to prevent any water loss through the bottom. The crocks were filled with the same quantity of soil by weight, and 100 ml of inoculum was mixed with the soil in each crock at the time they were filled.

TABLE I

CERTAIN PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE SOILS FROM NEAR DOVER (LOAMY SAND) AND TONKAWA (CLAY LOAM), OKLAHOMA[1]

Test		Loamy Sand	Clay Loam
Available Phosphorus		57.76 lb./A	104.96 lb./A
Available Potassium		620.00 lb./A	590.00 lb./A
Available Sodium		75.00 lb./A	110.00 lb./A
Cation Exchange Capacity		2.83	17.09
pH		6.15	5.65
Total Nitrogen		0.54%	0.12%
Organic Matter		0.33%	2.76%
Classification of Soil Particles			
	Sand	83.75%	29.25%
	Silt	10.50%	33.00%
	Clay	5.75%	36.25%

[1]Analysis made by Harold R. Myers, student, Department of Agronomy, Oklahoma State University, Stillwater, Oklahoma.

Each soil type was subjected to three moisture treatments; dry, wet, and variable. The dry treatment was accomplished by supplying the minimum quantity of water which would support growth of the plants. This amount was adjusted periodically as the plants grew. The wet treatment was in excess of the amount of water necessary for optimum plant growth. The total weight of the crock and soil was measured and water was added to each crock until an arbitrary preselected weight was reached which was dependent upon the soil type. Also, this preselected weight had to be adjusted upward from time to time due to increased plant growth. The variable treatment was accomplished by watering for one week in the same manner as the wet treatment, then allowing the crocks in this series to stand without water until the plants showed signs of water deficiency. They were then watered in the same manner as the dry treatment. This variable series was alternated between the wet and dry treatments at weekly intervals throughout the course of the experiment. The average quantities of water added to each crock of the dry and wet treatments are listed in Tables II and III. Maximum distribution of the water added to the soil was attained by inserting a plastic tube 1.5 cm in diameter and 21 cm long in the center of each crock and through which the water was added. The tubes were perforated with 10 holes approximately 2 mm in diameter punched at intervals in the lower third of the tube. The opening in the bottom of the tubes were plugged with a cork and the tube was placed in the soil when the crock was filled.

The wheat varieties Wichita and Improved Bluejacket were planted in each moisture treatment. The seed was carefully selected for clean plump kernels. Each variety was planted at two depths, $\frac{1}{2}$ inch and 1 inch. Three seeds of each variety were planted in each crock. There were 10 crocks of each soil type for each planting depth and moisture treatment,

TABLE II

AVERAGE QUANTITY OF WATER ADDED TO LOAMY SAND SOIL PER CROCK, PER DAY

Time Period	Treatment	Wet Planting Depth $\frac{1}{2}$"	Wet Planting Depth 1"	Dry[1]
July 13-Aug. 14	Inoculated	56.0 ml	54.0 ml	15.0 ml
	Check 1[2]	47.0	54.3	15.0
	Check 2	51.0	51.0	15.0
Aug. 15-Sept. 25[3]	Inoculated	6.4	6.6	4.3
	Check 1	5.5	11.5	4.3
	Check 2	3.7	7.1	4.3
Sept. 26-Oct. 31	Inoculated	44.8	46.9	6.0
	Check 1	40.9	49.3	6.0
	Check 2	50.4	71.0	6.0
Nov. 1-Nov. 30	Inoculated	46.9	68.8	6.0
	Check 1	72.1	93.0	6.0
	Check 2	79.8	57.7	6.0
Dec. 1-Dec. 31	Inoculated	63.8	83.8	8.0
	Check 1	63.6	93.6	8.0
	Check 2	129.0	98.1	8.0
Jan. 1-Jan. 31	Inoculated	70.8	84.9	13.3
	Check 1	74.0	84.5	13.3
	Check 2	79.8	121.1	13.3
Feb. 1-Feb. 28	Inoculated	78.8	90.8	22.5
	Check 1	80.7	92.1	22.5
	Check 2	156.9	146.2	22.5

[1]Both depths of planting in the dry treatment were given the same quantity of water.

[2]Check 1 = uninoculated check crock filled with loamy sand soil; check 2 = uninoculated check crocks filled with greenhouse soil mixture.

[3]The plants were vernalized during this period.

TABLE III

AVERAGE QUANTITY OF WATER ADDED TO CLAY LOAM SOIL PER CROCK, PER DAY

Time Period	Treatment	Wet Planting Depth ½"	1"	Dry[1]
Sept. 21-Oct. 18	Inoculated	38.0 ml	34.0 ml	6.0 ml
	Check 1[2]	41.0	33.0	6.0
	Check 2	41.0	41.0	6.0
Oct. 18-Dec. 5[3]	Inoculated	9.1	7.7	2.5
	Check 1	8.9	8.3	2.5
	Check 2	7.7	6.4	2.5
Dec. 6-Dec. 31	Inoculated	72.4	73.5	10.0
	Check 1	72.7	76.4	10.0
	Check 2	81.8	85.5	10.0
Jan. 1-Jan. 31	Inoculated	65.5	70.8	20.0
	Check 1	63.1	81.1	20.0
	Check 2	87.4	105.6	20.0
Feb. 1-Feb. 28	Inoculated	68.6	70.7	25.0
	Check 1	70.5	90.2	25.0
	Check 2	95.7	114.6	25.0
Mar. 1-Mar. 20	Inoculated	98.6	105.3	35.0
	Check 1	118.5	120.2	35.0
	Check 2	164.0	164.7	35.0

[1]Both depths of planting in the dry treatment were given the same quantity of water.

[2]Check 1 = uninoculated check crock filled with clay loam soil; check 2 = uninoculated check crock filled with greenhouse soil mixture.

[3]The plants were vernalized during this period.

or a total of 60 crocks. Uninoculated check crocks were planted in the two types of soil, previously discribed, and a check crock was also planted using a greenhouse soil mixture containing 4 parts silty clay loam soil, 1 part sand, 1 part peat moss, and 1 part dehydrated, pulverized cattle manure.

This experiment was conducted in an air conditioned greenhouse where the temperature was maintained at 20 degrees centigrade except during the summer months when the temperature rose to 28 or 32 degrees centigrade for a few hours at midafternoon.

When the plants were 30 days old half of the crocks were emptied and the soil was washed from the roots of the plants. The volume and weight of each plant was measured, and the number of disease lesions on each plant was counted. The plant volume was measured by partically filling a graduated cylinder with water and submerging the plant in the water and reading the increase in volume on the cylinder. The plant weight was measured on a beam balance. The lesions counted were those visable to the naked eye on the roots and mesocotyl tissue. The plants in the remaining crocks were vernalized for a period of 6 weeks at 3 to 4 degrees centigrade. The water added during the vernalization period was stored in the cold chamber so that it would not increase the temperature of the soil when applied.

After vernalization the plants were allowed to grow to maturity when data were taken on: (1) the days to heading; (2) root volume; (3) extent of visible infection; (4) degree of discoloration of the crown; (5) height of the plants; and (6) the number of sterile and dead tillers.

The number of days to heading was measured when the head was fully exerted. The root volume was measured by inserting those portions of the plant below the node of the first leaf into a graduated cylinder partially filled with water and reading the increase in volume on the cylinder. The extent of visible infection was a measure of the extent of discoloration of the plant from the base of the crown upward through the tillers. The degree of discoloration of the crown was recorded as shades of tan or black. The dead and living tillers were counted and the latter were classified as to whether a fertile head had or had not been produced. All measurments were recorded as an average per plant.

Subsequently, two variety tests were made with wheat and barley seedlings. The first test included 59 varieties or selections of wheat and 22 varieties of barley, these are listed in Table XVI. The seeds were planted in wood flats 20 x 15 x 3 inches in size. Ten seeds of each variety were planted in 4 inch rows. Each flat contained 30 rows. The soil used was a mixture of 4 parts silty loam soil, 1 part sand, 1 part peat moss, and 1 part dehydrated, pulverized cattle manure. The soil was sterilized for 6 hours at 15 lbs. steam pressure prior to planting. When the seedlings were approximately 3 inches tall they were sprayed with the previously described inoculum at a rate of 200 ml per flat. After inoculation the plants were incubated for 24 hours in a moist chamber. Two weeks following inoculation the percent of the first leaf killed by the organism was recorded.

The second variety test consisted of 20 varieties of wheat and 20 varieties of barley. There were 25 seeds of each variety planted in a 15 inch row at the rate of 10 rows per flat. The soil used was a mixture of one-half sand and one-half silty loam soil which was sterilized for 6 hours at 15 lbs. steam pressure. At the time of planting 30 ml of the inoculum was poured into each row. A second series was planted without the inoculum as a check. After 21 days the number of living plants was recorded for each variety.

RESULTS

An experiment was conducted to determine the effect of Helminthosporium sativum on two varieties of wheat planted at two depths in two contrasting soil types and subjected to three moisture conditions. The seedlings were allowed to grow for 30 days, at which time half of them were removed from the soil and measurments were taken on the plant weight and volume, and a count was made of the number of disease lesions per plant.

An examination of the 30 day old seedlings grown in loamy sand soil revealed that plant weight and volume increased with the amount of water applied. Plants in the variable and wet moisture treatments were much larger than those in the dry treatment. Depth of planting had little if any influence upon the size or weight of the seedlings at this stage. The number of lesions per plant also was found to have increased with the amount of water applied. In addition, the number of lesions per plant increased with the depth of planting. These data are reported in Table IV.

The seedlings grown in the clay loam soil attained the greatest plant weight and volume in the variable moisture treatment, with the least amount of growth shown in the dry moisture treatment. However, the number of lesions per plant increased with the amount of moisture and also with the depth of planting, in the same manner as the seedlings grown in the loamy sand soil. These data are listed in Table V.

The remaining half of the plants were vernalized and grown to maturity. When the plants were mature data were taken on: (1) the number of days required for heading; (2) the height of the plants; (3) the root volume;

TABLE IV

THE VOLUME, WEIGHT AND NUMBER OF LESIONS ON THE ROOTS OF 30-DAY OLD WHEAT SEEDLINGS GROWN IN LOAMY SAND SOIL INOCULATED WITH *Helminthosporium sativum*

	Moisture Treatment Average of 20 Plants		
	Dry	Variable	Wet
Weight Per Plant			
½" Planting Depth	2.4 gm	3.6 gm	3.9 gm
1" Planting Depth	2.4 gm	3.8 gm	3.8 gm
Volume Per Plant			
½" Planting Depth	1.8 ml	2.7 ml	3.1 ml
1" Planting Depth	1.8 ml	2.9 ml	2.6 ml
Lesions Per Plant			
½" Planting Depth	0.1	0.4	0.9
1" Planting Depth	0.2	0.5	2.1

TABLE V

THE VOLUME, WEIGHT AND NUMBER OF LESIONS ON THE ROOTS OF 30-DAY OLD WHEAT SEEDLINGS GROWN IN CLAY LOAM SOIL INOCULATED WITH *Helminthosporium sativum*

	Moisture Treatment Average of 20 Plants		
	Dry	Variable	Wet
Weight Per Plant			
½" Planting Depth	1.4 gm	3.1 gm	1.9 gm
1" Planting Depth	1.2 gm	2.1 gm	1.6 gm
Volume Per Plant			
½" Planting Depth	1.8 ml	4.0 ml	2.6 ml
1" Planting Depth	1.6 ml	2.8 ml	2.3 ml
Lesions Per Plant			
½" Planting Depth	0.4	0.6	1.3
1" Planting Depth	0.6	1.4	2.4

(4) the type of tillers produced (whether fertile or sterile, living or dead); (5) the intensity of discoloration of the crown; and (6) the extent of visable infection upward from the base of the crown.

The effect of planting depth and soil moisture condition on the days required for heading of the early variety Wichita and the late maturing variety Improved Blue Jacket planted in inoculated loamy sand soil is given in Table VI. These data indicate there was very little difference

TABLE VI

THE NUMBER OF DAYS REQUIRED FOR HEADING OF TWO VARIETIES OF WHEAT PLANTED IN LOAM SAND SOIL INOCULATED WITH *Helminthosporium sativum*

	No. of Days Required for Heading of:			
	Wichita		Improved Blue Jacket	
	Planting Depths		Planting Depths	
Moisture Level	½"	1"	½"	1"
Dry	215[1]	209	224	217
Variable	215	204	221	216
Wet	214	208	222	220

[1]Each figure is the average of 5 plants.

between the moisture levels used. However, both varieties when planted ½ inch deep required from 2 to 11 days more time to head than those planted 1 inch deep. The fact that those plants seeded at the 1 inch depth required less time to head may be correlated with the number of lesions found on the seedlings, indicating a hastening of maturity caused by infection by *H. sativum*.

The height attained by the plants grown in loamy sand soil increased generally with the amount of water applied (Table VII). No consistant difference was noted between the two planting depths. Wichita, which is normally a tall variety, attained more height in all treatments than Improved Blue Jacket.

TABLE VII

THE HEIGHT OF TWO VARIETIES OF WHEAT GROWN IN
LOAMY SAND SOIL INOCULATED WITH
Helminthosporium sativum

Moisture Level	Height in Centimeters of: Wichita Planting Depths ½"	1"	Improved Blue Jacket Planting Depths ½"	1"
Dry	43[1]	41	22	26
Variable	53	50	41	36
Wet	57	59	35	50

[1]Measured from the ground level to the tip of the head excluding the awns. Each figure is the average of 5 plants.

The root volume of the mature plants grown in loamy sand soil was found to be greatest when the plants were grown in the variable moisture treatment (Table VIII). Those plants grown at the dry moisture level were found to have the smallest roots. The root volume of plants seeded at the ½ inch level tended to be somewhat larger than those seeded at the 1 inch level, but this difference was minor and not consistant. The plants of Wichita, in spite of being early in maturity, had a uniformly larger root volume than the plants of Improved Blue Jacket.

TABLE VIII

THE ROOT VOLUME OF MATURE PLANTS GROWN IN
LOAMY SAND SOIL INOCULATED WITH
Helminthosporium sativum

Moisture Level	Root Volume in ml. of: Wichita Planting Depths ½"	1"	Improved Blue Jacket Planting Depths ½"	1"
Dry	4.8[1]	2.9	2.0	3.4
Variable	15.0	10.2	8.2	7.8
Wet	8.5	9.7	7.2	7.0

[1]Each figure is the average of 5 plants.

The total number of tillers per plants grown in loamy sand soil increased generally with the amount of water added (Table IX). Planting depth had little influence upon the total number or classification of tillers, and both varieties seemed to react the same in the total production of tillers. The proportion of dead tillers was higher in the variety Wichita than in Improved Blue Jacket, but the proportion of sterile tillers was higher in Improved Blue Jacket than in Wichita. This was particularly true at the higher moisture levels.

The crown discoloration and extent of visible infection also increased with the amount of moisture applied. These data are presented in Table X. At the dry moisture level there was more intensive and extensive discoloration of the plants grown at the 1 inch level than at the ½ inch level, but this difference was observed with increased amounts of moisture.

Similar data were taken on the plants grown in clay loam soil inoculated with H. sativum. These data are presented in Tables XI, XII, XIII, XIV, and XV.

The number of days required for Wichita and Improved Blue Jacket to head indicated that planting depth had a more consistant effect on maturity than the moisture level. Both varieties generally required less time to head when planted 1 inch deep, but the variety Improved Blue Jacket was more influenced by this factor than Wichita. Indeed, in the clay loam soil, Improved Blue Jacket headed earlier than Wichita when planted at the 1 inch level. This was contrary to the field maturity of these two varieties.

Much less time was required for heading of plants in the clay loam soil than in the sandy loam soil. This may have been due to the different times at which these experiments were made, or may again be an

TABLE IX

THE TILLER CLASSIFICATION OF TWO VARIETIES OF WHEAT GROWN IN LOAMY SAND SOIL INOCULATED WITH *Helminthosporium sativum*

	Average No. of Tillers Per Plant of:							
	Wichita				Improved Blue Jacket			
	Living		Dead	Ave. Total	Living		Dead	Ave. Total
	F[1]	S			F	S		
Dry								
½" Planting Depth	0.8[2]	1.8	0.4	3.0	0.6	0.8	0.8	2.2
1" Planting Depth	0.8	0.6	0.4	1.8	0.4	2.2	0.8	3.4
Variable								
½" Planting Depth	1.4	2.8	0.6	4.8	1.0	3.4	0.6	5.0
1" Planting Depth	1.6	2.0	1.6	5.2	0.8	5.0	0.6	6.4
Wet								
½" Planting Depth	2.0	2.6	2.2	6.8	1.4	4.4	0.2	6.0
1" Planting Depth	2.8	2.0	1.8	6.6	1.8	3.4	1.8	7.0

[1]F = Fertile tillers; S = Sterile Tillers.

[2]Each figure is the average of 5 plants.

TABLE X

THE CROWN DISCOLORATION AND EXTENT OF VISABLE INFECTION OF TWO VARIETIES OF WHEAT GROWN IN LOAMY SAND SOIL INOCULATED WITH *Helminthosporium sativum*

Average Extent and Intensity of Discoloration in:	Wichita		Improved Blue Jacket	
	Inf.[1]	Disc.[2]	Inf.	Disc.
Dry				
½" Planting Depth	2.0[3]	2.6	2.0	2.8
1" Planting Depth	3.0	3.4	2.2	2.8
Variable				
½" Planting Depth	3.6	3.4	3.8	3.8
1" Planting Depth	3.4	3.8	3.6	3.4
Wet				
½" Planting Depth	4.0	3.6	3.8	3.6
1" Planting Depth	4.0	3.1	3.8	4.0

[1]Inf. = Extent of visable infection: 1 = No discoloration; 2 = Crown discolored; 3 = discoloration into tillers less than 1 cm; 4 = discoloration into tillers more than 1 cm.

[2]Disc. = Crown discoloration: 1 = none; 2 = light tan; 3 = dark tan; 4 = chocolate brown or black.

[3]Each figure is the average of 5 plants.

indication of the hastening of maturity caused by infection with *H. sativum* since there was a greater degree of infection in the clay loam soil than there was in the sandy loam soil (Tables IV and V).

TABLE XI

THE NUMBER OF DAYS REQUIRED FOR HEADING OF TWO VARIETIES OF WHEAT PLANTED IN CLAY LOAM SOIL INOCULATED WITH *Helminthosporium sativum*

	No. of Days Required for Heading of:			
	Wichita		Improved Blue Jacket	
	Planting Depths		Planting Depths	
Moisture Level	½"	1"	½"	1"
Dry	170[1]	169	181	158
Variable	167	161	170	155
Wet	165	170	177	165

[1]Each figure is the average of 5 plants.

The height of the mature plants grown in the clay loam soil was found to increase with the moisture level. The variety Wichita was generally taller, and the depth of planting had little effect on the height of either variety.

TABLE XII

THE HEIGHT OF TWO VARIETIES OF WHEAT GROWN IN CLAY LOAM SOIL INOCULATED WITH *Helminthosporium sativum*

	Height in Centimeters of:			
	Wichita		Improved Blue Jacket	
	Planting Depths		Planting Depths	
Moisture Level	½"	1"	½"	1"
Dry	28[1]	57	41	37
Variable	54	60	53	58
Wet	58	49	38	41

[1]Measured from the ground level to the tip of the head excluding the awns. Each figure is the average of 5 plants.

The average root volume of mature plants grown in clay loam soil, listed in Table XIII, was found to vary with variety, moisture level and planting depth. The plants grown in the variable moisture treatment were found to have the greatest root volume with the least root volume in the dry moisture treatment. As with the height, the lack of water probably limited root development at the dry moisture level. However, the somewhat smaller root volume in the wet moisture treatment may be correlated with the amount of infection found in the seedlings and mature plants, where the infection increased with the amount of water added.

Data taken on the type of tillers produced by Wichita and Improved Blue Jacket grown in clay loam soil showed that the latter variety tended to have more tillers than Wichita. However, there were proportionately more dead tillers and somewhat more sterile tillers in Improved Blue Jacket than in Wichita. The total number of tillers increased and the proportion of dead tillers tended to increase with increased moisture. Planting depth seemed to have little or no effect upon tiller development.

TABLE XIII

THE ROOT VOLUME OF MATURE PLANTS GROWN IN CLAY LOAM SOIL INOCULATED WITH *Helminthosporium sativum*

	Root Volume in ml. of:			
	Wichita Planting Depths		Improved Blue Jacket Planting Depths	
Moisture Level	½"	1"	½"	1"
Dry	2[1]	4	2	2
Variable	4	5	5	5
Wet	5	4	4	3

[1]Each figure is the average of 5 plants.

grown in clay loam soil, summarized in Table XV, was found to be greatest at the 1 inch planting depth. There was little or no difference between varieties. The effect of depth of planting was more consistantly evident than the effect of moisture levels. However, the discoloration tended to increase with moisture, the wet level being more severely discolored than either the dry or variable levels.

There was apparently very little difference in the two varieties used in these studies, although they were thought to be quite different in reaction to H. sativum in the field. Consequently, an experiment was devised to test a large number of varieties for their reaction to H. sativum in the seedling stage. Table XVI shows the results of this test in which the inoculum was sprayed on the leaves of various wheat and barley strains. Several varieties of wheat showed some resistance to this organism in the seedling stage. Those showing the most resistance were Frisco, Westar, Cimarron, and selections of the crosses Triticum Sp.- Agropyron elongatum x Ponca and Ponca x Triticum Sp.-Agropyron elongatum - Pawnee. Several other varieties of wheat and barley had lesser degrees of resistance.

A second test was made in which the inoculum was spread over the seed at planting time. The most resistant wheat and barley strains in the study just reported were planted in this test, together with a few of the most susceptible strains. However, no differiential effect could be recognized and the inoculated flats were equally as healthy as the uninoculated check flats.

TABLE XIV

THE TILLER CLASSIFICATION OF TWO VARIETIES OF WHEAT GROWN IN CLAY LOAM SOIL INOCULATED WITH *Helminthosporium sativum*

	Average No. of Tillers Per Plant of:							
	Wichita				Improved Blue Jacket			
	Living F[1]	Living S	Dead	Ave. Total	Living F	Living S	Dead	Ave. Total
Dry								
½" Planting Depth	0.2[2]	0.4	0.8	1.4	0.6	1.0	0.0	1.6
1" Planting Depth	1.0	0.4	0.2	1.6	0.6	0.2	0.2	1.0
Variable								
½" Planting Depth	0.8	0.6	1.6	3.0	1.4	2.0	1.4	4.8
1" Planting Depth	1.4	0.0	2.0	3.4	1.2	0.6	2.0	3.8
Wet								
½" Planting Depth	1.6	1.0	1.6	4.2	1.4	1.0	2.2	4.6
1" Planting Depth	1.2	0.6	2.4	4.2	1.0	1.0	3.4	5.4

[1]F = Fertile tillers; S = Sterile Tillers.

[2]Each figure is the average of 5 plants.

TABLE XV

THE CROWN DISCOLORATION AND EXTENT OF VISABLE INFECTION OF TWO VARIETIES OF WHEAT GROWN IN CLAY LOAM SOIL INOCULATED WITH *Helminthosporium sativum*

Average Extent and Intensity of Discoloration in:	Wichita		Improved Blue Jacket	
	Inf.[1]	Disc.[2]	Inf.	Disc.
Dry				
½" Planting Depth	2.2[3]	1.8	1.2	1.2
1" Planting Depth	2.0	1.8	2.8	3.0
Variable				
½" Planting Depth	1.6	1.6	1.2	1.2
1" Planting Depth	2.8	2.2	1.8	1.4
Wet				
½" Planting Depth	2.2	2.2	3.2	3.2
1" Planting Depth	3.0	2.8	3.6	3.4

[1]Inf. = Extent of visable infection: 1 = No discoloration; 2 = Crown discolored; 3 = discoloration into tillers less than 1 cm; 4 = discoloration into tillers more than 1 cm.

[2]Disc. = Crown discoloration: 1 = none; 2 = light tan; 3 = dark tan; 4 = chocolate brown or black.

[3]Each figure is the average of 5 plants.

TABLE XVI

THE REACTION OF CERTAIN 4-WEEK OLD WHEAT AND BARLEY SEEDLINGS TWO WEEKS AFTER INOCULATION WITH *Helminthosporium sativum*

Variety of Selection	C.I. or Selection No.	% 1st Leaf Killed
Wheat		
Cheyenne	8885	80
Comanche	11673	50
Concho	12517	40
Ponca	12128	40
Westar Reselection	Stw55R876	50
Klein Aniversario	12578	70
Wab. x Am. Ban.	12757	30
Tr. sp.-Ag. el. x Paw.	13020	50
Frisco	13106	15
Kanred	5146	50
Crockett	12702	50
Mint. x Ill. #1-Chin.-Tr. timo-Rd. Tk.-Blkhk.	13225	90
Supr-Fultz-Kaw x Fultz-Hung-W38-Fair-Trum3-Hp.-Hus.	13170	50
Wab. x Kl. Aniv.	13227	50
Trum2-Ag. el. x Fultz-Trum-Hp.-Hus.	13228	100
Imp. Blue Jacket	40-H-10	95
Imp. Bl. Jkt. x Com.	13185	95
do.	13186	95
Paw. x Nebred	13015	100
Ponca x Chy.	13019	90
Kan-Hd. Fed.-Tq-Med.-Hp. x Cim	13023	95
Cim-Hp. x Chy-Com.	13024	100
Taylor	12461	95
Front. x Med-Hp.-Paw.	Stw57R6608	90
do.	Stw57R6615	95
Ponca x McMurachy-Exc.-Rdmn.	KS55-793	100
do.	KS55-794	100
4126A9-32-1 x 3848A5-1-36-1 x Rd. Egypt.	45201-1-1-1-5-1	95
Tr-sp-Ag. el. x Paw. Sel.	Stw57R6697	100
Knox2 x 39120A5-3-1-1-1	5324C2-1	95
Westar	12110	15
Ponca x Tr.-Ag. el.-Paw.	Stw56R1528	10
do.	Stw56R1535	95
do.	Stw57R6704	95
do.	Stw57R6711	100

TABLE XVI CON'T

Variety of Selection	C.I. or Selection No.	% 1st Leaf Killed
Com. x Tr. sp.-Ag. el. Paw	Stw56R1542	95
do.	Stw57R6718	100
Mqo.-Oro x Oro-Tq.	12406	100
Cim.-Hp. x Chy.	13022	80
Oro.	8220	95
Cimarron	12120	15
Avoca	13395	95
Kelo	---------	95
Rye x Wheat	WH44H4-9	95
Concho x Tr. sp.-Ag. el. F_8	Stw575604	95
do.	Stw575605	80
do.	Stw575606	95
do.	Stw575607	80
do.	Stw575611	75
do.	Stw575616	95
do.	Stw575625	50
Concho x Tr. sp.-Ag. el. F_8	Stw575639	80
do.	Stw575645	60
do.	Stw575648	50
do.	Stw575649	70
do.	Stw575651	90
do.	Stw575666	95
do.	Stw575667	50
Tr-Sp-Ag. el. x Ponca F_8	Stw575746	15
Barley:		
Harbine	7524	100
Ward	6007	60
Tenkow	646	80
Rogers	9174	90
Kearney	7585	100
Hudson	8067	100
Cordova	7576	95
Kenbar	7574	90
For. Intro. Sel.	Stw514701	40
Mo. B640	7572	60
Colonial #2	8062	50
Omugi	5144	100
Watuaga	9172	90
Mo. B-475	9168	95
Kenate	9570	100
Meimi	5136	95
Ward x Omugi-Ward	53RGD8	95
Ward x Omugi-Ward	53RGD9	95
Ward x Ward-Omugi	53RGD7	100
Dobaku	5238	100
Dayton	9517	40
Seibaku x Tenkow	Stw514604	95
M.E.B. x Texan	9528	100

DISCUSSION

Limitations of space where temperature could be adequately controlled prevented the use of a complete set of uninoculated controls. Consequently, the inoculated series could not be compared directly with uninoculated plants, but only with other inoculated treatments. However, plants were grown in the two field soils and in a sterilized greenhouse soil mixture which were not inoculated. From these controls it was evident that there were some root invading fungi, particularly Helminthosporium sativum, present in both of the field soils. The plants in these contaminated control crocks did not behave similarly to the inoculated series because they were held at an optimum soil moisture level. The plants in the sterilized greenhouse soil remained disease free and grew normally.

The amount of growth made by the seelings planted in inoculated loamy sand soil increased generally from the dry to the wet moisture treatment and with the depth of planting in the dry and variable moisture treatments. However, those plants grown in the wet moisture treatment planted 1 inch deep did not show as much growth as those planted $\frac{1}{2}$ inch deep. It would appear that the retarded growth in the deeper planting depth of the wet moisture treatment was caused by the disease, since the number of lesions of infection increased with both the amount of water added and planting depth. The measurements made on seedlings grown in inoculated clay loam soil indicates greater growth was made in the variable moisture treatment at $\frac{1}{2}$ inch planting depth, and that the greatest infection occured in the wet moisture treatment, and in the 1 inch planting depth of all moisture treatments.

A comparison of the seedlings grown in the two soil types shows that those grown in the loamy sand soil attained more growth and had fewer lesions per plant than those grown in clay loam soil. The number of lesions per seedling was found to be greater on those plants grown in the clay loam soil, with both the Wichita and Improved Blue Jacket varieties. In both soil types, the number of lesions per plant was greatest on the seedlings planted 1 inch deep, and in most treatments Improved Blue Jacket had more lesions per plant than Wichita.

The amount of time required for the heading of varieties was greater in loamy sand soil than in the clay loam soil. In either soil type, no great difference was noted in the effect of soil moisture level on the days required to heading. In general, the plants grown in inoculated soil required more time to head than those grown in soil where the organism was not added. Both varieties, when planted $\frac{1}{2}$ inch deep, required more time to head than when planted 1 inch deep. Since more infection was noted in those plants planted 1 inch deep, it was assumed that the organism was responsible for the earlier maturity.

The height of the plants grown in inoculated soil was somewhat less than those grown in soil free of the organism, and the variety Wichita was taller than Improved Blue Jacket. Increased moisture brought an increase in height, but soil type and depth of planting did not seem to influence plant height.

The root volume of plants grown in the variable moisture treatment was generally greater in both soil types than the root volume of the plants grown at the dry or wet moisture level, and it was noted that most of the plants planted ½ inch deep had more root volume than those planted 1 inch deep. Plants grown in the clay loam soil had considerably less root volume than those grown in the loamy sand soil.

The presence of H. sativum reduced the number of fertile tillers produced by both soil types when compared with plants grown in soil free of the organism. The greatest number of sterile and dead tillers was produced by those plants planted 1 inch deep, and Improved Blue Jacket generally produced less fertile and more sterile tillers than Wichita, but the latter variety had the higher proportion of dead tillers, particularly in the loamy sand soil.

The extent and intensity of discoloration of the crown area tissues was found to be greater in those plants grown in loamy sand soil, with the least amount being found in the variable moisture treatment of the clay loam soil. The nature and extent of the crown area discoloration is shown in Figure 1.

The paucity of seedling infections compared to the extent and intensity of infection of the mature plant crowns would indicate that seedling infection bears little relation to the development of root and crown rot in mature plants. In these studies, planting depth and soil moisture level seemed more critical in crown rot development than did the soil type or variety.

In spite of the amount of crown infection encountered in these plants, none of them died at flowering time as so often happens in the field. This type of plant symptom was not produced by this method of inoculation under the conditions of this experiment.

When inoculum of H. sativum was sprayed on the leaves of wheat and barley seedlings varying degrees of resistance between varieties was found. If the reaction of the seedling leaf is indicative of the reaction to crown and root rot caused by this fungus, this technique could be used to screen large numbers of varieties relatively easy and in a short length of time in the greenhouse. Further studies would be required to determine if seedling leaf reaction is indicative of the mature plant reaction to this disease.

Figure 1. The crown and major roots of mature wheat plants grown in the greenhouse. Upper row: healthy plants grown in sterilized soil. Lower row: Diseased plants grown in soil inoculated with Helminthosporium sativum.

SUMMARY

1. Wheat seedlings grown in a loamy sand soil attained more growth and had fewer root rot lesions per plant than those grown in a clay loam soil.

2. The number of lesions per seedling increased with the depth of planting and soil moisture level in both soil types.

3. Heading of both the Wichita and Blue Jacket varieties was delayed approximately one week when the seeds were planted $\frac{1}{2}$ inch deep instead of 1 inch deep in either loamy sand or clay loam soil.

4. The height of mature plants grown in inoculated soil was generally less than those grown in soil free of the organism.

5. Height of the mature plants increased with an increase of moisture level, but was not affected by depth of planting.

6. The root volume of mature plants was largest in the variety Wichita at the variable moisture level and planted $\frac{1}{2}$ inch deep.

7. Increased moisture increased the total number of tillers and also the proportion of dead tillers. The proportion of living sterile tillers was higher in the variety Improved Blue Jacket.

8. More infection was found in the mature plants grown in loamy sand soil than in clay loam soil. The least amount of infection noted in the loamy sand soil was in the dry moisture treatment, while the least amount noted in the clay loam soil was in the variable moisture treatment. Less infection was generally found in the $\frac{1}{2}$ inch planting depth than at 1 inch.

9. A test in which seedlings of several wheat and barley varieties were sprayed with the organism indicated there is a varietal difference in reaction to *H. sativum*.

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