STUDIES ON THE SEEDLING DISEASE OF BARLEY CAUSED BY *HELMINTHOSPORIUM SATIVUM* P. K. AND B.

By

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ABSTRACT

This investigation was concerned with host-parasite relations in the root-rotting phase of H. sativum on barley. The relative virulence of thirteen isolates of H. sativum obtained from the Canada Department of Agriculture, Research Station, Winnipeg was evaluated. The isolates showed a range from highly virulent to completely avirulent; isolate 514 was the most virulent of the group.

Two different types of inoculum, the cornmeal and the powder, or dried, inoculum were tested. The cornmeal inoculum gave consistent results within an experiment, but inconsistent results between experiments. It was the most satisfactory method available for testing small numbers of varieties. However, it was too laborious and time-consuming to permit testing large numbers simultaneously. Powder inoculum, on the other hand, was easy to prepare and applicable in a large experiment but the procedure requires improvement and standardization to reduce the chance of escape. The varieties Anoidium, Husky, Parkland and Olli were inoculated at temperatures of 54-56°F., 72-74°F., 80-82°F., 86-88°F. to determine the relative pathogenicity of the two isolates, 514 from Manitoba and 680 from Ottawa, at the various temperatures. The results indicated that there was no obvious variation in either the pathogenicity or the relative virulence of the two isolates due to temperature. Powder inoculum of the 514 isolate was used to screen approximately 6200 entries for resistance to root-rot caused by H. sativum. A group of 100 entries classified as resistant by the screening

test, were retested in cornmeal inoculum, along with 10 check varieties, using the two isolates 514 and 680. The results indicated that only seven entries demonstrated resistance to both isolates with reasonable consistency. Their C. I. numbers are as follows: 2550, 8873, 10241, 8969, 1343, 5435, 2355. The highly virulent isolate 514 was crossed to the avirulent isolate 499 to determine the segregation of mating type, growth and virulence. The results showed monogenic segregation for mating type, and indicated segregation of two gene pairs for growth rate. The genetic segregation for virulence could not be clearly defined. During the course of this investigation it was shown that H. sativum produces a toxin which inhibits growth in Pythium aristosporum Vanterpool.

INTRODUCTION

Helminthosporium sativum P. K. and B., the conidial stage of Cochliobolus sativus (Ito and Kuribayashi) Drechsl., produces seedling blight, foot and root-rot, head blight and leaf spot of cereals and grasses (4, 5, 8, 9). These various phases of the disease are responsible for a steady annual loss in crop yield. During the years 1921-22, Hayes, Stakman, Griffee and Christensen (12) conducted studies in Minnesota to determine the injury caused by H. sativum. It was found that susceptible smooth - awned barley varieties in artificially induced epidemic conditions, had average yields of 42 bushels per acre, while resistant varieties of the same type yielded an average of 54 bushels per acre. During 1930-1941 a survey was conducted by Machacek (20) in Manitoba to estimate the reduction in yield in wheat due to root-rot caused by H. sativum. It was found that during this period, no wheat field in the province was absolutely free from the disease. The average percentage of diseased plants and the average reduction in yield for the three years 1939, 1940, and 1941 was estimated at 38.3 percent and 12.1 percent respectively. In 1954, Wood et al. (28) reported that the commercial varieties, Kindred, Mars and Barbless, originally resistant to H. sativum, became susceptible, and in 1953 reduction of the potential yield in Kindred amounted to 50 percent in some areas.

The pathogen is either seed or soil borne but is not a soil saprophyte, and, under dry conditions at least, soil invasion would seem to be limited to dormant conidia and infested plant debris (4, 5, 13, 22). The breeding of resistant varieties offers the best practical

means of controlling this pathogen (10, 12). Attempts to find resistant material were conducted by various investigators (1, 2, 10, 12, 16, 17, 28). It was found by Arny (1, 2) that the difference between susceptibility and resistance to spot blotch in barley seedlings appeared to be due to a single factor pair with susceptibility dominant, whereas the studies of Hayes et al. (12) indicated that more than one genetic factor pair was involved. Loiselle (17) reported on two genes involved in the resistance of Br 3962-4 toward root-rot caused by H. sativum. Recently a thorough test for resistance to H. sativum was conducted by Hamilton et al. (10) and Loiselle (16). These investigators followed the method of inoculation established by Ludwig et al. (18) and reported several varieties resistant to isolates prevalent in the Ottawa area.

The work reported in this thesis was concerned with three main objectives:

I. To establish methods and conditions appropriate for testing a large number of barley entries for resistance to seedling root-rot caused by H. sativum.

II. To find new sources of resistance to a highly pathogenic strain of H. sativum occurring in western Canada, which might be useful in a plant breeding programme.

III. To obtain information concerning the inheritance of pathogenicity, rate of growth and mating type of a highly pathogenic strain of H. sativum isolated in Manitoba.

REVIEW OF LITERATURE

Several names have been given to Helminthosporium sativum by different investigators at different times. The synonymy of this pathogen is as follows:

Cochliobolus sativus (Ito and Kurib.) Drechsl. Perfect stage (Ophiobalus sativus Ito and Kurib.).

Helminthosporium sativum, Pam. King and Bakke. Conidial stage.
(Helminthosporium acrothecioides, Lindf.)
(Helminthosporium inconspicuum Peck)
(Helminthosporium sorokinianum Sacc.)

In 1910 Pammel, King and Bakke, described Helminthosporium sativum as a new pathogen on barley (9). Previously Sorokin, in Russia, described a new Helminthosporium disease on heads of wheat and rye; Saccardo lists pathogen as Helminthosporium sorokinianum Sacc. Luttrell, as reported by Dickson (9) examined the Saccardo type-specimens of H. sorokinianum and H. sativum and reported their synonomy.

Ito and Kuribayashi in 1929, as reported by Tinline (24), described an ascigerous fungus obtained in a culture as the perfect stage of H. sativum under the binomial Ophiobolus sativum. He also reported that Drechsler, in 1934, erected the new genus Cochliobolus to include the helicoid ascigerous species, with conidia belonging to Helminthosporium, that had previously been referred to the genus Ophiobolus.

Tinline (24) produced the perfect stage by mating compatible lines of the fungus, and studied the complete life cycle in culture. He used

the binomial Cochliobolus sativus (Ito and Kuribayashi) Drechsl., and confirmed the description given by Kuribayashi in 1929 (Figs. 1-7).

Tinline, (24) in his study of the perfect stage of H. sativum established a method for the induction of perithecial development in laboratory cultures, and reported that isolates of H. sativum were hermaphroditic, self-sterile, intragroup sterile and intergroup fertile. Subsequently, Tinline and Dickson (26) extended the studies on perithecial development and reported on inheritance of spore color and mating type. Hrushovetz (15), in cytological studies of the ascus and Shoemaker (23) in studies of the biology, cytology, and taxonomy of Cochliobolus sativus, confirmed the findings of Tinline. They also shed more light on the conditions and the processes of the perfect stage in H. sativum. Shoemaker (23), through his discovery of the nature of sexuality in the fungus, described in detail the development of spermagonia, ascogonia, Protothecium and Pseudothecium of Cochliobolus sativus. He pointed out that a temperature of 24°C. matured spermagonia, while ascosporogenesis was favoured by a temperature of 20°C. Hrushovetz (15) has shown that the two parental nuclei from a cross fused in the ascus, and this was soon followed by a fusion of their nucleoli. In meiosis, two divisions occurred, with pairing between highly contracted chromosomes early in the first division. A third division, which was a mitotic division, followed, and resulted in the formation of eight haploid nuclei. The haploid chromosome complement, as determined from pachytene and metaphase stages consisted of seven or eight chromosomes. Later, Tinline and Dickson (25), reported on heterocaryosis, and Tinline (27) reported heterocaryosis and parasexuality in H. sativum. It was found that heterocaryosis was achieved through hyphal anastomosis,

which occurred rarely between the hyphae of morphologically distinct isolates, but frequently between the hyphae of a single isolate.

Hrushovetz (14) in cytological studies of H. sativum, confirmed the findings of Tinline on heterocaryosis in H. sativum. Moreover, Hrushovetz showed that hyphal fusion with subsequent nuclear migration through the bridging hyphae can occur among different isolates, and suggested that the high mutation rate of H. sativum can, in principle, be interpreted in terms of heterocaryosis with occasional nuclear dissociation.

Wood (29, 30) reported on the relation of variation in H. sativum to seedling blight of small grains. Of 103 isolates tested, 28 percent were virulent on barley, wheat and oats, 19 percent on barley and wheat, one percent on wheat and oats, 15 percent on wheat only, five percent on barley only, one percent on oats only, and 31 percent were nonpathogenic or caused no apparent damage on any of the three hosts. It was found that progenies from a single conidium differed strikingly in pathogenicity. In contrast, Christensen and Davies (6) and Christensen and Schneider as reported by Dickson (9) demonstrated that a monosporous line passed through Marquis wheat for ten successive generations was relatively stable with a mutation frequency of 1:2900.

In studies of temperature effects on pathogenicity, Morton (21) and Clark et al. (7) found differences in expression of the spot blotch disease. However Clark et al. (7) and Wood (29, 30) in similar experiments on the

root-rot phase, found no variation in either the pathogenicity or the relative virulence of the isolates due to the effect of temperature.

Ludwig et al. (18) and Ludwig (19) reported on a toxin produced by H. sativum, and its significance in disease development. The toxin was evaluated by a biossay technique based on spore germination of Sclerotinia fructicola and seed germination of barley. A loose correlation was found to exist between toxin production and pathogenicity in a limited number of H. sativum strains. Even in the presence of abundant toxin, these strains differed in their ability to invade barley, indicating that factors other than toxin production were involved in pathogenicity.

Tinline and Dickson (25) studied genetic segregation in H. sativum. It was found that segregation ratios could not be determined accurately for pathogenicity, growth rate, or conidial production, since segregation was largely indistinguishable from normal variation. Some isolates derived from randomly selected ascospores differed significantly in pathogenicity from one another and from the common parental isolates. The data obtained, when four pairs of isolates derived from the eight ascospores of an ascus were compared with parental isolates for differences in pathogenicity, suggested that multiple factors controlled pathogenicity.

In 1952, dried inoculum was tested by Kreitlow and Sherwin (11) for effectiveness in determining reaction to some foliar pathogens. It was found that an excellent infection was obtained in the greenhouse with dried inoculum of Rhizoctonia on Lotus spp. and Bromus inermis;

with Sclerotinia homeocarpa on numerous species of grasses, and with Cercospora sojina and Corynespora cassiicola on soybean in both greenhouse and field. The dried inoculum was prepared as follows - cultures of each pathogen were grown on wheat-oat mixtures, then the material was dried at room temperature, ground and stored at 5°C. Host plants were inoculated by dusting the finely pulverized inoculum over moistened leaves.

Various methods for use in the production of artificial inoculum of H. sativum were tested by Ludwig et al. (18). These tests have shown that a cornmeal inoculum induces uniform plant disease development when thoroughly incorporated with the planting medium. The medium for the cornmeal inoculum was prepared by mixing 5 percent cornmeal with 95 percent sand and moistening this mixture with a nutrient solution made from inorganic salts in Czapsek's medium. This medium was inoculated with spore suspension of H. sativum.

Hamilton et al. (10) studied the reaction of barley varieties and selections to root-rot and seedling blight incited by H. sativum, using cornmeal inoculum as a soil infestation. A total of 600 varieties and selections was tested. Of these, 51 were classified as resistant. The varieties Anoidium, Br. 3962-4, Lenta and Opal B were rated the highest. In 1962 two hundred and seventy-two entries were tested by Loiselle (16) for reaction to H. sativum. Fourteen entries were classified as resistant to H. sativum.

MATERIALS AND METHODS

I. ISOLATES OF *HELMINTHOSPORIUM SATIVUM*

Thirteen isolates of *H. sativum* were obtained from the Canada Department of Agriculture, Research Station, Winnipeg. Their origins are shown in Table I. All isolates were grown on V8 juice agar and were sub-cultured when necessary by transferring single conidia produced on water agar; cornmeal inoculum was prepared from each isolates to test the relative pathogenicity of the thirteen isolate.

II. VARIETIES AND STRAINS OF BARLEY

The following selections of barley were tested for reaction to *H. sativum* in the seedling stage:

(a) Approximately 5,560 entries from the U.S.D.A. World Barley collection. Seed of these entries was obtained from Dr. K. W. Buchannon of the C.D.A. Research Station, Winnipeg.

(b) 160 lines numbered 001618 to 001778 from a U.S.D.A. collection from Peru. Seed provided by Dr. S. B. Helgason, University of Manitoba.

(c) 143 lines numbered 011336-0011479 from the United States. Seed provided by Dr. S. B. Helgason, University of Manitoba.

(d) 300 lines from the cross *H. polystichum* x Parkland. Seed provided by Dr. S. B. Helgason, University of Manitoba.

(e) 8 strains of *H. spontaneum* collected in Israel (Table II). Seed provided by Dr. J. Moseman, U.S.D.A.

(f) 35 lines of miscellaneous *Hordeum* spp. and genetic stocks (Table II). Seed provided by Dr. S. B. Helgason.

TABLE I

ORIGIN OF THE ISOLATES OF *Helminthosporium sativum* TESTED FOR RELATIVE PATHOGENICITY

Isolate Number		Date initial isolation	Oil	V8 Medium
499	Lesion on basal sheath of Merion blue grass Winnipeg	30-10-62	5-11-62	12-2-63
501	Bent grass roots from Winnipeg	30-10-62	1-11-62	12-2-63
514	Barley, Manitoba	30-10-62	5-11-62	12-2-63
533	Barley, Winkler, Manitoba	30-10-62	5-11-62	12-2-63
599	Vantage leaf spots, Christie, Manitoba	22-10-62	26-10-62	12-2-63
606	H.D. 2200 Barley Glenlea, Manitoba	30-10-62	5-11-62	12-2-63
607	H.N. 2242 Barley Glenlea, Manitoba	30-10-62	5-11-62	12-2-63
626	Exeter oats Quebec Normandin	21-9-62	1-10-62	12-2-63
629	Phalaris cananary grass Morris, Manitoba	2-10-62	12-10-62	12-2-63
641	Barley Elyakim Israel	4-1-63		12-2-63
642	Bet Gamlial, Israel	4-1-63		12-2-63
671	U. of Man. Co-op Betzes seed R.K.	1-10-62		12-2-63
627	Parkland, Quebec Normandin	21-9-62	26-9-62	12-2-63

TABLE II

SPECIES AND GENETIC STOCKS

R1	H. disticon nudum
2	H. polystichum 2H6
3	H. intermedium rimpaui
4	H. spontaneum 2H18
5	H. spontaneum 2H24
6	H. deficiens triceros
7	H. nudideficiens
8	H. nigrinudum
9	H. deficiens steudelii
10	H. vulgare ethiops
11	H. vulgare nigrum
12	H. distichon angustispicatum
13	H. intermedium subcornutum
14	H. vulgare pallidum
15	H. trifurcatum
16	H. horsfordianum
17	H. distichum rimpaui
18	H. distichum vimpani
19	H. distichum pamella
20	H. distichum nigrilaxum
21	H. distichum nudum
22	H. distichum laxum
23	H. intermedium haxtoni
24	H. intermedium mortoni
25	H. intermedium atricornutum
26	H. intermedium nudihaxtoni
27	H. intermedium cornutum
28	H. intermedium nudimortoni
29	H. intermedium subaethiops
30	H. deficiens sublanum
31	H. deficiens decorticatum
32	H. deficiens nudideficiens
33	H. deficiens tridax
34	Genetic K B1 Yellow head
35	Brachytic

Group of Hordeum species collected in Israel

PI 268242
PI 249983
PI 227301
PI 227091
PI 268244
PI 283423
CI 6912

(g) 10 varieties or lines on which the reaction to infection by H. sativum had been reported (Table III).

III. DISEASE INDUCTION

Two methods of inoculation were selected for inducing the disease caused by H. sativum in the seedling stage of barley.

(1) Sand-cornmeal - Nutrient salt inoculum

This inoculum was described by Ludwig et al. (18) in their studies of the seedling disease caused by H. sativum. The following description is based on their studies. One part by weight of cornmeal was mixed with 20 parts, clean, dry, white sand, and the mixture was dispensed into 500 ml. Erlenmeyer flasks at approximately 200 grams per flask. This was moistened with a nutrient solution based on Czapek's medium* and sterilized by autoclaving for 1/2 hour. Each flask was then inoculated with a heavy spore suspension of H. sativum prepared from 7 to 10-day old slant cultures and was incubated for 10 days at 24°C.

The plants under test were grown for 21 days in a mixture of 90 percent white silica sand and 10 percent inoculum as the planting medium, contained in wax coated paper cups 3.5 inches wide and 2.5 inches deep. Water was added as required to bring the moisture content to an optimum level. Ten seeds were planted in each cup.

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* Czapek's medium

Distilled water		1000.00 gms.
Sodium nitrate	$NaNO_3$	2.00 "
Potassium dibasic phosphate	K_2HPO_4	1.00 "
" chloride	KCl	0.50 "
Magnesium sulphate	$MgSO_4.7H_2O$	0.50 "
Ferrous sulphate	$FeSO_4.6H_2O$	0.01 "

TABLE III

CHECK VARIETIES TESTED WITH CORNMEAL INOCULUM

Varieties	Reaction to *H. sativum*
Anoidium	Resistant to root rot [Hamilton *et al.* (10)]
N.D.B112	Resistant to spot blotch [Morton (21)]
Husky	Resistant to root rot [Hamilton *et al.* (10)]
Swan	Intermediate resistance to root rot [Hamilton *et al.* (10)]
Olli	Susceptible to root rot [Hamilton *et al.* (10)]
Parkland	Susceptible to root rot [Hamilton *et al.* (10)]
Br. 3962-4	Resistant to root rot [Hamilton *et al.* (10)]
Br. 7212-10-2	Field resistance to root rot*
Br. 7212-39-1	" " " "
Br. 7212-42-1	" " " "

* D.R. Metcalfe C.D.A. Experimental Farm, Brandon, Man. (personal communication)

(2) Powder Inoculum

Powder inoculum or dried grain inoculum was tested by Kreitlow and Sherwin (11) and described by them as a method which offers certain advantages over other methods for screening large numbers of clover plants for resistance to foliar pathogens. The necessity of testing this type of inoculum arose when hundreds of varieties and selections were to be tested for resistance to H. sativum. Powder inoculum prepared as follows, was tried in this work and found satisfactory.

Approximately 110 grams of barley grain were dispensed into 550 ml. Erlenmeyer flasks, soaked for an hour in water, and then sterilized by autoclaving for one hour. The grain in each flask was then inoculated with a heavy spore suspension of H. sativum from a slant culture and incubated for fourteen days at 24°C. After the incubation period, the infected seeds were dried on blotting paper for 2-3 days, and ground in a Wiley mill to a powder which would pass a 20 mesh sieve. This was mixed with Talcum to increase the volume and to absorb any moisture present.

Ten kernels of each barley variety to be tested were sown in a sand bed, and the surface of the seeds was inoculated by scattering powdered inoculum of the 514 isolate of H. sativum over them. The amount of powder inoculum for 10 kernels was approximately 1.8 grams. The inoculum and seed were covered by one inch of sand. In each bed, 500 entries were tested. In addition to the inoculated entries in the bed, un-inoculated

entries were used as control. The controls were arranged to form a "T", configuration in each bed as shown in Fig. 10.

IV. DISEASE EVALUATION

(1) Disease Evaluation in the Powder Inoculum Test

The entries in the powder inoculum test, also referred to as the screening test for resistance to H. sativum, were classified for reaction to root-rot by means of a visual comparison between the height of plants from inoculated seed and that of the un-inoculated controls, and also by means of the number of seeds which emerged out of a total of ten kernels. Entries in which 5 or more of the seeds emerged, and the height of the first leaves was 50 percent or more of the control, were classified as resistant in the screening test.

(2) Disease Evaluation in the Cornmeal Inoculum Test

At the end of the growing period in the planting medium containing cornmeal inoculum, the seedlings were removed from the planting medium and washed free of sand and inoculum. The length of the roots and first leaf of the inoculated plants was compared with that of the un-inoculated control. Entries were classified as resistant when the length of the first leaves and roots exceeded 50 percent of the control. When less than 50 percent, they were classified as intermediate or susceptible. The first test was made without replication, but those entries classified as resistant or intermediate in the first test were retested until reliable information was obtained on their root-rot reactions.

V. MEDIA AND METHODS FOR THE CROSSING PROGRAM WITH *H. SATIVUM* ISOLATES

The following description of the media and methods used for the crossing program in this study are based on the studies by Tinline on the perfect stage of *H. sativum* (24).

(1) Medium for Perithecia Development

Barley grains were soaked for 1 to 4 hours in water, surface sterilized with 1 percent $HgCl_2$ in 10 percent Javex, washed with sterile distilled water, and boiled in water for one or two minutes to kill the embryos; the seeds were placed on Sach's nutrient agar (S.N.A.) in petri dishes, and inoculated by immersion in an aqueous suspension of conidia from isolates of the required mating type. The plates were wrapped in paper to eliminate light, and the cultures were incubated for 7 days at 24°C. and for an additional 14 days at 20°C.

(2) Method for Isolating the Spores from an Ascus

The method used to isolate all the spores from an ascus was as follows: Perithicia that were freed of debris and adhering spores by rolling them across an agar surface, were crushed in a water droplet. If examination showed abundant ascospores, the material was washed from the slide to an agar surface in a petri dish, an ascus containing spores was selected and moved apart from the perithecial material. The ascus wall was ruptured with a sharp needle. The spores were withdrawn from the ascus case, uncoiled, and distributed singly on a free agar surface in the

dish. After spore germination (4 to 12 hours), blocks of agar that contained individual spores were transferred to a fresh medium in petri dishes. Following further incubation, single conidia from each isolate were transferred to V8 agar slants.

VI. PREPARATION OF *HELMINTHOSPORIUM SATIVUM*, *HELMINTHOSPORIUM TERES* AND *ALTERNARIA TENUIS* EXTRACT

The necessity of preparing extracts of *H. sativum*, *H. teres* Sacc. and *Alternaria tenuis* Nees, arose when the uninoculated control seeds in a sand bed were found to be infected by *Pythium* spp., while seeds planted with powder inoculum of *H. sativum* were not. This suggested that a *H. sativum* toxin behaved as an inhibitor to Pythium spp. To test this hypothesis the Pythium was isolated in pure culture and transferred to V8 media containing an extract of one of the three mentioned organisms. The extracts of *H. sativum*, *H. teres* and *Alternaria tenuis* were prepared as follows.

Individual lots of potato sucrose liquid medium were inoculated by heavy suspensions of *H. sativum*, *H. teres* or *Alternaria tenuis*, and incubated for 5 days at 24°C. After the incubation period, each lot of inoculum was transferred to a Waring blendor to cut the mycelium, and to get a homogenous substance. Using suction, the extract was obtained through a millipore filter, which did not permit the spores and cells to pass through.

RESULTS

Relative Pathogenicity of Thirteen Isolates of H. sativum

Selection for resistance in the host requires that highly pathogenic isolates be used. The thirteen isolates from the Canada Department of Agriculture Research Station, Winnipeg (Table I) were increased on V8 media and cornmeal inoculum was prepared from each one. Three varieties, Anoidium, Husky, N.D.B112, were inoculated to determine the pathogenicity of the isolates. The results showed great differences in pathogenicity between the isolates (Table IV).

The results presented clearly demonstrated an advantage for isolate 514 in making further tests for resistance among variants of the host. Isolate 514 represents the most pathogenic type obtained in Manitoba. To broaden the scope of the tests the most pathogenic isolate used in Ottawa, No. 680, was obtained from Dr. R. V. Clark, of the Genetics and Plant Breeding Institute. Subsequent cornmeal tests for verifying the resistance among entries selected by the screening test, were conducted by using the two isolates 514 and 680.

Type of Inoculum for Testing Root-Rot and Seedling Blight

The reliability of the cornmeal inoculum method of inducing disease in the seedling stage of barley was tested. The seedling reactions were evaluated by the means previously described. It was shown that the reactions of the varieties were constant in the replicates within an experiment but were not constant between experiments as shown in Table V and Table IX.

TABLE IV

RELATIVE PATHOGENICITY OF THIRTEEN ISOLATES OF H. SATIVUM

Isolate Number	Reaction to seedling root-rot			Pathogenicity
	Anoidium	Husky	N.D.B112	
514	S*	S	S	Highly pathogenic
626	mS	mS	mS	Pathogenic
629	mS - mR	mS - mR	mS - mR	Moderately pathogenic
607	mS - mR	mS - mR	mS - mR	" "
501	mR	mR	mR	Slightly pathogenic
533	mR	mR	mR	" "
671	mR	mR	mR	" "
627	mR	mR	mR	" "
599	R	R	R	Non-pathogenic
606	R	R	R	"
499	R	R	R	"
641	R	R	R	"
642	R	R	R	"

* Designation referring to seedling reaction of host varieties to pathogen isolates.

TABLE V

REACTION OF ANOIDIUM, HUSKY AND HERTA IN TWO TESTS IN DUPLICATE USING CORNMEAL INOCULUM OF ISOLATE NO. 514

Varieties	Reaction in Test I		Reaction in Test II	
	I	II	I	II
Anoidium	S	S	S	S
Husky	R	R	mR	mR
Herta	R	R	S	S

Entries which showed resistance grew faster and greater height and root length, than the susceptible entries in all the replicates within one experiment (Figs. 8 and 9). This result confirms that obtained by Ludwig et al. (18), who stated that considerable reliance can be placed on treatment comparisons within an experiment, but that comparisons between experiments are much less accurate. The variability among the experiments in the cornmeal test could be due to (1) variation in the progeny of the isolates, as was found by Wood (29, 30); (2) variation due to the environment; (3) the influence of H. sativum toxin on the response of the seedlings in cornmeal inoculum as was shown by Ludwig et al. (18) and Clark et al. (7). Moreover, cornmeal inoculum was found to require much labour and time for preparation, and the number of tests possible with the inoculum which could be handled in one experiment was small. To overcome these difficulties powder rich in spores was prepared and tried for it's ability to differentiate

varietal reaction. The test showed that the powder inoculum was more severe than the cornmeal inoculum in affecting the host. Most of the entries failed to emerge as is seen in Figs. 10 and 11. A high proportion of entries which did emerge proved to be escapes when re-tested in the cornmeal test. However, some of these have shown a reasonable resistance to the pathogen as seen in Table IX. The powder inoculum is easy to prepare, can be prepared in large amounts, and may be kept in a refrigerator for a long period of time. The last test for reliability of the spores in the powder inoculum was done recently. Most of the spores germinated after a seven month period in a refrigerator.

In this work, the two methods were used to select plants resistant to H. sativum, using isolate 514 in the powder inoculum, and isolates 514 and 680 in the cornmeal inoculum. The powder inoculum was used in testing hundreds of entries in a long sand bed as shown in Fig. 10. This test was called the screening test for selecting for varietal resistance to H. sativum. The entries which showed resistance in this test were selected and re-tested with cornmeal inoculum. The tests in cornmeal inoculum were repeated till reliable classifications of the seedling reaction to root-rot were obtained.

Comparison of Temperatures for Testing Barley to Root-Rot and Seedling Blight Caused by H. sativum

An experiment was conducted at temperatures of 54-56°F., 72-76°F., 80-82°F. and 86-88°F., and at a relative humidity of 100 percent, using

cornmeal inoculum of the two isolates 680 and 514. Four varieties were used as indicators of the relative pathogenicity: Anoidium, Husky, Parkland, and Olli. Tests were made in duplicate. From the data in Tables VI and VII it appears that at 54-56°F., the pathogenicity of isolate 680 was more severe than at the other temperatures. This may be due to slow germination and growth of the host plants at this temperature which subjected the seedling to a longer period in the accumulating toxin, as suggested by Clark and Dickson (7). At 80-82°F., the pathogenicity of isolate 514 was slightly more severe than at the other temperatures. In general, there was no obvious variation in either the pathogenicity or the relative virulence of the 514 and 680 isolates associated with temperature levels.

A comparison of the un-inoculated controls at each temperature showed that the extreme temperatures had more effect on the plants than on the pathogen. This experiment confirmed the results obtained by Wood (29, 30) and Clark et al. (7). The average height of both controls and the inoculated plants at 72-76°F. was comparatively greater than at the other temperatures. As a result of this test, subsequent experiments for detecting resistance among varieties were conducted at the 72-76°F. temperature, and relative humidity of 100 percent.

TABLE VI

REACTION OF ISOLATE 514 AT DIFFERENT TEMPERATURES

Isolate 514								
Temperature	54°F. -56°F.		72° - 76°F		80° - 82°F		86° - 88°F	
No. of duplicate	I	II	I	II	I	II	I	II
Varieties								
Anoidium	S	S	S	S	S	S	S	S
Husky	S	mR	mR	mR	S	S	S	mR
Parkland	S	mR	S	mR	S	S	mR	mR
Olli	S	S	S	S	S	S	S	S

TABLE VII

REACTION OF ISOLATE 680 AT DIFFERENT TEMPERATURES

Isolate 680								
Temperature	54°- 56°F		72° - 76°F		80° - 82°F		86° - 88°F	
No. of duplicate	I	II	I	II	I	II	I	II
Varieties								
Anoidium	mR	mR	mR	mR	mR	mR	mR	mR
Husky	S	S	mR	mR	mR	mR	S	S
Parkland	S	S	mR	mR	mR	mR	mR	mR
Olli	S	S	S	S	S	S	S	S

Results of the Screening Test

Approximately 6200 entries were tested for resistance to H. sativum using powder inoculum of isolate 514. Out of the 6200 entries, one hundred were classified as resistant in the screening test. The names, CI numbers, sources and entry numbers of these strains are listed in Table VIII.

As was mentioned previously, the powder inoculum was more severe than the cornmeal inoculum. The seed available of most entries was limited, and sufficient for only one replicate. Moreover, the viability of the seeds of some entries was low, and as a consequence, some of the entries that were classified susceptible in this test may in fact have some resistance.

Results of the Cornmeal Test

The test using cornmeal inoculum in sand was applied to one hundred entries classified as resistant based on the screening test, and to the check entries listed in Table III. The cornmeal inoculum was prepared from the two isolates 514 and 680, and the test for determining seedling reaction was made to both isolates. The seedling reaction of the varieties is shown in Table IX. Out of 110 entries evaluated by cornmeal inoculum from 1 to 4 times, only seven entries showed a considerable resistance to 680 and 514 isolates in a consistent manner. It was found that six entries among the seven were different sources. The source of the seventh was unknown. These results suggest the possibility that these resistant entries may

TABLE VIII

LIST OF ENTRIES CLASSIFIED AS RESISTANT TO ROOT ROT AND SEEDLING BLIGHT IN THE SCREENING TEST

Variety	C.I.	Source	Entry number
Golden Grain	588	England	8
Kitzing	1134	"	15
Chevalier	1142	"	16
Goldthorpe	2264	"	27
	7270	"	49
	2339	Engledow	55
Varde	10033	Norway	92
Chevalier II	200	Svalof	174
Princess	529	"	175
Shed	1389	Denmark	196
	2531	"	205
Mahrisohe	921	Germany	230
Cruzat	6482	"	262
Piroline	9558	"	291
	9692	"	304
	6423	Poland	365
Bohemia	1837	Bohemia	435
Baronne	8295	France	519
Isaria	7595	Austria	598
Trabut	5055	N. Africa	802
	2550	Algeria	825
	3378	"	838
	3341	Egypt	894
	3664	Mariout Egypt	971
	3668	"	974
	3701	"	983
	3737	"	995
Hank	3558	Minia Egypt	1040
	3562	"	1043
Abyssinian	1216	Ethiopia	1058
Abyssinian	2384	"	1096
	4356	"	1210
	4375-1	"	1237
	5849	"	1307
	8139	Turkey	2067
	8149	"	2077
	8239	"	2155
	8527	"	2185
	8528	"	2186
	8529	"	2187
	8549	"	2206

Cont'd

CONT'D

Table VIII

List of Entries Classified as Resistant to Root Rot and Seedling Blight in the Screening Test

Variety	C.I.	Source	Entry number
	8622	Turkey	2277
	8643	"	2297
	8720	"	2373
	8721	"	2374
	8740	"	2389
	8742	"	2391
	8873	"	2440
	8993	"	2468
	7101	Iran	2685
	3347	Caucasus	2725
	5627	Kubanska	2756
	5631	"	2760
	5634	"	2763
	10241	Afganistan	3007
	10242	"	3008
	10160	Pakistan	3021
	10163	"	3023
Nushera	3406	India	3060
	4217	"	3074
	4624	"	3081
	5023	"	3082
	7644	"	3145
	7645	"	3146
	7693	"	3191
Bander	2354	Russia	3570
	4012-3	"	3607
	4954-2	"	3660
	4956	"	3662
	5760	Valkie	3843
	5764	"	3847
	4422	Manchuria	4372
	4471	"	4427
Hayakiso No. 2	8944	Japan	4947
Bakata No. 2	8969	"	4958
	6198	Argentina	5082
Forrajera de Invioruo	8159	"	5099
	4208	Venezuela	5135
	9746	Guatamala	5150
	5070	Canada	5210
York	6090	"	5223
Early white Turkestan	3095	California	5300

Cont'd

CONT'D

Table VIII

List of Entries Classified as Resistant to Root Rot and Seedling Blight in the Screening Test

Variety	C.I.	Source	Entry number
Tenn. Winter smooth awn B5-14	6570	Tennessee	5458
Mornobarb	6120	Maryland	5472
Hero	4602	Hybrid USDA	5551
Rufflyn	6374	" Wash.	5622
Gem	7243	" Idaho	5649
Otis	7557	" Colorado	5677
Lonhals	1343	" Minn.	5741
	2578	" Minn.	5782
Spartan	5027	" Mich.	5834
	5287	C.C. Sel. 4116	5871
	5322	" " "	5906
	5355	" " "	5938
	5418	" " "	6001
	5422	" " "	6005
	5435	" " "	6018
	2355	Unknown	6089
Hiland	9530	Hyb. Wyo.	6115
R_2 Hordeum-polystichum	-	-	-

TABLE IX

REACTION OF ENTRIES CLASSIFIED AS RESISTANT IN SCREENING TESTS, AND THE CHECK VARIETIES, TO TWO ISOLATES OF *H. sativum* IN CORNMEAL INOCULUM

	514 Replicates				680 Replicates			
Entry number	I	II	III	IV	I	II	III	IV
8	S	mR			mR	mR		
15	S	mR			mR	mR		
16	S	mR			S	mR		
27	S				S			
49	S				S			
55	S				S			
92	S				S			
174	mR	S	S		R	mR	S	
175	S	S	S		R	mR	S	
196	mR	S	S	S	mR	S	S	
205	mR	S	S	S	mR	S	S	mR
230	S	S			mR	S		
262	S	S			mR	mR		
291	S				S			
304	S				S			
365	S				S			
435	S				S			
519	S				S			
598	S	S			mR	S		
802	R	R	mR	mR	S	mR	S	mR
825	R	R	R		R	R	R	
838	S				S			
894	R	R	mR	S	S	mR	mR	mR
971	mR				S			
974	mR				S			
983	mR				S			
995	mR				mR			
1040	mR	mR	S		mR	mR	mR	
1043	mR	mR			mR	mR		
1058	S				S			
1096	S				S			
1210	S				S			
1237	mR				S			
1307	S				S			
2067	S				S			
2077	S				S			
2155	S				S			
2185	S				S			

Cont'd

CONT'D

Table IX

Reaction of Entries Classified as Resistant in Screening Tests and the Check Varieties to Two Isolates of *H. sativum* in Cornmeal Inoculum

Entry number	514 Replicates I	514 Replicates II	514 Replicates III	514 Replicates IV	680 Replicates I	680 Replicates II	680 Replicates III	680 Replicates IV
2186	S				S			
2187	mR				S			
2206	mR				S			
2277	mR				S			
2297	S				S			
2373	S				S			
2374	mR				S			
2389	S				S			
2391	S				S			
2440	R	R	mR	R	R	mR	S	R
2468	R	S			S	S		
2685	S				S			
2725	S				S			
2756	mR	S			S	S		
2760	S				S			
2763	R	mR			S	S		
3007	R	R	mR	R	mR	S	mR	mR
3008	mR	mR	mR	mR	mR	S	mR	mR
3021	mR				S			
3023	mR				mR			
3060	mR				mR			
3074	S				S			
3081	S				mR			
3082	mR				mR			
3145	R	mR			S	mR		
3146	S				S			
3191	S	mR	mR		S	S	mR	
3570	S				S			
3607	mR				S			
3660	mR	S			S	S		
3662	mR	S			mR	S		
3843	mR	S			S	S		
3847	S				S			
4372	mR				mR			
4427	S				S			
4947	S				S			
4958	R	mR	R		R	mR	R	
5082	mR	mR	mR		mR	mR	mR	
5099	S				S			
5135	R	mR			S	mR		
5150	mR				S			
5210	S				S			
5223	S				S			
5300	S				S			

Cont'd

CONT'D

Table IX

Reaction of Entries Classified as Resistant in Screening Tests and the Check Varieties to Two Isolates of *H. sativum* in Cornmeal Inoculum

	514 Replicates				680 Replicates			
Entry Number	I	II	III	IV	I	II	III	IV
5458	R	mR			mR	mR		
5472	mR				S			
5551	mR				mR			
5622	mR	S			mR	S		
5649	mR	mR			mR	mR		
5677	mR	mR			mR	mR		
5741	R	R	R		S	mR	mR	
5782	S				S			
5834	mR				mR			
5871	mR				mR			
5906	mR				S			
5938	mR	S			S	S		
6001	mR	mR			S	mR		
6005	mR				S			
6018	R	R	R		mR	R	R	
6089	R	R	R		R	R	R	
6115	mR	mR			mR	mR		
Anoidium	S	mR	S	S	mR	mR	S	S
N.D.B112	mR	mR	S	S	mR	mR	mR	S
Husky	mR	mR	S	S	mR	S	S	S
Swan	S	S			S	S		
011i	S	S	S	S	S	S	S	S
Parkland	mR	S	S	S	mR	mR	S	mR
Br-3962-4	S	S			S	S		
Br-7212-10-2	mR	mR			S	S		
Br-7212-39-1	mR	mR	S	mR	S	S	S	S
Br-7212-42-1	mR	mR			S	mR		
R_2	mR	mR	S	mR	mR	mR	S	mR

contain different genes for resistance to H. sativum. The reaction of these entries to root-rot, their entry number, C.I. number, and source are submitted in Table X.

C.I. 10241 has been found resistant also to net blotch (H. teres) by Buchannon and McDonald (Personal communication).

Helminthosporium sativum Toxin as Inhibitor to Other Fungi

It was observed, during the screening test, that the un-inoculated seedlings of control plants were infected by Pythium spp. in one of the sand-bed series. The fungus was isolated in pure culture and transferred to petri plates, containing V8 agar media to which extracts from H. sativum, H. teres or Alternaria tenuis had been added. The results showed that the Pythium spp. grew normally on the check containing only V8, on V8 containing H. teres extract or Alternaria tenuis extract, but was inhibited by H. sativum extract, as shown in Fig. 12. The Pythium spp. isolate was identified by Dr. T. C. Vanterpool, University of Saskatchewan, as a Pythium aristosporum Vanterpool.

In 1957 an evaluation H. sativum toxin effects on barley was made by Ludwig (19). He found that many symptoms characteristic of seedling blight, such as stunting and chlorosis, were produced by the application of toxin culture filtrates to seedlings. The toxin also was shown to predispose the plant to invasion. In this study, the extract of H. sativum acted as an inhibitor of growth in Pythium aristosporum Vanterpool, but this does not necessarily imply that the same substance was responsible as that which produced the toxic effect

TABLE X

ROOT-ROT REACTION OF SELECTED ENTRIES IN SUCCESSIVE TESTS SHOWING CI NUMBERS, VARIETY NAMES, AND SOURCES

CI Number	Sources	Variety Name	Reaction to Isolate Number 514	680
2550	Algeria	-	R,R,R	R,R,R
8873	Turkey	-	R,R,mR,R	R,mR,S,R
10241	Afghanistan	-	R,R,mR,R	mR,S,mR,mR
8969	Japan	Bakalia No. 2	R,mR,R	R,mR,R
1343	Hybrid Minn.	Lonhals	R,R,R	S,mR,mR
5435	C.C. Sel. 4116	-	R,R,R	mR,R,R
2355	unknown	-	R,R,R	R,R,R

on the host in Ludwig's experiments (19).

The Inheritance of Pathogenicity

It was considered useful to obtain information concerning the genetic control of the high level of pathogenicity expressed in isolate 514. To obtain compatible isolates, crosses were attempted between 514 and each of the non-pathogenic isolates (Table IV). The cross with isolate 499 produced perithecia readily. The avirulence of isolate 499 was verified in a test using cornmeal inoculum in four replicates applied to the varieties Anoidium, N.D.B112 and Parkland. The results are shown in Table XI.

TABLE XI

THE RATE OF PATHOGENICITY OF ISOLATE 499

Duplicate Varieties	Reaction to 499 Isolate I	II	III	IV
Anoidium	R	R	R	R
N.D.B112	R	R	R	R
Parkland	R	R	R	R

The data shown in Table XI confirmed the results previously obtained and presented in Table IV concerning the avirulence of isolate 499. Eight ascospous from a single ascus from the cross 514 X 499 were isolated and designated by Roman numerals from I to VIII. Each isolate was sub-transferred by single conidia to V8 slants. Cornmeal

inoculum was prepared from each isolate and used to infest sand. The three varieties, Anoidium, N.D.B112 and Parkland, were chosen to test the relative virulence of the progeny isolates. They were chosen on the basis of their susceptible reaction to 514 as was shown in Table IX and their resistance to isolate 499 as was shown in Table XI. They were inoculated in 4 replicates to detect segregation for virulence among the eight isolates.

The results are shown in Table XII and illustrated in Figs. 13-21. The results showed obvious segregation in a ratio of 1:1:1:1, which suggested that two genes were involved in segregation of pathogenicity in this cross. Later, the mating types of the eight isolates were determined by crossing each with the parent 499. Isolate 514 was arbitrarily designated as (+) and the mating type of 499 as (-). The eight isolates were designated according to their behaviour in the crosses with 499. The results are shown in Table XIII.

TABLE XIII

MATING TYPES OF THE EIGHT ISOLATES DERIVED FROM THE CROSS 514 X 499

Mating type	
+	-
II	I
VI	VII
VIII	V
III*	IV

*Isolate III, for an unknown reason, was sterile and failed to produce fertile perithecia with either parent or with the other isolates. By elimination it should belong to the (+) group.

TABLE XII

REACTION OF THE EIGHT ISOLATES FROM 514 X 499 CROSS IN CORNMEAL TEST USING ANOIDIUM, N.D.B112 AND PARKLAND AS TESTERS FOR THE RELATIVE VIRULENCE OF THE PROGENY ISOLATES

	Isolate I - Replicates				Isolate VII - Replicates			
	a	b	c	d	a	b	c	d
Anoidium	R	R	R	R	R	R	R	R
N.D.B112	R	R	R	R	R	R	R	R
Parkland	R	R	R	R	R	R	R	R
	Isolate VIII - Replicates				Isolate IV - Replicates			
Anoidium	R	R	mR	mR	mR	mR	mR	mR
N.D.B112	R	R	R	R	R	R	mR	R
Parkland	R	R	mR	mR	R	R	R	R
	Isolate II - Replicates				Isolate V - Replicates			
Anoidium	S	mR	S	S	mR	S	S	S
N.D.B112	mR	mR	mR	S	mR	mR	mR	S
Parkland	S	S	mR	S	S	S	S	S
	Isolate III - Replicates				Isolate VI - Replicates			
Anoidium	S	S	S	S	S	S	S	S
N.D.B112	S	S	S	S	S	S	S	S
Parkland	S	S	S	S	S	S	S	S

The results indicate that:
I and VII - avirulent
VIII and IV - slightly virulent
II and V - virulent
III and VI - highly virulent

Two conclusions may be drawn from Table XIII

(1) The segregation of four isolates (+) and four isolates (-) or in the ratio 1:1 indicates that one gene pair is involved in the determination of mating type.

(2) The results of the test which concerned the relative virulence of the progeny isolates, showed identical classification of isolates VIII and IV, and of II and V. Identification for mating type, which is more positive, showed that isolate VIII and IV belong to different groups of mating type; VIII belongs to (+) and IV to (-). The same was true of isolates II and V. Thus neither isolates VIII and IV nor II and V are identical and the results of Table XII, which are concerned with the relative virulence of the progeny isolates and their segregation for pathogenicity can not be considered authentic.

Another attempt to determine segregation for pathogenicity was made through a new crossing program. Isolate VI, which showed a high degree of virulence (Table XII), was crossed with the avirulent parent, 499. Two sets of eight ascospores were isolated. One set of eight ascospore progenies was increased individually in V8 media, and the eight isolates were designated as A, B, C, D, E, F, G and H. Cornmeal inoculum was prepared from each isolate and used to infest sand for virulence tests. Three varieties, Anoidium, N.D.B112 and Parkland, were inoculated with cornmeal inoculum in four replicates. The results showed no clear segregation among the eight isolates.

The contradictory results in segregation of pathogenicity could be explained in a number of ways.

(1) Segregation was largely indistinguishable from normal variation.

(2) As suggested by Tinline and Dickson (25), multiple factors control pathogenicity in *H. sativum*.

(3) Segregation ratios were distorted by interference from the toxin affecting seedling reaction.

(4) From the genetic viewpoint the varieties that were chosen to detect segregation ratios were inadequate.

Segregations Involved in the Growth Rate of Isolates 514 and 499

Single spores from isolates 514 and 499 were transferred to petri dishes containing potato sucrose agar (P.S.A.) with duplicates for each. After seven days of growth, the two isolates were compared. The results showed that isolate 514 was slow-growing and isolate 499 was fast-growing.

The eight isolates from the cross 514 X 499 were transferred to potato sucrose agar to detect segregation ratios which might indicate the number of genes involved in growth rate. The results are shown in Table XIV.

TABLE XIV

GROWTH RATE OF EIGHT ISOLATES DERIVED FROM THE CROSS 514 BY 499 AND THE PARENTS

Isolate No.	Growth Rate
514	Slow
499	Fast
III and VI	Very slow
V and IV	Slow
II and VIII	Fast
I and VII	Very Fast

The results from Table XIII and XIV are combined for comparing growth rate and mating type in Table XV.

TABLE XV

GROWTH RATE AND MATING TYPE OF THE ISOLATES DERIVED FROM THE CROSS 514 BY 499 AND THE PARENTS

Isolate Number	Growth Rate	Mating Type
514	S	+
499	F	-
III	V.S.	+
VI	V.S.	+
V	S	-
IV	S	-
II	F	+
VIII	F	+
I	V.F.	-
VII	V.F.	-

From these results three conclusions may be drawn:

(1) The isolates classified similarly for growth rate are of the same mating type.

(2) The growth rate ratio of 1:1:1:1 indicates that 2 gene pairs are involved.

(3) Growth rate and mating type appeared to be inherited independently.

Another experiment was conducted to detect the segregation in the growth rate of the two sets of eight ascospores derived from the cross VI X 499. Each isolate was transferred to potato sucrose agar medium. The growth rate was determined after seven days. The results are shown in Table XVI, Table XVII and Figs. 22-23.

TABLE XVI

THE GROWTH RATE OF THE EIGHT ISOLATES DERIVED FROM VI X 499 CROSS, ASCUS NUMBER I, AND THE PARENTS ON POTATO SUCROSE AGAR MEDIA

Isolate No.	Growth Rate
VI	S
499	F
E and C	S
B and F	S
A and G	F
D and H	F

TABLE XVII

THE GROWTH RATE OF THE EIGHT ISOLATES FROM VI X 499 CROSS, ASCUS NUMBER II AND THE PARENTS

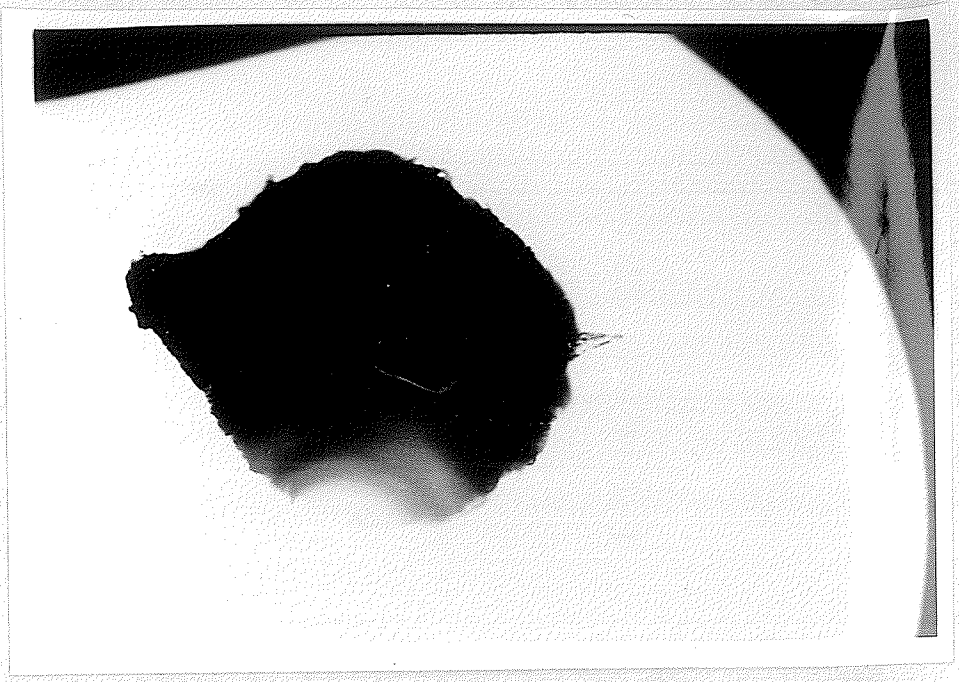
Isolate Number	Growth Rate
VI	S
499	F
S and K	S
T and O	S
P and N	F
R and V	F

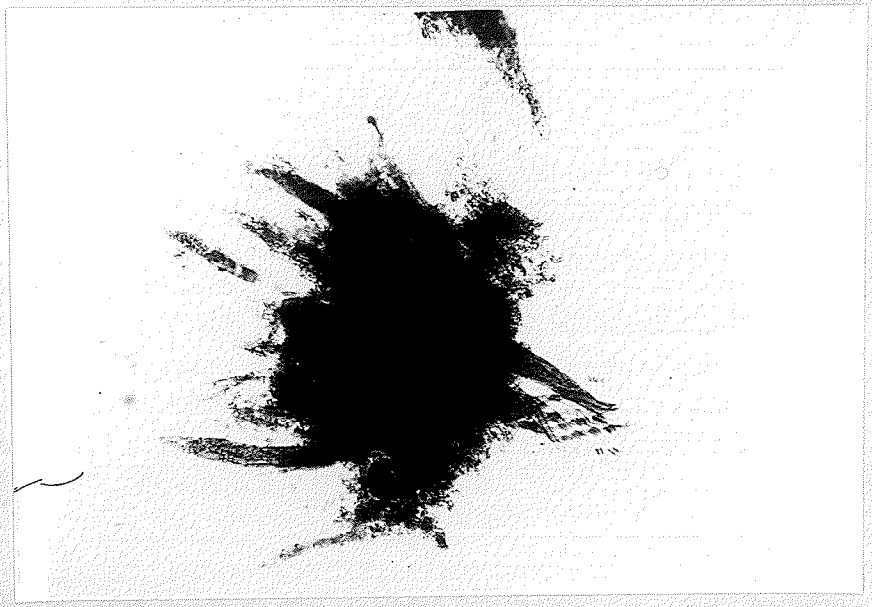
The data from the two backcross asci agreed in showing a 1:1 segregation for growth rate. This result can be explained on the basis that isolate VI, which was very slow growing, carried neither of the genes favourable to growth. The transgressive segregation in the original cross of 514 X 499 (Table XV) suggests that each parent carried a gene favourable to growth, a strong one in 499, a weaker one in 514. Hence, monogenic segregation is expected in the backcross asci. The results obtained from all three asci are in agreement, supporting the hypothesis of two genes determining growth rate in these crosses.

Perfect stage of Helminthosporium sativum

Fig. 1. Beaked perithecium X 10.

Fig. 2. Section of perithecium with asci and paraphysis X 54.

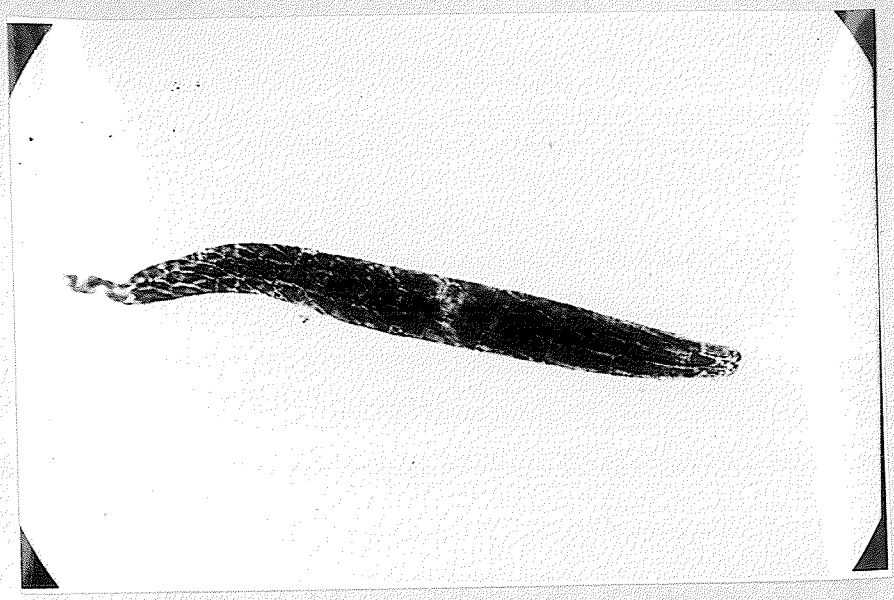


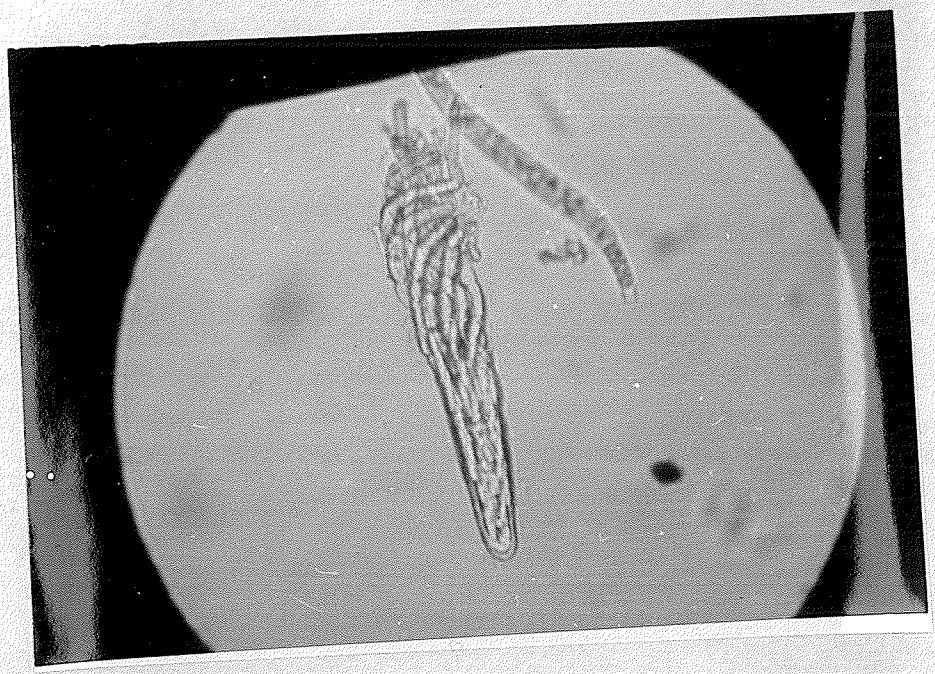


Perfect stage of Helminthosporium sativum

Fig. 3. Ascus showing helicoil arrangement of the ascospores X 340.

Fig. 4. A ruptured ascus with coiled ascospores X 240.

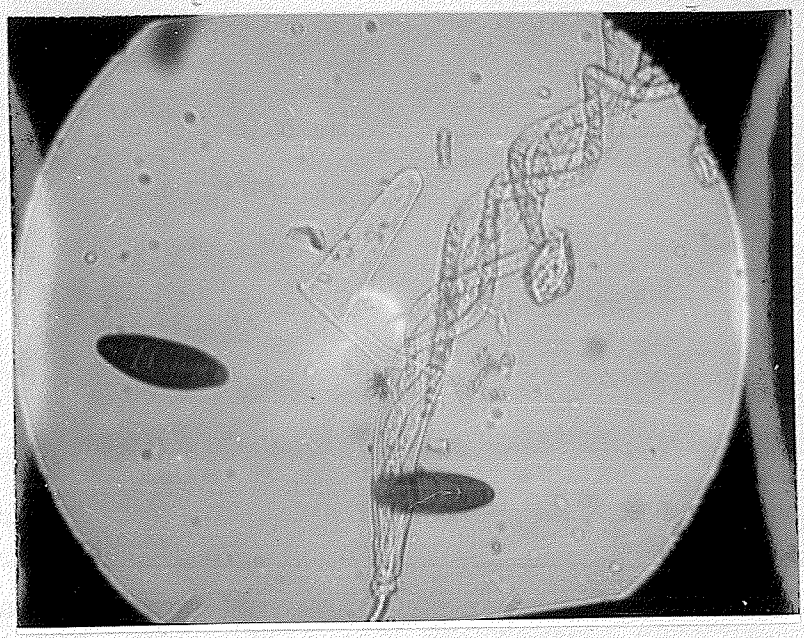


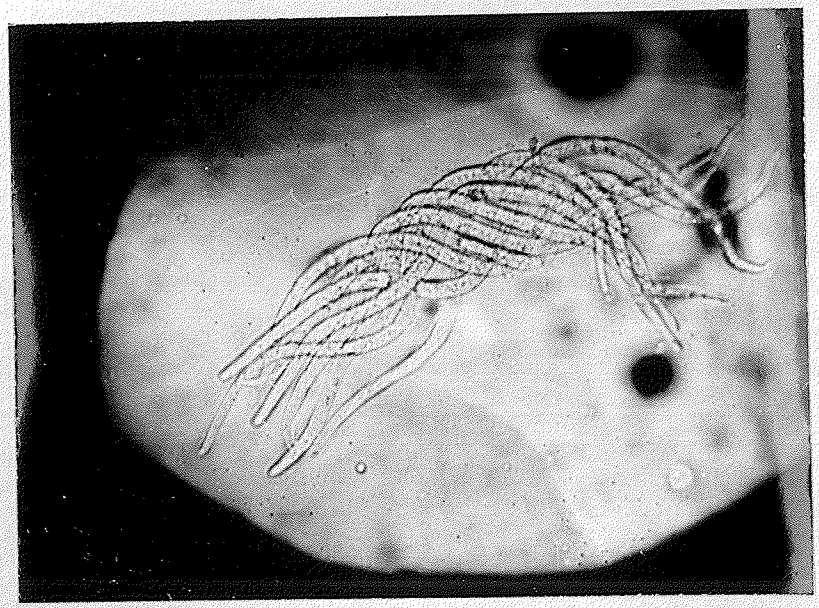


Perfect stage of *Helminthosporium sativum*

Fig. 5. Two conidia with ruptured ascus which has ascospores emerging X 340.

Fig. 6. Eight coiled ascospores after emerging from the ruptured ascus X 340.





Perfect stage of Helminthosporium sativum

Fig. 7. Septate ascospore X 560.

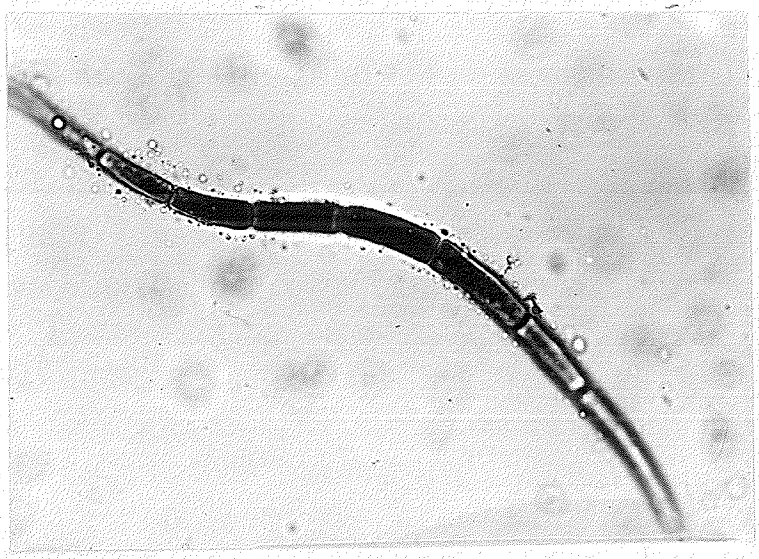


Fig. 8. Resistant and susceptible entries in cornmeal inoculum test, with uninoculated check.

Fig. 9. Resistant, susceptible entries and check, washed free of sand, in cornmeal inoculum test.


Check
Resistant
Susceptible

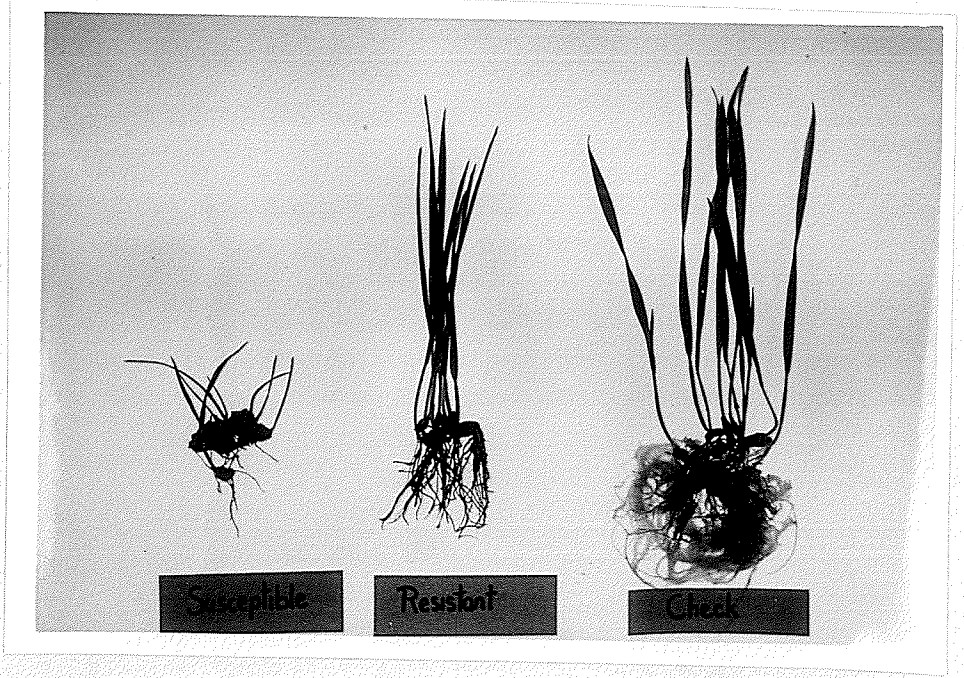

Susceptible
Resistant
Check

Fig. 10. View of sand bed in the screening test for resistance to Helminthosporium sativum with check's forming T. configuration.

Fig. 11. Section of the sand bed showing the check and susceptible entries.

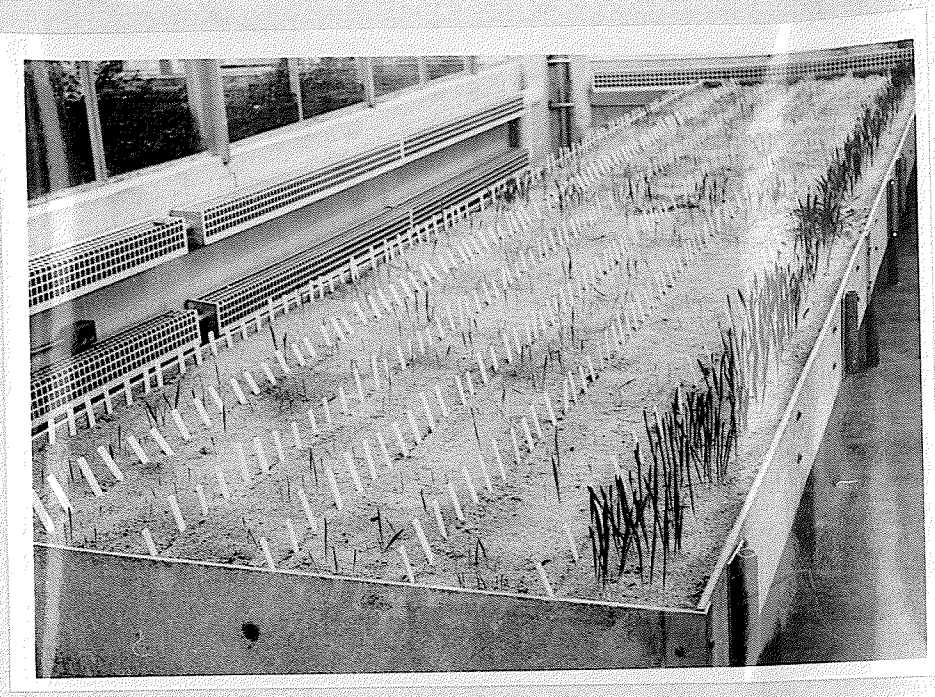


Fig. 12. Growth of Pythium aristosporum on V8 medium with Helminthosporium sativum extract (top left), on Alternaria tenuis extract (top right), nothing added (bottom left) and on Helminthosporium teres extract (bottom right).

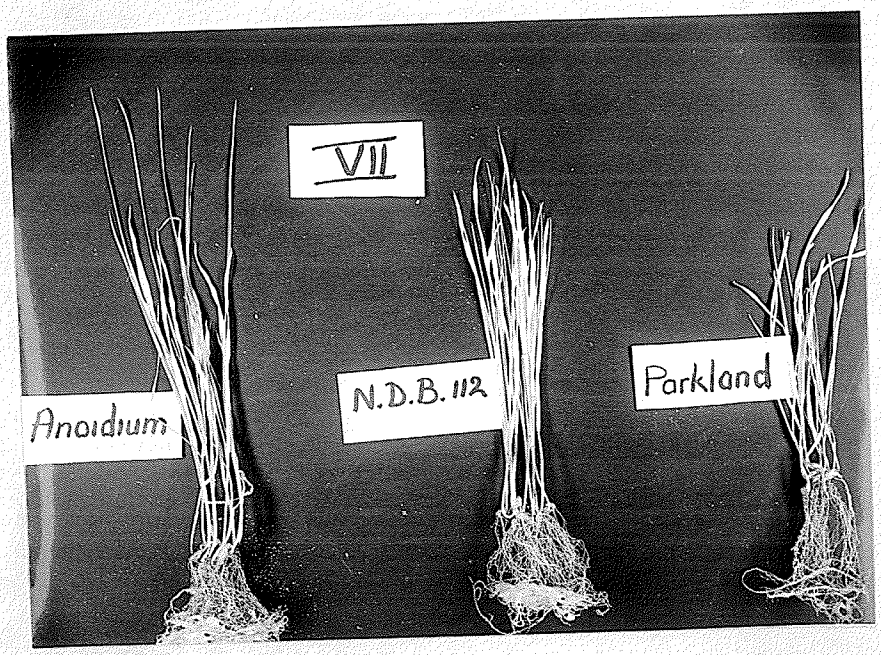
Segregation in pathogenicity of the eight isolates, I, II, III, IV, V, VI, VII, VIII from 514 X 499 cross in cornmeal inoculum test using Anoidium, N.D.B112 and Parkland as testers.

Fig. 13. Test varieties uninoculated as a check.

Segregation in pathogenicity of the eight isolates, I, II, III, IV, V, VI, VII, VIII from 514 X 499 Cross in cornmeal inoculum test using Anoidium, N.D.B112 and Parkland as testers.

Fig. 14. Reaction of the testers to isolate I avirulent.

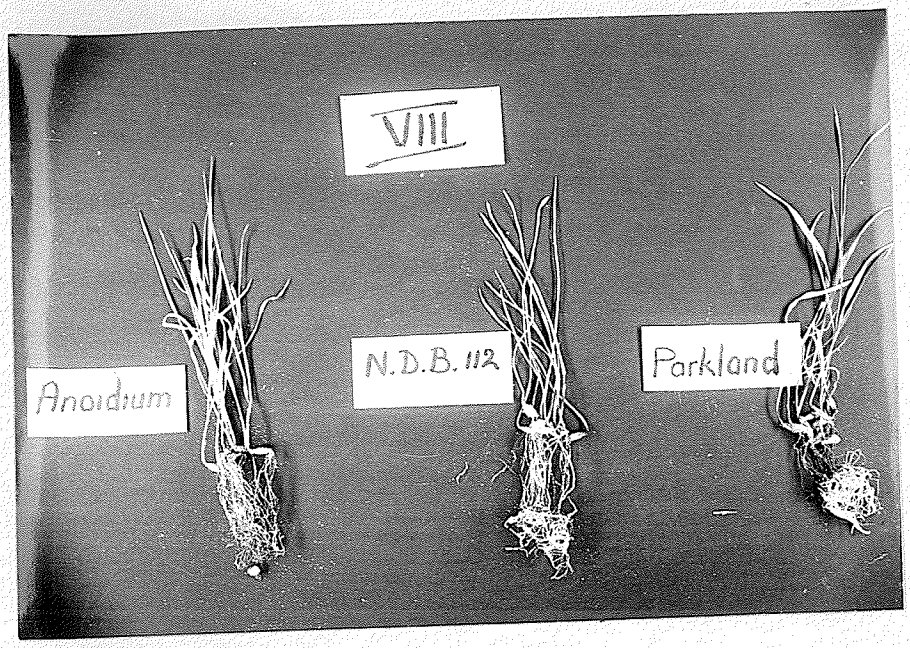
Fig. 15. Reaction of the testers to isolate VII avirulent.


VII
Anoidium
N.D.B. 112
Parkland

Segregation in pathogenicity of the eight isolates, I, II, III, IV, V, VI, VII, VIII from 514 X 499 cross in cornmeal inoculum test using Anoidium, N.D.B112 and Parkland as testers.

Fig. 16. Reaction of the testers to isolate VIII slightly virulent.

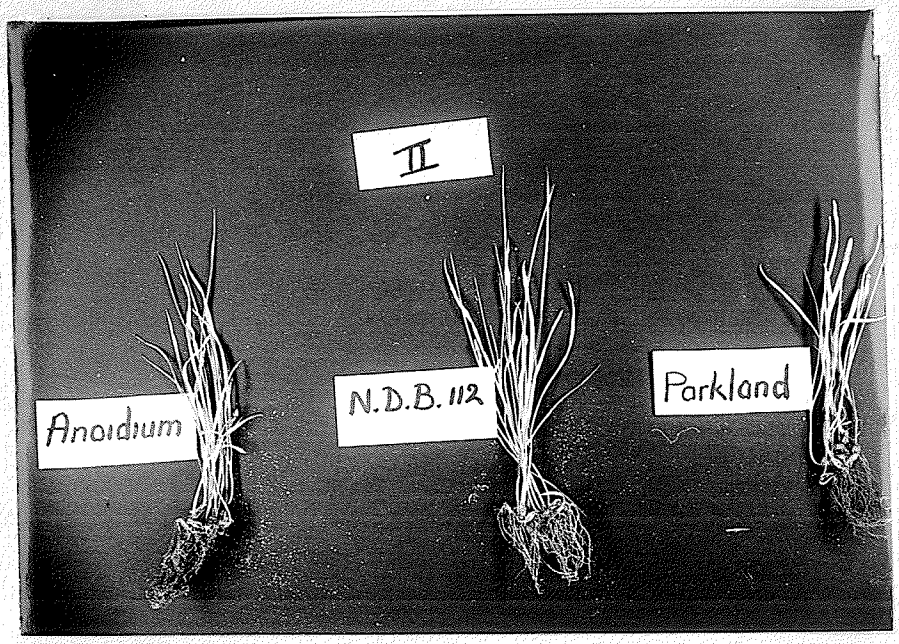
Fig. 17. Reaction of the testers to isolate IV slightly virulent.

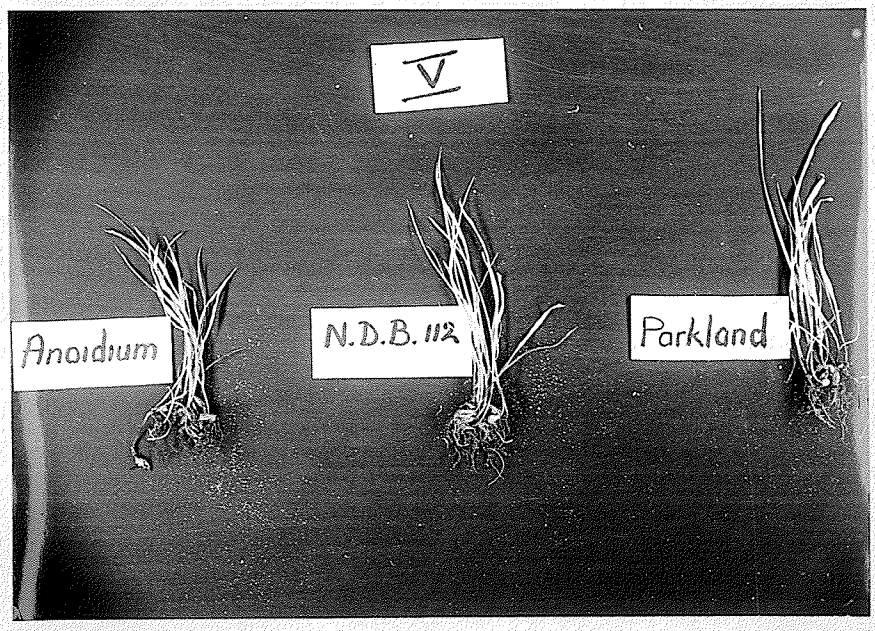

VIII
Anoidium
N.D.B. 112
Parkland

Segregation in pathogenicity of the eight isolates, I, II, III, IV, V, VI, VII, VIII from 514 X 499 cross in cornmeal inoculum test using Anoidium, N.D.B112 and Parkland as testers.

Fig. 18. Reaction of the testers to isolate II virulent.

Fig. 19. Reaction of the testers to isolate V virulent.


II
Anoidium
N.D.B. 112
Parkland


V
Anoidium
N.D.B. 112
Parkland

Segregation in pathogenicity of the eight isolates, I, II, III, IV, V, VI, VII, VIII from 514 X 499 cross in cornmeal inoculum test using Anoidium, N.D.B112 and Parkland as testers.

Fig. 20. Reaction of the testers to isolate III highly virulent.

Fig. 21. Reaction of the testers to isolate VI highly virulent.


VI
+ maidium
VI
N. D. 13. 112
VI
Parkland

Fig. 22. Segregation in growth rate of the eight isolates, A, B, C, D, E, F, G, H, derived from 499 X VI cross, ascus number I, and the parents on P.S.A. media.

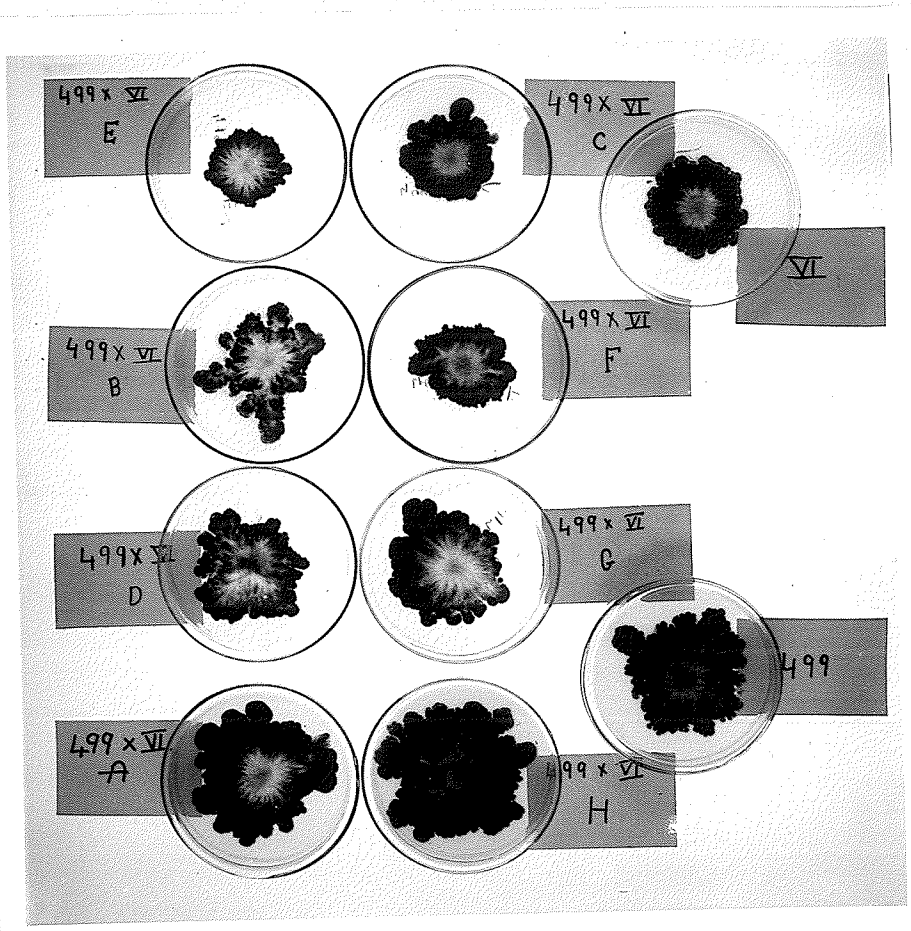
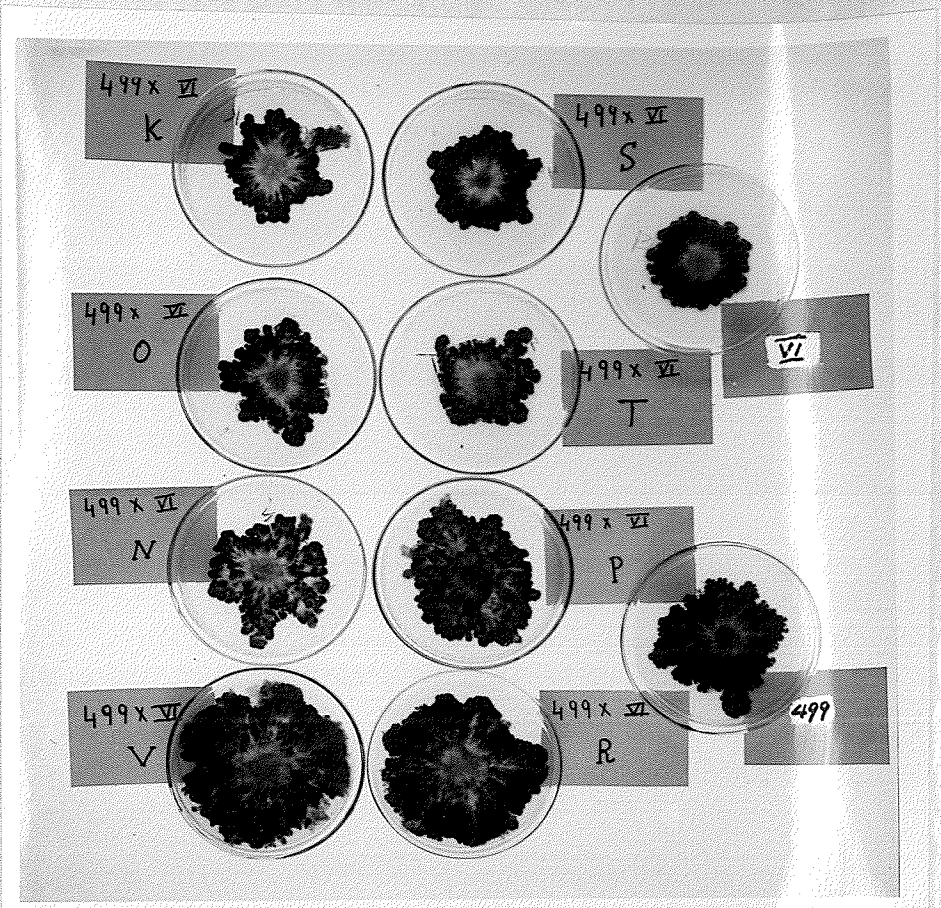

499 x VI
E
499 x VI
C
VI
499 x VI
B
499 x VI
F
499 x VI
D
499 x VI
G
499
499 x VI
A
499 x VI
H

Fig. 23. Segregation in growth rate of the eight isolates, K, N, O, P, R, S, T, V, derived from 499 X VI cross, ascus number II, and the parents on P.S.A. media.


499 x VI
K
499 x VI
S
499 x VI
O
499 x VI
T
VI
499 x VI
N
499 x VI
P
499 x VI
V
499 x VI
R
499

DISCUSSION

Great differences in pathogenicity were found among thirteen isolates of H. sativum, from the highly pathogenic isolate 514 to the non-pathogenic group 599, 606, 499, 692. These results indicate that selection for resistance should be based on an annual survey of the isolates which are prevalent in the region. Only by this means can effective selection for resistance in the host, to the highly pathogenic isolates which may occur under field conditions, be carried on.

Two methods of inoculation were tested. Both of these require improvement in order to make them highly effective in selecting for resistance to root-rot caused by H. sativum. The reaction of the entries in the cornmeal inoculum were relatively constant among replicates within an experiment, but were not constant in comparisons between experiments. In cornmeal, various factors can influence the reaction of the entries toward root-rot in the seedling stage. The factors could be environmental, genetic variation in the progeny of the isolates, and/or the effect of the toxin on the seedling reaction. Various devices could be tried in an effort to decrease the effect of these factors. The effect of the toxin might be reduced by pregermination of the seed before planting in the infested sand, as well as by washing the cornmeal inoculum to decrease accumulation of the toxin, as suggested by Ludwig et al. (18). Uniformity of the environment should be stressed in experiments. The seed should be sized and tested for vigour of germination to reduce to a minimum the effect of seedling vigour on the reaction of the entries in different experiments. But even if it were possible to greatly improve

the cornmeal inoculum method, it will not be applicable to experiments on a large scale. In such experiments, the great amount of labour and time involved in preparation and handling of the inoculum makes this method impractical. The powder inoculum, on the other hand, is applicable to experiments on a large scale, since it can be prepared very easily in a large amounts, and may be kept in a refrigerator for a prolonged period. However, much improvement is required to refine the techniques utilizing this type of inoculum. The powder inoculum was very severe, and probably many entries which were in fact resistant gave a susceptible reaction due to the heavy infection produced by this type of test. Moreover, a large number of entries were found to have escaped, as shown by subsequent tests. Precise experiments are required to determine the most critical amount of inoculum to be used for differentiating reaction among host varieties, and to determine the best way of applying the inoculum. Methods to be tested might include applying the powder above the seed after planting or under the seed before planting. Alternatively, the seed could be mixed with powder before planting or the powder mixed directly with the sand. Moreover, the entries could be planted with replication in randomized blocks to permit a more precise estimation of environmental effects on the seedling reaction.

The effect of temperatures, in the range from 54°F. to 88°F., on disease development in the seedling stage of barley was tested, using the isolates 514 and 680. The results showed that there was no major variation in either the pathogenicity or the relative virulence of the 514 or 680 isolates due to temperature. This confirmed the results

obtained by Wood (29, 30) and by Clark et al. (7). From the data which concern isolate 680 at the 54-56°F. temperature, it appeared that the pathogenicity of this isolate was greater than at the other temperatures. This may be due to slow germination of the seed because of low temperatures, resulting in a longer period of exposure to the accumulating toxin in the planting medium. Comparisons between tables VI and VII indicated that isolate 514 was more virulent than the 680 isolate at all the temperatures on these testers. Thus the severity of the infection of isolate 514 might mask the influence of the accumulating toxin at the 54° - 56° temperature. At the 80-82°F. temperature, the pathogenicity of isolate 514 was slightly greater than at the other temperatures. The significance of this variation is in some doubt, and only further experiments would make it possible to determine the consistency of the effect and the nature of it.

The effect of H. sativum toxin on disease development in the host was described by Ludwig (19). The toxin was evaluated through a bioassay technique, using seed germination of barley and spore germination of Sclerotinia fructicola as criteria. The suggestion was made that the toxin of H. sativum predisposed the plant to invasion. In the present studies, H. sativum extract inhibited the growth of Pythium aristosporum Vanterpool. Further tests would be required to determine whether the toxin which inhibited the growth of Pythium aristosporum is identical to the one studied by Ludwig.

Approximately 6200 entries were screened using powder inoculum and 100 of these were retested by the cornmeal inoculum procedure. Only seven varieties showed a desirable level of resistance to both of

the isolates, 514 and 680. An examination of the resistant entries showed that the roots were brownish as in the susceptible host varieties, but had grown longer. It was found also, that the resistant types had developed new white roots in addition to the original ones which had turned brown. Such roots were never observed in the susceptible types. This suggests that the susceptible plants were restricted by the presence of the pathogen and failed to develop new roots to replace the ones destroyed by infection. The occurrence of browning in both types may indicate that the toxin of H. sativum, which developes in the medium, is non-specific and affected both resistant and susceptible types. This observation is in accord with the finding of Ludwig (19) that toxin, even in the absence of the organism, produced symptoms similar to those resulting from infection by H. sativum. The exact nature of resistance to this complex of toxin and pathogen requires further study. It is not known whether the symptoms on resistant varieties are caused entirely by the toxin, or are partly due to invasion by the pathogen.

Experiments were conducted to study segregation of pathogenicity in a cross of the highly pathogenic isolate 514 with the non-pathogenic isolate 499. It was not possible to establish the mode of genetic segregation for relative pathogenicity among the eight isolates from this cross. One possible explanation of this result is multiple gene control of pathogenicity, as was suggested by Tinline and Dickson (25). It could also be due to the differential effect of toxin on the reaction of the seedlings towards specific isolates. A third possibility is that the genetic constitution of the host varieties used as testers was inadequate to reveal the segregation of virulence genes of the pathogen.

Although one or more of these factors may have influenced the results, it is mostly likely that the range of the variation usually encountered in using the cornmeal inoculum was the main hindrance for detecting segregation which would explain the genetics of virulence in this cross. The extremes in virulence, high or low, were differentiated successfully in relation to mating type (Table XII, XIII). The intermediate isolates could not, however, be classified correctly, based on the same criterion. Ludwig (19) has pointed out that toxin production in isolates of intermediate virulence was less closely related to pathogenicity and more variable between experiments than in highly virulent strains. Since accumulation of toxin is characteristic of the cornmeal inoculum procedure, this is likely an important factor in masking the true virulence of the intermediate isolates. It is likely that a more sensitive method of inoculation, with adequate testers would have made possible a more precise classification of segregates for virulence, leading to a genetic explanation of the segregation. Growth rate classes appeared to be more readily distinguishable. The variations which were found within classes were considered to be the normal ones influenced by environment or the segregation of modifiers with minor effects. Typical variation are shown by progeny of the cross VI X 499 (Fig. 22-23), in which the major growth differences in the ascospore cultures were considered to fit a 1:1 ratio. It is recognized that further investigations are required to establish definitely the validity of assumptions concerning the minor fluctuations in growth rate.

LITERATURE CITED

1. ARNY, D. C., 1948. Inheritance of resistance to spot blotch of barley. Phytopathology 38:1 Abstract.

2. ARNY, D. C., 1951. Inheritance of resistance to spot blotch in barley seedlings. Phytopathology 41: 691-698.

3. ASHWORTH, L. J. Jr., K. A. LAHR, and R. J. COLLINS. 1960. Pathogenic specialization of Helminthosporium sativum. Phytopathology 50: 627. Abstract.

4. CHINN, S. H. F., B. J. SALLANS and R. J. LEDINGHAM. 1962. Spore populations of Helminthosporium sativum in soils in relation to the Occurrence of Common Root-Rot of Wheat. Can. J. Plant Sci., 42: 720-727.

5. CHINN, S. H. F., and R. D. TINLINE. 1963. Spore germinability in Soil an Inherent Character of Cochliobolus sativus. Phytopathology 53: 1109-1112.

6. CHRISTENSEN, J. J. and F. R. DAVIES. 1937. Nature of variation in Helminthosporium sativum. Mycologia 29: 85-99.

7. CLARK, R. V. and J. G. DICKSON. 1958. The Influence of Temperature on Disease Development in Barley Infected by Helminthosporium sativum. Phytopathology 48: 305-310.

8. DICKSON, J. G. 1946. Helminthosporium Foot Rot of Barley. Phytopaghology 36: 397. Abstract.

9. DICKSON, J. G. 1956. Diseases of Field Crops. McGraw-Hill Book Company. Inc., N. Y., Toronto. London.

10. HAMILTON, D. G., R. V. CLARK, A. E. HANNAH and R. LOISELLE. 1960. Reaction of Barley Varieties and Selections to Root-Rot and Seedling Blight Incited by Helminthosporium sativum P. K. and B. Can. J. Plant Sci. 40: 713-720.

11. KREITLOW, K. W., and Helen S. SHERWIN. 1952. Infectivity of Dried Inoculum of Some Foliar Pathogens. Phytopathology 42: 469 Abstract.

12. HAYES, H. K., E. C. STAKMAN, F. GRIFFEE and J. J. CHRISTENSEN. 1923. Reaction of Barley Varieties to Helminthosporium sativum. Part I. Varietal Resistance. Part II. Inheritance Studies in a Cross Between Lion and Manchuria. Minn. Agr. Exp. Sta. Bull. 21

13. HENRY, A. W., 1931. Occurrence and Sporulation of Helminthosporium sativum P. K. and B. in the Soil. Can. J. Research 5: 407-413.

14. HRUSHOVETZ, S. B., 1956. Cytological Studies of Helminthosporium sativum. Can. J. Botany 34: 321-327.

15. HRUSHOVETZ, S. B., 1956. Cytological Studies of Ascus development in Cochliobolus sativus. Can. J. Botany 34: 641-651.

16. LOISELLE, R., 1962. Note on Additional Sources of Resistance to Helminthosporium sativum P. K. and B. Can. J. Plant Sci. 42: 368-369.

17. LOISELLE, R., 1962. Root-Rot Inheritance Studies. Barley Newsletter 93.

18. LUDWIG, R. A., R. V. CLARK, J. B. JULIEN and D. B. ROBINSON. 1956. Studies on the Seedling Disease of Barley Caused by Helminthosporium sativum P. K. and B. Can. J. Botany 34: 653-673.

19. LUDWIG, R. A., 1957. Toxin Production by Helminthosporium sativum P. K. and B. and Its Significance in Disease Development. Can. J. Botany 35: 291-303.

20. MACHACEK, J. E., 1943. An Estimate of Loss in Manitoba From Common Root-Rot in Wheat. Sci. Agr. 24: 70-79.

21. MORTON, Donald J., 1962. Influence of Temperature, Humidity and Inoculum Concentration on Development of Helminthosporium sativum and Septoria passerinii in Excised Barley Leaves. Phytopathology 52: 704-708.

22. SIMMONDS, P. M., B. J. SALLANS and R. J. LEDINGHAM. 1950. The Occurrence of Helminthosporium sativum in Relation to Primary Infection in Common Root-Rot of Wheat. Sci. Agr. 30: 407-417.

23. SHOEMAKER, R. A., 1955. Biology, Cytology, and Taxonomy of Cochliobolus sativus. Can. J. Botany 33: 562-575.

24. TINLINE, R. D., 1951. Studies on the Perfect Stage of Helminthosporium sativum. Can. J. Botany 29: 467-478.

25. TINLINE, R. D. and J. G. DICKSON. 1954. Genetic Segregation and Heterocaryosis in Helminthosporium sativum. Phytopathology 44: 508. Abstract.

26. TINLINE, R. D. and J. G. DICKSON. 1958. Cochliobolus sativus. I. Perithecial Development and the Inheritance of Spore Color and Mating Type. Mycologia 50: 697-706.

27. TINLINE, R. D., 1962. Cochliobolus sativus. V. Heterokaryosis and Parasexuality. Can. J. Botany 40: 425-437.

28. WOOD, L. S., J. J. CHRISTENSEN and J. W. LAMBERT. 1954. Helminthosporium sativum Becomes Destructive on Hitherto Resistant Varieties of Barley. Phytopathology 44: 511 Abstract.

29. WOOD, L. S., 1958. Genetic Variation of Helminthosporium sativum in Relation to Seedling Blight of Small Grains. Ph.D. Thesis. U. of Minnesota.

30. WOOD, L. S., 1962. Relation of Variation in Helminthosporium sativum to Seedling Blight of Small Grains. Phytopathology 52: 493-498.