STUDIES ON THE PARASITISM OF PLANTS BY THE FUNGUS *HELMINTHOSPORIUM SATIVUM*

Thesis for the Degree of M. S.
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A THESIS

Submitted to the College of Science and Arts
Michigan State University of Agriculture
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Department of Botany and Plant Pathology

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To a graduate student undertaking his first problem in scientific research, inspiration and insight play a vital role. This inspiration and insight were provided by my major professor Dr. Richard L. Kiesling. I am pleased to have been his student.

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ABSTRACT

Host range studies showed that _Helminthosporium sativum_ Pam., King, and Bakke is capable of parasitizing a wide range of plant species outside of the grain and grass families.

Several barley varieties were tested in three locations and showed no resistance to _H. sativum_. There was no correlation in the degree of susceptibility among these three barley nurseries. Small grain and beans used in crop rotation increased the amount of infection. Michelite bean was shown to be a natural host of _H. sativum_. Sporulation of _H. sativum_ on barley straw during the growing season is the main source of inoculum for kernal infection. Sporulation of _H. sativum_ on barley straw appears where the straw is exposed to direct sunlight.

Amylase, cellulase, and sucrase were present in acetone powder preparations of _H. sativum_. Barley plants infected with _H. sativum_ show a higher amount of sucrase activity than plants not infected. The presence of alpha amylase, diastase, hemicellulase, and invertase separately correlates with the inhibition of germination of barley seeds. Culture filtrates from _H. sativum_ cultures contained amylase, cellulase, and sucrase. The culture filtrate had a toxic effect on barley seeds (inhibited germination). The toxic effect correlated positively with the presence of amylase and sucrase, and correlated negatively with the presence of cellulase.

TABLE OF CONTENTS

TABLE OF CONTENTS - CONTINUED

LIST OF TABLES

LIST OF PLATES

LIST OF GRAPHS

I. INTRODUCTION

The fungus Helminthosporium sativum Pam., King, and Bakke has presented perplexing problems to plant pathologists since it was first discovered to be a cereal pathogen in 1910. Since then an enormous amount of research has been conducted and hundreds of papers have been published on parasitism by H. sativum. In all, a great deal of knowledge concerning H. sativum has been uncovered, but no solution or partial solution has ever been found. The variability of H. sativum is one of the most important and hardest to understand aspects of this problem.

In approaching the problem in this study, an enzyme theory of parasitism was proposed. It was postulated that in order for a conidium of H. sativum to invade a plant, it must first germinate and then produce exoenzymes to aid invasion by breaking down the plant constituents. The breakdown products are then used for food by the fungus along with other constituents of the plant. Infection results when the composition of the plant and the enzyme system of the fungus are compatible, assuming environmental factors are favorable to enzyme reactions.

In view of the theory outlined, host range, etiological, and enzyme studies were made and are reported in this thesis.

II. LITERATURE REVIEW

Helminthosporium sativum was first described as a fungus disease of barley in 1910 by Pammel, King, and Bakke (22). Since this first description, much of the disease damage found in barley and in wheat can be attributed to *H. sativum*. The fungus attacks mainly the foot of grain plants (foot rot) but is also important in many areas as a leaf parasite (spot blotch) and a kernel blight.

During the period from 1920 to 1930 an extensive amount of research was carried on in an effort to understand and control parasitism caused by *H. sativum* (3, 4, 5, 6, 9). This research produced some hope of finding resistance to the disease through a grain breeding program. These hopes were diminished when in 1954 Wood, Christensen, and Lambert published a paper entitled, "*Helminthosporium sativum* becomes destructive on hitherto resistant varieties of barley" (35).

Extensive host range studies have been made on grain and grass varieties (1, 2, 11, 12, 16, 23, 26, 28, 29, 33, 34). The only plants, other than grains and grasses, reported as having been parasitized by *H. sativum* are cotton, opium poppy, pea cotyledons, and seeding flax (8, 13, 21, 32).

Natural inoculum is known to be present in the soil and in the air (18, 20, 24, 25, 27, 31). The importance of the inoculum in the air and in the soil in relation to infection of grain plants during their development is not fully understood.

Recent research indicates that a toxin produced by H. sativum predisposes plant tissue to infection. Ludwig (14, 15) evaluated the toxin produced by means of a barley germination test and suggested that the toxin limits the growth of H. sativum as well as host plants. Ludwig postulates that the toxin produced by the fungus affects susceptible hosts by conditioning them to infection.

Hess (10) found that certain culture filtrates of H. sativum had a harmful effect on the development of barley and that the filtrates were inactivated by a change in pH or by being heated. Barley proved to be an unsuitable host in these tests because its susceptibility was altered by even slight changes in the nutrient solution and by retarded development. Earhart (7) found a positive correlation between pathogenicity of H. sativum and toxin in the culture filtrate as evaluated on seedlings of Victorgrain 48-93 and Southland oats.

III. MATERIALS AND METHODS

The experiments reported in this work were all made using one isolate (H. S. 101) of *H. sativum* that was a result of single sporing through six generations. Because of the large number of types of the fungus and the indefinite limits of strains, no other method of insuring stability or reproducibility in tests was practical.

Host Range Studies

To obtain a better understanding of the capability of *H. sativum* as a parasite, inoculations of 24 plant species and varieties were made. The plants were selected to give a range of plant families as well as structural and compositional differences.

The seeds (Table IV.) were planted in four inch pots in the greenhouse. When the plants had developed sufficiently (several well developed leaves) they were placed in a moist chamber and sprayed with a water spore suspension of *H. sativum* (H. S. 101). The inoculum was prepared by scraping spores from dried potato dextrose agar (P.D.A.) Petri plates into sterile distilled water. The spore suspension was filtered through cheesecloth to remove mycelial fragments. After inoculation of the plants the humidity in the chamber was kept relatively high (70-90 per cent) for two days. After this incubation period, the plants were returned to normal greenhouse conditions. Uninoculated controls were included in all cases.

In nine days the plants were examined for lesions and where lesions were found, reisolations were made to recover H. sativum and verify the cause.

Three separate inoculation trials were made in this experiment differing only in the plants used and the reisolation techniques employed. The reisolation techniques used are as follows:

Trial I: A small section of tissue was removed from the edge of a lesion and dipped in a solution of one part commercial sodium hypochlorite (Chlorox) to, one part 95 per cent ethyl alcohol for one minute. The section of tissue was then placed on a sterile P.D.A. plate and incubated.

Trial II: Leaves containing lesions were removed and washed in running tap water for 48 hours. The leaves were then dipped in a 20 per cent commercial sodium hypochlorite (Chlorox) solution for fifteen seconds followed by a rinse in sterile water. Small sections of the tissue were removed from the edge of the lesions and placed on acidified P.D.A. (5 drops of lactic acid per 200 ml of P.D.A.) and incubated.

Trial III: Leaves containing lesions were removed from the plant. A 3/8 inch cork borer was used to remove a plug of tissue from the edge of a lesion. The plugs were placed in a Gooch crucible and dipped in a solution of 1 part 95 per cent ethyl alcohol, 1 part commercial sodium hypochlorite (Chlorox), 1 part distilled water for five seconds. The plugs were then placed on acidified P.D.A. and incubated.

Etiological Studies

1. Reaction of Several Barley Varieties to Natural Infection

In the spring of 1957 a barley nursery to study H. sativum distribution was established in Michigan at three separate locations. Most of the varieties used in this study were reported by the Canadians to have some resistance to H. sativum (Table II). The three locations, East Lansing, Standish, and Tuscola represent three different areas in Michigan where barley is grown. The soil practices on the sites chosen were good; the crop rotation is shown in Table I. The varieties were read for infection by H. sativum on culms, leaves, lower nodes and upper nodes after the plants had headed. In addition, sporulation on the straw was also read in the East Lansing nursery. The data are shown in Table V.

2. The Natural Infection of Michelite Bean

Eight leaves were selected at random from a field of Michelite beans adjacent to the barley nursery at Tuscola. This was done in an effort to correlate the pathogenicity of H. sativum in the greenhouse to the field. Fifty-eight isolations were made of lesions on the leaves using the technique of Trial III. The field in which the beans were growing had the same crop rotation as the Tuscola barley nursery (Table I).

3. Air-borne Inoculum in Relation to Infection of Barley in Michigan

TABLE I

Crop Rotation at the Three Locations Used to Test the Resistance to *Helminthosporium sativum* of Several Barley Varieties

Year	East Lansing	Standish	Tuscola
1957	Barley Nursery	Barley Nursery	Barley Nursery
1956	Soy Beans	Corn	Spring Barley (Erie)
1955	Corn	Corn	Navy Beans
1954	Small Grain	Hay	Oat and Barley Plots
1953	Summer Fallow	Hay	Navy Beans
1952	---	Hay	---

TABLE II

Varieties[a] of Barley Grown in Nurseries at Three Locations in Michigan to Test Their Reaction to Naturally Occurring Inoculum of *Helminthosporium sativum*.

ROW I	ROW II
187	1245
148	1907
691	1517
711	4578
731	6969
739	691
1245	2276
1367	198
1517	4979
1907	7269
2276	731
4578	187
4979	5105
7269	6969
5105	711
6969	1367

Each variety is planted in an eight-foot row.

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[a] Each variety is listed according to its United States Department of Agriculture Cereal Investigation (C.I.) number.

To investigate the disease cycle a study of air-borne inoculum in a field of barley was made. Soil and seed-borne inoculum of *H. sativum* account for most of the foot rot and seedling blight infection which occurs in Michigan. However, the source of inoculum which gives rise to kernal blight had not been determined in Michigan.

A twenty acre field of barley which included at one end the East Lansing barley nursery was chosen for the site of this study. Six microscope slides were placed vertically on stakes six inches above the ground. Each slide had a thin coat of vaseline and was placed on the side of the stake facing the prevailing wind. The stakes were spaced in the field to obtain a random sample of the foreign spores which might pass through the field as well as those produced in the field. The slides were changed periodically during the season. After the slides were removed from the field, a one inch square cover slip was placed on the vaseline in the center of each slide. The slides were then read under the low power of the microscope (10x) by passing across the cover slip three times at 5 mm intervals. The average number of spores per slide for each group of six slides for each period was recorded. The experiment ran from April 27 to August 26, 1957.

Enzyme Studies

These studies were undertaken to determine if amylase, cellulase, and sucrase were produced by *H. sativum* and to correlate the production of these enzymes with the pathogenicity of the isolate and the effects of such parasitism on the host. Amylase, cellulase, and sucrase, as referred to in this thesis, include any or all of the enzymes which could be present and could hydrolyze soluble starch, alpha cellulose, and sucrose respectively.

1. The Preparation and Reaction of Acetone Powders from H. sativum

To determine the presence of amylase, cellulase, and sucrase, acetone powders were made in the following way: three Fernbach flasks containing 200 ml of potato dextrose solution were inoculated with H. S. 101. The flasks were then incubated a week at 28° C. The mycelial mat which had formed was harvested and made into an acetone powder (Table III). The acetone powder was then frozen and used as needed for reactions. To test the acetone powder for amylase, cellulase, and sucrase, a portion of the powder was added to 40 ml of glass distilled water. The solution was then placed in a cellophane membrane and dialyzed for twelve hours at 1° C. against one liter of glass distilled water. After dialysis, the solution was centrifuged to remove debris (a few minutes in a small centrifuge is sufficient). The supernatent is poured off and added to a substrate to test for enzymes. This is accomplished by adding 2 ml of the enzyme solution to 10 ml of substrate (1 per cent wt./ vol. soluble starch, alpha cellulose or sucrose) and incubating the reaction for four hours at 28° C. The reactions are best if run in 100 ml volumetric flasks and contamination may be excluded by adding a small layer of toluene. At the end of the

TABLE III

Preparation of Acetone Powder from a Mycelial Mat of

Helminthosporium sativum

1. Filter off the mycelial mat from the liquid media using a suction filter without filter paper.

2. Place the mat in 200 ml of sterile glass distilled water in a beaker and shake a few seconds to dissolve particles from the media which may be adhering to the mat. Filter off the mat with suction. Repeat the process three times.

3. Place the mat in 200 ml of cold acetone for 24 hours.

4. Filter off acetone by means of suction and suck air through mat for 20 seconds (if desired, mat may be washed with 200 ml of C.P. ethyl ether before sucking air through the mat).

5. Dry mat in vacuo for two days at 1° C. (sulfuric acid or some other drying agent may be placed in the bottom of the dessicator).

6. Grind mat in mortar and pestle machine for a minimum of two hours, add sterile sea sand and glass distilled water as needed to facilitate grinding. This operation should be carried out at 1° C. if possible.

7. Pour ground mat into an airtight pyrex container and freeze. The ground mat is the acetone powder. Remove portions of the acetone powder as needed for enzyme reactions.

four hour reaction period, 2 ml of Somogyi's reagent (30) are added and the flasks are placed in boiling water. The boiling water stops enzyme reaction and speeds the reaction between Somogyi's reagent and reducing sugars. Flasks in which sucrose is used as a substrate are boiled 10 minutes, soluble starch and alpha cellulose flasks 20 minutes. The flasks are cooled and 2 ml of Nelson's reagent (19) are added to bring out a stable color. The final solution is diluted to 100 ml with distilled water. Checks of 2 ml of enzyme solution and 10 ml of substrate solution are made at the same time. After the reaction solutions have been diluted to 100 ml, they are read in a Klett-Summerson colorimeter. The readings of all solutions are recorded and then converted to equivalent mg of glucose which would produce the same color under the conditions of the reaction minus the check values.

2. The Production of Sucrase during Parasitism

If amylase, cellulase, and sucrase are necessary for parasitism by H. sativum, then possibly infected plants would contain a greater amount of such enzymes as compared to noninfected plants. This idea was tested on Odessa barley (C.I. 934) grown in the greenhouse. Half of the plants were inoculated with H. sativum and half remained as uninoculated checks. Two days later lesions had appeared on the inoculated plants and leaves of infected and uninfected plants were harvested. Samples were taken from leaves which were fairly uniform in size by cutting out sections approximately 1/2 cm square. From

this material .2 g of infected tissue and .2 g of uninfected tissue were weighed out. Each weighed sample was then placed in a sterile 200 ml Erlenmeyer flask. After freezing the samples for 24 hours, 30 ml of glass distilled water was added to each flask. The flasks were placed on a reciprocal shaker for 17 hours at 25° C. The supernatent was filtered off and used in these studies. Each solution was dialyzed and tested for sucrase activity as described in the preceding section except that 2 ml of 1 per cent sucrose and 2 ml of enzyme solution were reacted at 37° C. Checks were made of the enzyme solutions and the substrates. Results are recorded as equivalent mg of glucose which would give the same color minus the check values.

3. The Effect of Extra Enzymes on Barley Seed Germination

a. Effect of purified enzymes

The effect of enzymes on the germination of barley seed was tested using the method described by Ludwig (14, 15). The purpose of this test was to determine whether enzymes would actually inhibit the germination of barley seeds. Twenty-five seeds of a bright Montcalm barley sample (C.I. 7149) were placed in a test tube. 1.0 and .1 per cent wt./vol. solutions of enzymes were made using glass distilled water as a solvent. Five ml of solution (enzyme preparation or culture filtrate) were added and the seeds were allowed to soak in this solution for 4 hours. At the end of the 4 hour soaking period, the seeds were placed on moist filter paper in a Petri dish and incubated at 23° C. for 3 days. Each treatment was repeated six times. After three days the seeds which had germinated were counted and the percentage germination was computed. Checks were made using water and water covered with toluene because the enzyme solutions used were preserved under toluene.

b. Effect of amylase, cellulase, and sucrase produced by *H. sativum*

This experiment was designed to show whether *H. sativum* produces amylase, cellulase, and sucrase as exoenzymes in culture and whether these enzymes are related to the toxicity of the culture filtrate to barley seeds in the germination test.

Five 250 ml Erlenmeyer flasks containing 25 ml of sterile potato dextrose solution were inoculated with 1 ml of a spore suspension of H. S. 101 and incubated at 23° C. Starting on the first day and each successive day for 4 days, the following data were recorded:

1. pH of media after dialysis
2. ml of media after growth
3. weight of the mycelia (dry weight)
4. increase in weight of mycelia (growth increment)
5. toxicity of the media to barley seeds
    a. filtered media (1:0)
    b. media diluted 50 per cent (2:1)
    c. media diluted 100 per cent (1:1)

d. dialyzed media

6. activity of enzymes in dialyzed media

a. amylase

b. cellulase

c. sucrase

The pH of the filtered media was determined by colorimetric methods after the media was dialyzed for 4 hours at 1° C. against 800 ml of glass distilled water.

Each flask was harvested by filtering the mycelia on a pre-weighed filter paper. The volume of filtrate was measured. The mycelia and filter paper were dried in an oven for 2 days at 60° C., and then weighed.

The toxicity of the filtered media was tested on 25 barley seeds as described in part a. above. The culture filtrate was tested in series of 1:0, 2:1 and 1:1 dilutions. The toxicity of the filtrate was also tested after dialysis. Results are recorded as percentage germination.

After dialysis the media was tested for amylase, cellulase, and sucrase activity. Enzyme reactions were incubated at 37° C. and reaction mixtures were protected from contamination by adding a small amount of toluene. Sucrase reactions were incubated for 4 hours while amylase and cellulase reactions were incubated for 168 hours. To improve the quantitative results in the cellulase reaction the solution was filtered after incubation to remove the cellulose particles which cause the solution to be turbid and to disturb the colorimetric readings. The filtered cellulose was washed with distilled water to assure that all the reduction products were present for the color test. Somogyi and Nelson reagents were added and the final volume diluted to 100 ml. The colorimeter readings for each solution are recorded as equivalent mg of glucose which would give the same color as the reduction products of the enzyme reaction. Four ml of enzyme solution (dialyzed media) was run as a colorimetric check and this value was subtracted from the value obtained in the enzyme reaction.

IV. RESULTS

Host Range Studies

All of the species and varieties tested proved to be hosts under the conditions of the experiment except for cabbage, radish, wild cucumber, and winter oats. Winter oats are known to be susceptible to H. sativum under certain environmental conditions. The lesions which appeared in Trial I and Trial II were not successfully reisolated in many cases because the sterilization techniques were too harsh and killed the pathogen (Table IV). The modified sterilization technique used in Trial III was highly successful.

Etiological Studies

1. Reaction of Several Barley Varieties to Natural Infection

The varieties tested showed no correlation in resistance from one location to another. None of the varieties displayed a high amount of resistance to infection. In the East Lansing nursery sporulation of H. sativum on the straw seemed to correlate to an extent with infection of the culm. Generally, infection was in the form of culm rot. Nodes were infected in almost every variety. Although the leaves were usually infected with H. sativum, the damage was not extensive and sporulation did not occur on the leaves. Infection was generally heavier at the Tuscola location.

2. The Natural Infection of Michelite Bean

Of the 58 isolations made, four (3.5 per cent) proved to be

TABLE IV

Results of the Host Range Study Showing the Plants Inoculated in Each Trial, the Appearance of Lesions, and the Reisolation of *Helminthosporium sativum* from the Lesions.

		Trial I		Trial II		Trial III	
	Plant Host	L[a]	R[b]	L	R	L	R
Bean:	Blue Lake	+	+	+	–	+	+
	Fordhook No. 242	+	–	+	–	+	+
	Great Northern	+	–	+	–	+	+
	Idaho	+	+	+	–	+	+
	Michelite	+	+	+	–	+	+
	Pencil Pod Black Wax	+	–	+	–	+	+
Crucifers:	Chinese Mandarin	+	–			–	–
	Danish Baldhead	+	–			–	–
	Premium Flat Dutch					–	–
	Wisconsin Hollander					–	–
	Early Scarlet Radish	+	–	–	–	–	–
Corn:	Golden Cross Bantam	+	+	+	–	+	+
Cow Pea:	Black	+	+			+	+
Cucurbits:	National Pickling	+	+	+	–	+	+
	Mammoth King Pumpkin	+	+	+	+	+	+
	Wild Cucumber					–	–
Horse Bean		+	–	+	–	+	+
Peas:	Alderman	+	–	–	–	+	+
Small Grain:	Barley 934					+	+
	Barley 5105	+	+			+	+
	Winter Oats					–	–
Sunflower						+	+
Tomato:	Bonnie Best	+	–	+	+	+	+
	Rutgers					+	+

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[a] L (+) indicates lesion produced by *H. sativum*

[b] R (+) indicates reisolation of *H. sativum* from lesions

PLATE I

Infection of Four Plant Species by Artificial Inoculation with Spores of *Helminthosporium sativum*.

1. Michelite Bean 2. Black Cow Pea

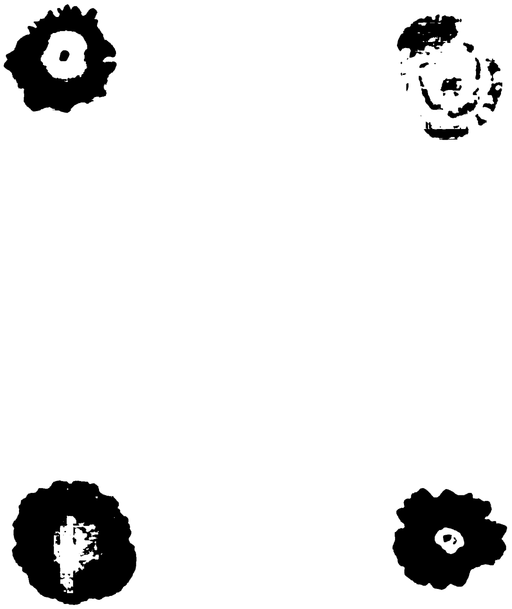
3. National Pickling Cucumber 4. Rutgers Tomato

PLATE II

1. Four isolations of Helminthosporium sativum from artificially inoculated leaves of Mammoth King Pumpkin. Note the variations among the colonies.

2. Nine isolates of <u>Helminthosporium</u> <u>sativum</u> from artificially inoculated plants. Reading left to right, top row, Black Cow Pea, Odessa Barley, and National Pickling Cucumber; Middle row, Mammoth King Pumpkin, Golden Cross Sweet Corn, and Blue Lake Bean; Bottom row Michelite Bean, Idaho Stringless Refugee Bean, and Michelite Bean.

PLATE II



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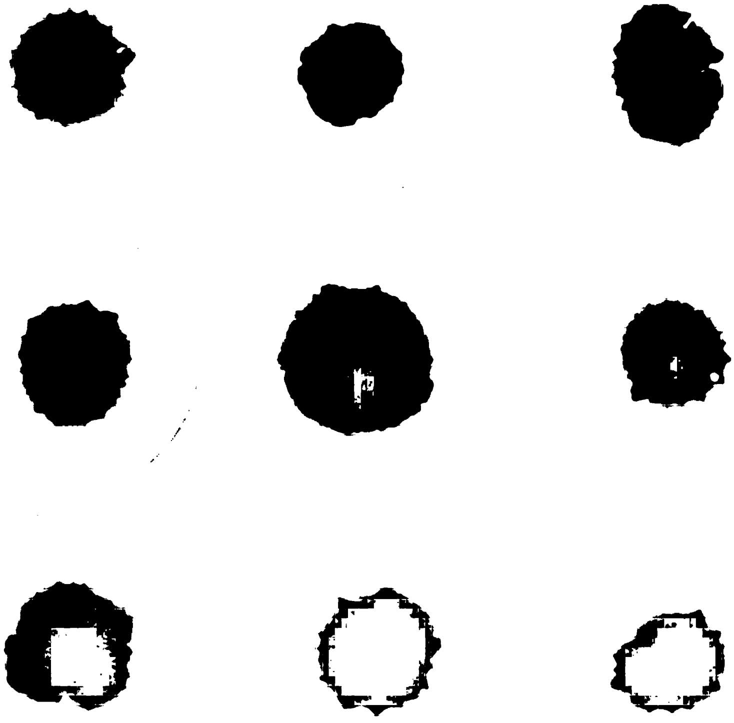

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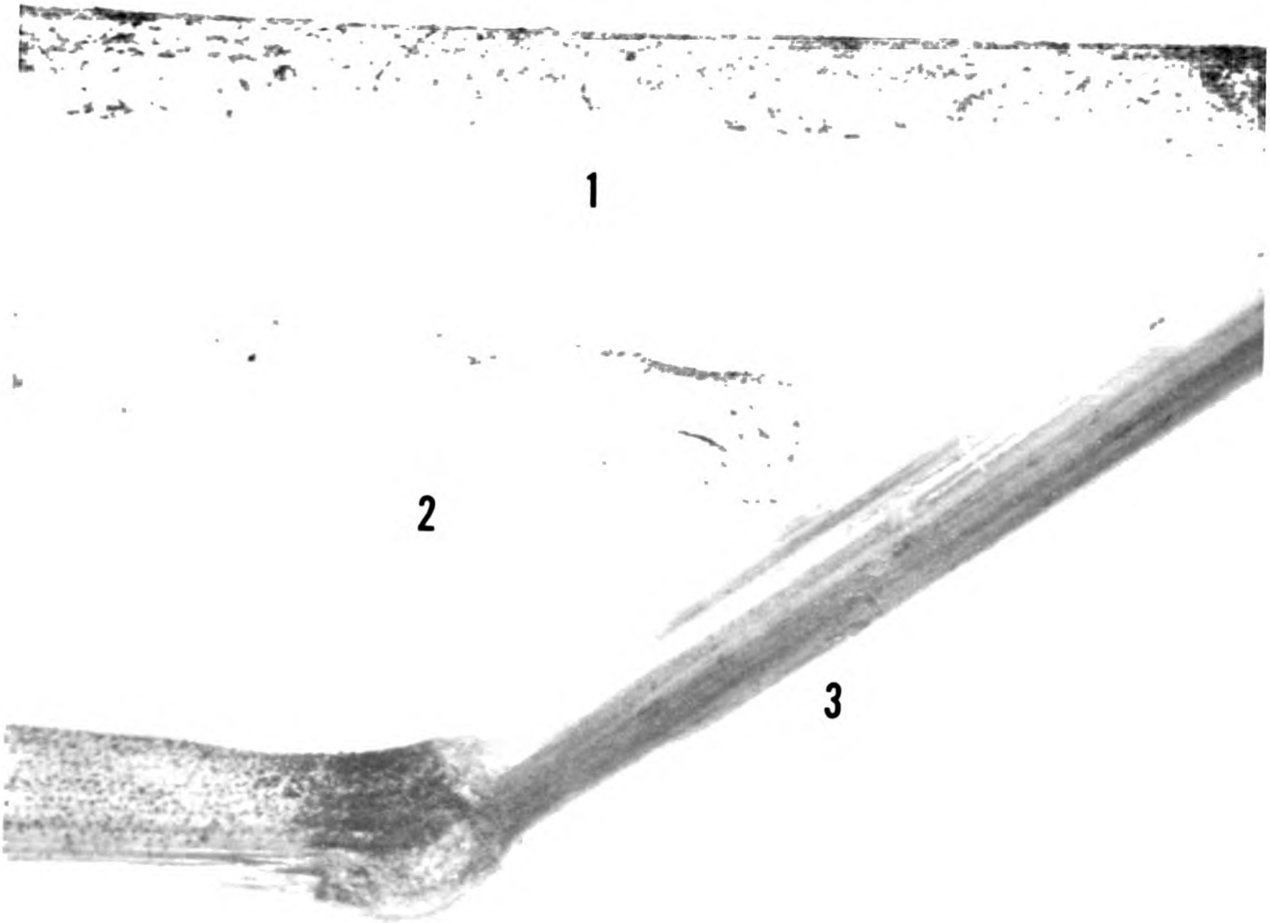
TABLE V

Reaction of Barley Varieties to Natural Infection by *Helminthosporium sativum*. Comparisons Are Made among Locations of Nurseries, Varieties, and Infected Plant Parts. Sporulation on the Straw of Infected Plants at East Lansing Is Compared to Plant Infection.

Barley Varieties	East Lansing			Standish		Tuscola		East Lansing		Standish	
	Sporulation	Culms	Lvs	Culms	Lvs	Culms	Lvs	UN	LN	UN	LN
6969	L	M	L	L	L	M	L			M	M
5105	L	L		M	L	H	M			L	M
7269	M	H	L	M	L	M	M	H	H	M	M
4979	L	H	H	L	L	H	M	H	H	L	L
4578	L	M	L	M	M	M	M			M	H
2276	L	L	L	M	M	H				M	H
1907	L	L	M	L	L	H	M	L		L	L
1517	M	M	L	M	M	M	L			M	M
1367	L	M	L	M	M	H	M	L		M	M
1245	L	L	L	M	L	L	M	0	0	L	L
739	L	L	L	L	L	M	L	0	M	L	L
711	M	H	L	M	L	H	M	0		M	M
691	M	M	M	M	M	H	M			H	H
193	L	L	L	L	L	H	L		L	M	M
187	L	M	L	L	L	M	L	H		L	L
731	L	L	L	M	L	M	L	0		M	M
Date Read:	Aug. 13	July 15		July 17, Aug. 5		July 9		July 15		July 17, Aug. 5	

H = Heavy Infection
L = Light Infection
0 = No Infection
M = Medium Infection
UN = Upper Nodes
LN = Lower Nodes
Lvs = Leaves

The infection rating in this table is the average infection of each variety at each location.



Sporulation of Helminthosporium sativum on Barley Straw Stimulated by Sunlight

1. Heavy sporulation of Alternaria sp. and Helminthosporium sativum.
2. Moderate sporulation of Helminthosporium sativum.
3. Heavy sporulation of Helminthosporium sativum.

H. sativum. It was interesting to note that *Alternaria* sp. was the most frequently isolated organism.

3. Air-borne Inoculum in Relation to Infection of Barley in Michigan

Conidia of *H. sativum* did not appear on the spore slides until late in the growing season. The barley headed on June 20. Sporulation of *H. sativum* on the straw was first noted on July 31. The first slides which showed conidia of *H. sativum* were removed from the field on August 3. These slides averaged 4.5 spores per slide. All slides thereafter, until the experiment terminated on August 26, averaged 2 spores per slide.

It was noticed that sporulation of *H. sativum* in the field on barley straw was affected by sunlight. Sporulation always appeared on the straw in view of direct sunlight. Careful examination of the straw eliminated the possibility that the reaction was caused by humidity, gravity or temperature. *Alternaria* sp. was found sporulating with *H. sativum* on the straw in many instances. Practice enables one to distinguish the two types of sporulation.

Enzyme Studies

1. Reaction of Acetone Powders from *H. sativum*

Experiments with acetone powders prepared from *H. sativum* revealed that amylase, cellulase, and sucrase were present. While the reactions all showed the presence of the enzymes, no quantitative estimation of the enzymes was made. A typical reaction gave the following results:

Substrate	Enzyme Tested For	(Mg Equiv. of Glucose) Reduction Products
Soluble Starch	Amylase	4.8
Alpha Cellulose	Cellulase	5.2
Sucrose	Sucrase	8.0

In every reaction using acetone powder as an enzyme source and a four-hour incubation period at 28° C.; sucrose was hydrolyzed the most, soluble starch was usually second and alpha cellulose third.

2. The Production of Sucrase during Parasitism

Substrate	Enzyme Source	(Mg Equiv. of Glucose) Reduction Products
Sucrose	Infected Barley Leaves	5.94
	Noninfected Barley Leaves	3.64

These results indicate that the infected barley plants had a 63.19 per cent greater sucrase activity than the noninfected plants.

3. The Effect of Extra Enzymes on Barley Seed Germination

a. Effect of purified enzymes

Alpha amylase, diastase, hemicellulase, and invertase inhibited germination (Table VI). None of the other enzymes tested showed any appreciable inhibition of germination.

b. Effect of amylase, cellulase, and sucrase produced by _H. sativum_

The data obtained in this experiment are shown in Table VII and are illustrated by Graphs I, II. The pH and mycelial weight increased each day while the volume of media decreased slowly. The filtered media had a toxic effect on barley seeds in the barley seed germination test. The 50 per cent (2:1) and

(1:1)
100 per cent dilutions as well as the dialyzed media were also toxic to barley seeds. Amylase, cellulase, and sucrase were present in the media as exoenzymes produced by *H. sativum*. The results of the enzyme reactions day by day correlate with the toxicity of the media in the barley seed germination test. The toxicity of the media to barley seeds increased with an increase of amylase and sucrase activity in the media. The opposite effect was observed for cellulase activity.

TABLE VI

The Effect of Extra Enzymes on the Germination of Barley

Solutions Tested	Per Cent Germination
Distilled Water Check	74
Distilled Water-Toluene	62
0.1% Alpha Amylase	47
1.0% Alpha Amylase	60
0.1% Diastase	38
1.0% Diastase	13
0.1% Hemicellulase	74
1.0% Hemicellulase	33
0.1% Invertase	33
1.0% Invertase	46
0.1% Pectinase	59
1.0% Pectinase	67
0.1% Protease	71
1.0% Protease	73
0.1% Proteinase	75
1.0% Proteinase	71

All of the above solutions were made up on a weight/volume basis and were preserved with a small amount of toluene. The enzymes were produced by the Nutritional Biochemicals Corporation.

TABLE VII

Data Recorded from Liquid Culture of *H. sativum*

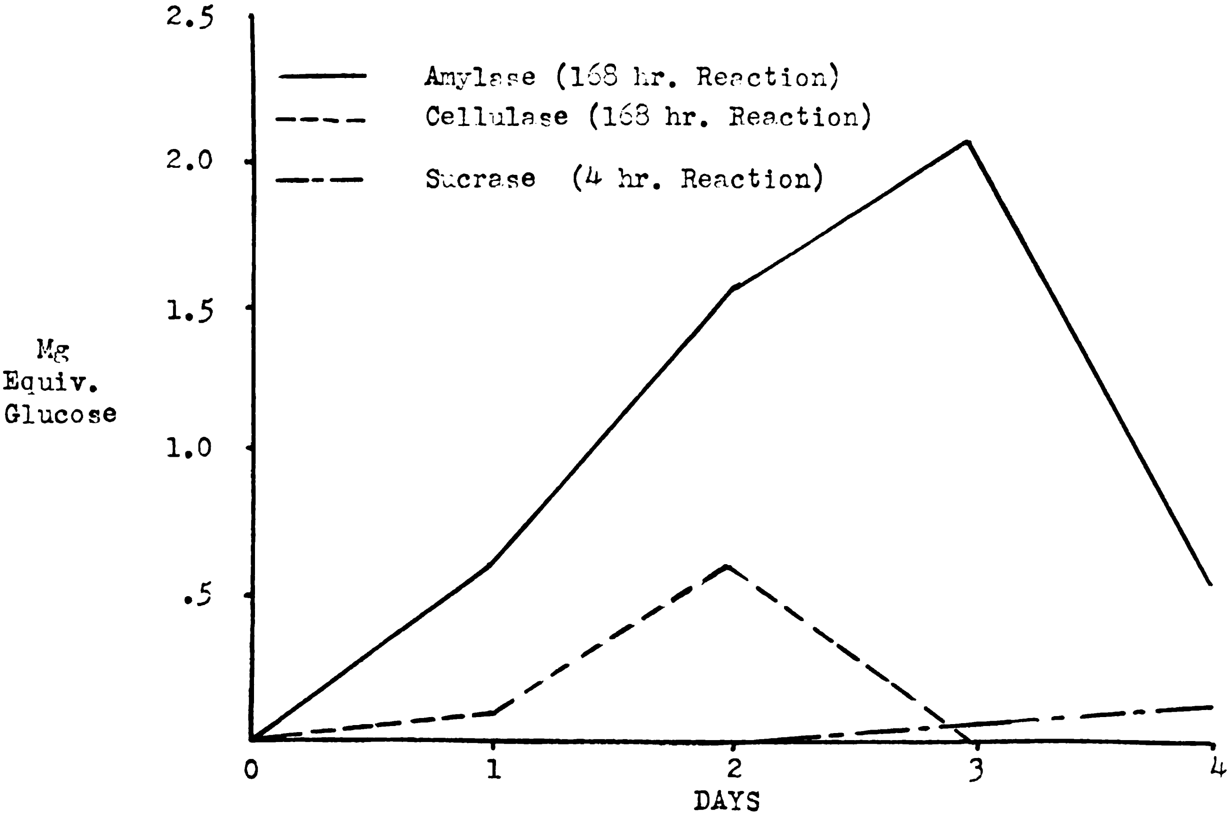
Day	pH of Filtered Media	Ml of Media after Growth	Grams of Mycelial Dry Weight/Rep.	Grams Mycelial Dry Weight Increase	Enzyme Activity (Mg Equiv. of Glucose) Amylase	Cellulase	Sucrase
0	5.8	41.5	0.0000	0.0000	0.00	0.00	0.00
1	6.0	43.0	0.0030	0.0030	0.62	0.12	0.00
2	6.6	41.5	0.0075	0.0045	1.56	0.64	0.00
3	6.4	38.0	0.0680	0.0605	2.16	0.00	0.06
4	7.0	36.0	0.0815	0.0135	0.58	0.00	0.10

Toxicity of Culture Filtrate to Barley Seeds (Per cent Germination)

Day	Filtered Media (1:0)	2:1 Dilution	1:1 Dilution	Dialyzed Media
0	40	52	36	60
1	60	48	56	80
2	88	96	76	88
3	28	88	64	48
4	0	12	12	32

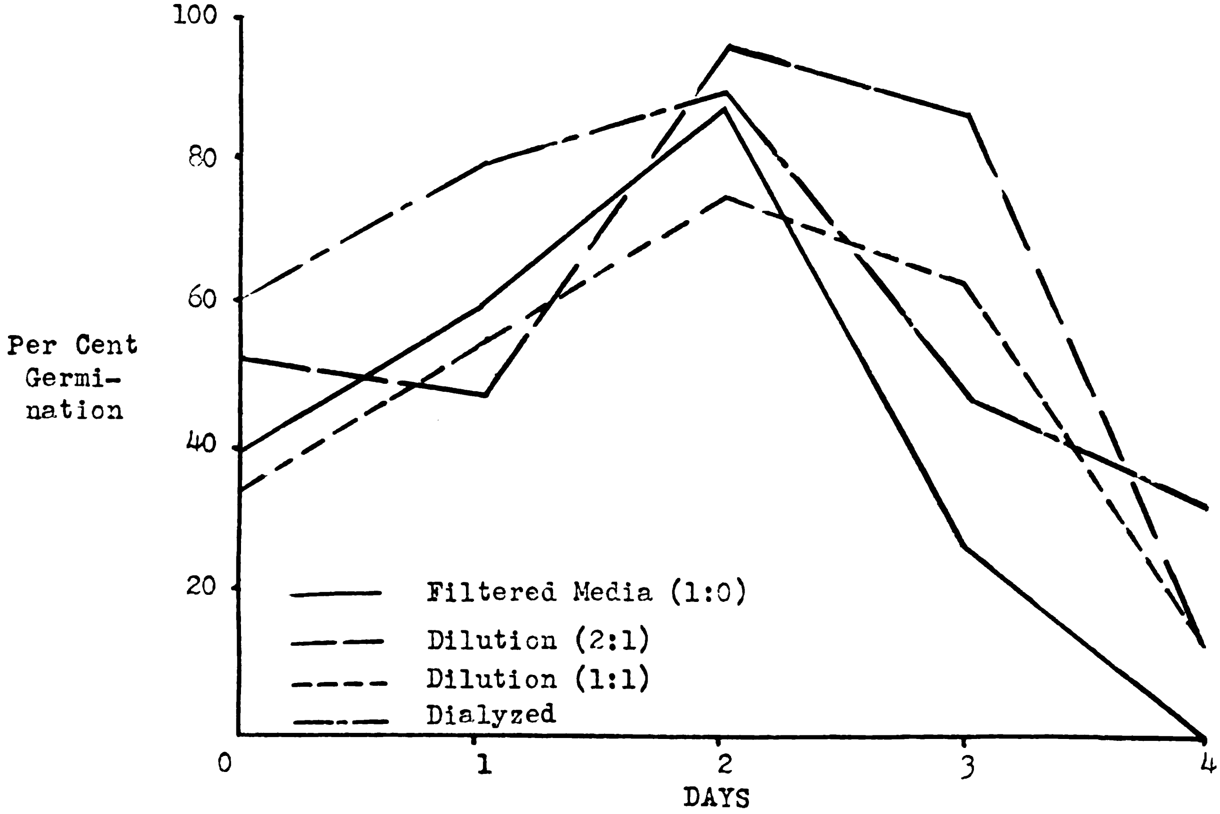
GRAPH I

Amylase, Cellulase, and Sucrase Activity of Dialyzed Culture Filtrate



GRAPH II

Toxic Effect of Culture Filtrates on Barley Seeds



V. DISCUSSION AND CONCLUSIONS

Host Range Studies

H. sativum is a capable parasite which can attack a wide range of plant species. In the case of the members of the Cruciferae which were not parasitized in this study, the reason may lie within the plant, i.e., perhaps the plant has a toxic compound which inhibits the fungus or perhaps the surface of the plant contains a compound which the enzymes produced during parasitism by H. sativum cannot break down. This isolate (H. S. 101) may not be able to parasitize members of the Cruciferae but some other isolate may be able to.

Etiological Studies

1. Susceptibility in Barley Varieties

While none of the barley varieties tested was resistant enough to encourage a breeding project, the nurseries served a useful purpose in pointing out that some other method of control may be more desirable. The incidence of a heavier infection at the Tuscola nursery coupled with the rotation data indicate the importance of crop rotation in relation to the build-up of inoculum. This is also emphasized by the isolation of H. sativum from Michelite bean at Tuscola. Environment and the genetics of H. sativum, as they effect the important enzymes used in parasitism, are more important to the etiology of this disease than the resistance of barley.

2. Air-borne Inoculum in Relation to Infection of Barley in Michigan

The principal source of inoculum for kernal infection is air-borne inoculum which appears after the barley has headed. This was clearly indicated in this study and confirms a study which Machacek and Greaney made in Canada in 1935 (17). A good deal of the air-borne inoculum which is found during the time the head is open to infection is from the barley straw which hosts abundant conidia of H. sativum.

Enzyme Studies

Amylase, cellulase, and sucrase are produced by H. sativum and may be found in the acetone powder preparations or as exoenzymes in culture filtrate. This information coupled with the increase in sucrase activity in infected barley plants and the toxic effect of amylase and sucrase on barley seeds support the proposed enzyme theory of parasitism. Of all the enzymes which H. sativum probably contains in its mycelia, none can be important or even useful without the enzymes which provide the initial food. It is proposed here that amylase, cellulase, and sucrase are among the principal enzymes which provide food and establish parasitism of the plant.

This research does not prove that the toxic effect which shows up in the barley seed germination test is the direct cause of toxicity, but rather that the presence of enzymes correlates with the toxic effect. The actual toxicity may be due either directly to the reaction of an enzyme or indirectly to an enzyme reaction product.

VI. SUMMARY

1. *H. sativum* has a wide host range and is capable of parasitizing many species of plants.

2. No source of resistance to *H. sativum* has been observed in barley varieties. In barley the susceptibility of a particular variety cannot be evaluated because of the wide range of variation in the isolates of *H. sativum* in one location.

3. Sporulation of *H. sativum* on barley straw correlates with varietal infection. Sporulation is stimulated by sunlight.

4. The isolation of *H. sativum* from Michelite bean and the general increase in infection where beans and grain are grown in succession point out the importance of rotation.

5. Air-borne inoculum in Michigan is the principal source of inoculum for kernal infection. Most of this inoculum is from the sporulation of *H. sativum* on the straw of the current season.

6. Acetone powders prepared from *H. sativum* contain amylase, cellulase, and sucrase.

7. The activity of sucrase in infected barley is greater than in noninfected barley plants. The additional sucrase in infected plants is probably caused by *H. sativum* activity.

8. Alpha amylase, diastase, hemicellulase, and invertase have a toxic effect on barley seeds and thus inhibit germination to an extent.

9. Amylase, cellulase, and sucrase are present as exoenzymes in culture filtrate. The production of these enzymes can be correlated to the toxicity of the culture filtrate to barley seeds.

VII. LITERATURE CITED

1. Andersen, H. 1955. Species of *Helminthosporium* on cereals and grasses in Denmark. Friesia 5: 80-89.

2. Andrews, E. A. 1953. Seedling blights and root rot of forage grasses. Diss. Abstr. 13: 962.

3. Christensen, J. J. 1922. Studies on the parasitism of *Helminthosporium sativum*. Minn. Agr. Exp. Sta. Tech. Bull. 11.

4. Christensen, J. J. 1926. Physiologic specialization and parasitism of *Helminthosporium sativum*. Minn. Agr. Exp. Sta. Tech. Bull. 37. R. A. M. 6: 473.

5. Christensen, J. J. 1929. The influence of temperature on the frequency of mutation in *Helminthosporium sativum*. Phytopathology 19: 155-162.

6. Dosdall, L. 1923. Factors influencing the pathogenicity of *Helminthosporium sativum*. Minn. Agr. Exp. Sta. Tech. Bull. 17.

7. Earhart, R. W. 1957. Comparison of two techniques for evaluating pathogenicity of *Helminthosporium* cultures. From Phytopathological meetings, Jan. 1957.

8. Greene, H. C. 1944. Notes on Wisconsin parasitic fungi. V & VI. Trans. Wis. Acad. Sci. Arts Lett. 36: 225-268. R. A. M. 25: 365.

9. Hayes, H. K., E. C. Stakman, F. Griffee and J. J. Christensen. 1923. Reaction of barley varieties to *Helminthosporium* sativum. Minn Agr. Exp. Sta. Tech. Bull. 21.

10. Hess, H. 1949. Ein Beitrag zum Problem der induzierten Abwehrreaktionen in Pflanzreich. (A contribution to the problem of induced defence reactions in the plant kingdom). Phytopathology 16: 41-70.

11. Ho, W. C. 1944. Soil-inhabiting fungi attacking the roots of maize. Res. Bull. Ia. Agr. Exp. Sta. 403-446. R. A. M. 24.

12. Ho, Wen-Chun and I. E. Melhus. 1940. Succession of soil-inhabiting fungi attacking the roots of maize. Phytopathology 30: 10.

13. Khristou, A. 1943. Fungi causing spots on the balls and moulding the seed of opium poppy. Jour. Agr. Exp. Sta. Bulgaria, 13: 13-19. R. A. M. 25: 417.

14. Ludwig, R. A. 1957. Toxin production by *Helminthosporium sativum* and its role in pathogenicity. From Phytopathological meetings, Jan. 1957.

15. Ludwig, R. A. 1957. Toxin production by *Helminthosporium sativum* P., K. & B. and its significance in disease development. Canad. Jour. Bot. 35: 291-303.

16. Luttrell, E. S. 1953. Spot blotch of brome grasses, tall fescue grass, and barley. Plant Dis. Reptr 37: 150-151.

17. Machacek, J. E. and F. J. Greaney. 1938. The "Black Point" or "Kernal Smudge" disease of cereals. Canad. Jour Res. 16 Sec. C-D: 84-113.

18. Machacek, J. E. and H. A. H. Wallace. 1952. Longevity of some common fungi in cereal seed. Phytopathology 42: 13.

19. Nelson, N. 1944. A photometric adaption of the Somogyi method for the determination of glucose. Jour. Biol. Chem. 153: 375-380.

20. Padwick, G. W. 1935. Influence of wild and cultivated plants on the multiplication, survival, and spread of cereal foot-rotting fungi in the soil. Canad. Jour. Res. 12: 575-589.

21. Padwick, G. W. 1938. Complex fungal rotting of pea seeds. Ann. Appl. Biol. 25: 100-114. R. A. M. 17: 431.

22. Pammel, L. H., C. M. King and A. L. Bakke. 1910. Two barley blights, with comparison of species of *Helminthosporium* upon cereals. Ia. Agr. Exp. Sta. Bull. 116: 176-190.

23. Patel, M. K. and Y. S. Kulkarni. 1949. Nitrogen utilization by *Xanthomonas malvacearum* (Sm.). Dowson, Indian Phytopathology 2: 62-64. R. A. M. 29: 508.

24. Polunin, N. 1951. Arctic aerobiology: pollen grains and other spores observed on sticky slides exposed in 1947. Nature, Lond., 168, 4278: 718-721.

25. Report of the Director of Science Service, Canada Department of Agriculture, for the year ended March 31, 1952. 51 pp.

26. Robles, Leonell H. 1949. The pathogenicity of species of *Helminthosporium* on corn. Phytopathology 39: 1020-1028.

27. Simmonds, P. M., B. J. Sallans and R. J. Ledingham. 1950. The occurrence of *Helminthosporium sativum* in relation to primary infections in common root-rot of wheat. Sci. Agr. 30, 10: 407-417.

28. Slykhuis, J. T. 1947. Studies on *Fusarium culmorum* blight of crested wheat and brome grass seedling. Canad. Jour. Res. Sec. C 25: 155-180. R. A. M. 27: 241.

29. Smith, J. D. 1953. Turf disease notes. Jour. Spots Turf Res. Inst. 8: 259-260.

30. Somogyi, Michael. 1952. Notes on sugar determination. Jour. Biol. Chem. 195: 19-23.

31. Stakman, E. C., A. W. Henry, G. C. Curran and W. N. Christopher. 1923. Spores in the upper air. Jour. Agr. Res. 24: 599-606.

32. Tervet, I. W. 1934. An experimental study of some fungi injurious to seedling flax. Phytopathology 27: 531-546.

33. Ullstrup, Arnold J. 1940. Observations on a noteworthy *Helminthosporium* disease of corn. Phytopathology 30+ 25.

34. Ullstrup, A. J. 1944. Further studies on species of *Helminthosporium* parasitizing corn. Phytopathology 34: 214-22.

35. Wood, L. S., J. J. Christensen and J. W. Lambert. 1952. *Helminthosporium sativum* becomes destructive on hitherto resistant varieties of barley. Phytopathology 44: 511.

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