A STUDY OF FACTORS INFLUENCING THE GENETICS OF REACTION OF BARLEY TO ROOT-ROT CAUSED BY *HELMINTHOSPORIUM SATIVUM* P.K. and B.

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ABSTRACT

The inheritance of seedling reaction to root-rot, caused by the 514 isolate of Helminthosporium sativum, was studied in crosses and backcrosses among the barley varieties, CI.8969 and CI.8873, which show resistance, and the variety Olli, which is susceptible. The study was carried out under a controlled environment in growth-cabinets. The data from reaction tests of F_3 and backcross families were analysed by Elston's procedure for testing a one-locus hypothesis, and by Powers' partitioning method. Polygenic inheritance for seedling reaction was indicated, but subsequent experiments showed that seed weight strongly influenced the reaction, which could account for the polygenic inheritance. Based on a drastic reduction of resistance in greenhouse-produced seed, irrespective of seed weight, a high positive correlation of seed weight with resistance in field-produced seed, and other evidence of a similar nature, it was assumed that resistance was related to a principle, present in the seed or on the seed surface, the quantity of which is modified by environment. Whether there is a genotypic influence on the quantity of the resistance principle, aside from that affecting seed weight, was not established conclusively.

A field study, made in an area known to produce natural root-rot infection, involving the same materials, showed that no correlation existed between the disease indices of the entries in the seedling stage in the growth-cabinet tests, and their reaction to the disease in the field. CI.8873 showed field resistance, whereas CI.8969 did not.

The implications of these findings with respect to breeding for field resistance to root-rot were discussed.

An attempt to establish a nursery for root-rot study at the University Farm failed. As the level of inoculum in the soil was found to be adequate, the lack of infection was attributed to antagonism of the soil environment.

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 (5,6,8,13,14). 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 (21) 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 (39) 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, were estimated at 38.3 percent and 12.1 percent, respectively. In 1954, Wood et al. (67) 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 (6,8,22,54). The breeding of resistant varieties offers the best practical means of

controlling this pathogen (20,21). Attempts to find resistant material were conducted by various investigators (3,4,20,21,33,34,67). It was found by Arny (3,4) 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.* (21) indicated that more than one genetic factor pair was involved. Loiselle (34) 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.* (20) and Loiselle (33). These investigators followed the method of inoculation established by Ludwig *et al.* (38) and reported several varieties resistant to isolates prevalent in the Ottawa area. Cohen (12) reported seven varieties resistant to the virulent isolate 514 of *H. sativum*.

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

1). Study of the inheritance of reaction to root-rot and seedling blight caused by *H. sativum* in the seedling stage in crosses between the resistant varieties, CI. 8873 and CI. 8969, and the susceptible variety Olli.

2). Study of other factors which contribute to the resistance of CI.8873 and CI.8969 in the seedling stage.

3). To determine the relationship between seedling resistance and field resistance.

4). To determine the effectiveness of a field disease nursery for testing disease reaction.

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.) *Bipolaris sorokiniana* (Sacc. in Sorok.)

In 1910 Pammel, King and Bakke described *Helminthosporium sativum* as a new pathogen on barley (14). 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 (14) examined the Saccardo type-specimens of *H. sorokinianum* and *H. sativum* and reported their synonomy.

Ito and Kuribayashi in 1929, as reported by Tinline (61) described an ascigerous fungus obtained in a culture as the perfect stage of *H. sativum* under the binomial *Ophiobolus sativus*. 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 (61) 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.

Tinline (61), 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 (59) extended the studies on perithecial development and reported on inheritance of spore color and mating type. Hrushovetz (23), in cytological studies of the ascus and Shoemaker (53) 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 (53), through his discovery of the nature of sexuality in the fungus, described in detail the development of spermagonia, ascogonia, prototheciam and pseudotheciam 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 (23) 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 (60) reported on heterokaryosis, and Tinline (58) reported heterokaryosis and parasecuality in H. sativum. It was found that heterokaryosis 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 (24) in sytological studies of H. sativum, confirmed the findings of Tinline on heterokaryosis 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 heterokaryosis with occasional nuclear dissociation.

Wood (65,66) 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 (9) and Christensen and Schneider, as reported by Dickson (14), 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 (45) and Clark et al. (10) found differences in expression on the spot blotch disease. However, Clark et al. (10) and Wood (65,66) 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.

Tinline and Dickson (60) 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 (28) 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. (38). 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 93 percent sand and moistening this mixture with a nutrient solution made from inorganic salts in Czapek's medium. This medium was inoculated with spore suspension of H. sativum.

Resistance to common root-rot in barley has been reported by several investigators. Hamilton et al. (20) studied the reaction of

barley varieties and selections to root-rot and seedling-blight incited by H. sativum, using cornmeal inoculum. 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 (34) for reaction to H. sativum. Fourteen entries were classified as resistant to H. sativum. Cohen (12) screened approximately 6,200 entries from the World Barley Collection for resistance to seedling-blight and root-rot, and found that only seven entries demonstrated resistance: they were CI.2550, CI.8873, CI.10241, CI.8969, CI.1343, CI.5435 and CI.2355. Lange de la Camp (29) evaluated the reaction of barley and wheat hosts to root-rot disease caused by H. sativum. After several years of experimentation in the greenhouse, she concluded that reproducible differences in resistance do not exist between host varieties within the same genus, whereas differences in virulence and aggressiveness were found between different isolates of the pathogen.

Loiselle (35) studied the inheritance of resistance to an Ottawa isolate of H. sativum using two varieties of barley, Anoidium and Br. 3962-4, resistant to the root-rot and seedling-blight phases of the disease. From the results of crosses between the two resistant varieties with the susceptible parent Olli, he concluded that Anoidium and Br. 3962-4 each possessed two dominant genes for resistance over Olli. The cross, Anoidium X Br. 3962-4 showed that the genes in Anoidium were allelic or closely linked with those of Br. 3962-4. Clark (11) studied the resistance to root-rot, leaf-spot, and head-blight caused by H. sativum. Certain lines from the cross Hordeum leporinum X H. vulgare and from subsequent crosses and backcrosses to common barley were evaluated. All lines were susceptible to the leaf-spot

phase of the disease. There was a wide range in the reaction of the lines to root-rot and to head blight; resistance to root-rot was found primarily in the winter types, whereas resistance to head blight was found largely in the spring types. There was no apparent relationship between root-rot and head blight resistance, as none of the lines showed good resistance to both.

Sallans et al. (51), in an attempt to select for resistance in wheat to root-rot caused by H. sativum in the segregating material of different crosses, indicated that there was no clear-cut evidence in the distribution of the segregating progenies of simple ratios of resistant and susceptible classes, such as would be expected if a single gene controlled resistance to H. sativum. They presumed that the inheritance of resistance to the fungus was quantitative, but expressed the belief that the development of resistance in wheats to H. sativum was amenable to breeding and selection methods.

Arny (3,4) found 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. (21) indicated that two genes were responsible for resistance to the spot blotch phase of the disease caused by H. sativum.

Griffee (18) studied resistance to spot blotch caused by H. sativum in the cross Svanhals X Lion. The distribution of the F_3 lines fitted a normal frequency curve and, based on linkage relations, he concluded that at least three genetic factors were concerned in producing resistance of the type possessed by Svanhals.

The "floatation method" and the "floatation viability method" were described by Ledingham et al. (31) and Chinn et al. (6), for obtaining spores of H. sativum from soil and determining their viability. According

to these authors (6,7,31), spore numbers in the different samples which were taken from two locations in each of 100 cultivated fields in Saskatchewan ranged from a count of less than 8 to 893, with an average of 118 per gram of soil.

The toxin theory of disease caused by H. sativum has received strong support in the work of Ludwig et al (38) and Ludwig (36). They reported that invasion of cereal seedlings by H. sativum was facilated by toxic substances produced by the fungus. The toxins were non-specific in their action, affecting wheat, oats, and barley approximately equally. Many of the symptoms characteristic of seedling-blight, such as stunting, chlorosis, and loss of normal tropic responses, were produced by the application of toxic culture filtrates to barley seedlings. In the presence of abundant toxin, strains of H. sativum differed in their ability to invade barley, which indicates that factors other than toxin production were involved in pathogenicity.

Wibe et al (64) found that extracts from cultures of H. sativum, when applied to barley leaves prior to inoculation with the fungus, appeared to be toxic toward Septoria passerinii. Cohen (12) found that H. sativum produces a toxin which inhibits growth in Pythium aristosporum Vanterpool. Donald et al (15) reported on the toxicity of a culture filtrate of H. sativum to uredospora of a culture of Puccinia graminis var. tritici race 32. Filtrates from cultures with many conidia inhibited development of rust more than did filtrates from cultures with few conidia. Storing these filtrates 5 weeks at 38°F. reduced their toxicity only slightly.

The chemical structure of the toxin of H. sativum, named Helminthosporol, has been described by de Mayo et al (40,41,42) as a compound of molecular weight 234 and formula $C_{15}H_{22}O_2$.

Tamura et al (57), Kato et al (25,26) and Okuda et al (47), reported that Helminthosporol, the metabolic product of H. sativum, is a new plant growth promoting substance. They found that Helminthosporol stimulated the elongation of the second leaf sheath of both dwarf and tall seedlings of barley, producing an effect similar to that of GA_3 (gibberellic acid), but its activity was lower than that of GA_3. The elongation was due to both cell elongation and cell division, as in the case of GA_3-induced growth.

Kato et al (25) also studied the effect of Helminthosporol on various dwarf mutant strains of corn, and found that it did not promote growth of the dwarf corn seedlings at any concentration tested. In other tests, they found no effects of Helminthosporol on callus formation, root initiation or lateral bud growth, nor was there any effect upon dark germination of lettuce seed, dwarf pea growth, or elongation of epicotyls of dwarf morning glory and dwarf bean.

The physiological effect of Helminthosporol was compared with that of GA_3 by Mori et al (44). Their study revealed that the amount of reducing sugars in the leaf sheaths of rice seedlings was remarkably increased by both Helminthosporol and GA_3. Their experiment with the endosperm of rice seeds showed that, as with GA_3 (62), Helminthosporol stimulated at least the de novo synthesis of amylase, along with possible activation of preformed anylase in the aleurone layer of the endosperms of rice seeds.

Roberts et al (50) studied the possibility of substituting Helminthosporol for gibberellic acid in xylogenesis under in vitro conditions. Using tissue slices of Coleus bulmini (Benth), it was found that, contrary to the action of GA, Helminthosporol inhibited the xylogenesis response in the isolated stem tissue slices of Coleus blumini (Benth).

Kaufman et al (27) studied the effect of seed size on plant development in barley. The results of greenhouse tests with two barley varieties showed that plants grown from large seed were superior to those grown from small seed in the rate of seedling growth and size of the first two leaves.

MATERIALS AND METHODS

Source of Materials

The parents in this investigation were the resistant lines CI.8873 and CI. 8969 and the susceptible variety Olli. CI.8873 and CI. 8969 were described by Cohen (12) as the entries most resistant of 6200 tested to isolate 514 of H. sativum, while Olli was one of the most susceptible. Isolate 514 was described by Cohen (12) as highly virulent and was used throughout the study.

The following populations were produced for carrying out the investigation:

F_3 seed of CI.8873 X Olli; Olli was used as the male parent.

F_3 seed of CI.8969 X Olli; " " " " " " "

F_3 seed of CI.8969 X CI.8873; CI.8873 was used as the male parent.

(All of the F_3 seed was produced by growing the F_2 plants in the field).

Backcrosses of the F_1 of CI. 8969 X Olli to each parent, F_1 was used as the female parent.

Backcrosses of the F_1 of CI. 8873 X Olli to each parent, F_1 was used as the female parent.

B.C.S-1 was the self-pollinated seed harvested from plants grown from backcrossed seed. Part of the B.C.S-1 seed was produced in the field, but some was produced in the greenhouse. Since resistance was not expressed to any degree in greenhouse grown seed (see results in section on condition of seed production), the B.C.S-1 seed produced in the greenhouse was sown in the field in plant-progeny rows, to obtain B.C.S-2 seed in a field environment. Five plants from each row were harvested

individually, at random, and the progeny tested for root-rot reaction to obtain the mean disease indices of each of the five entries from each row. The frequency distribution of the mean disease indices of the B.C.S-2 lines closely resembled that of B.C.S-1 lines, the seed of which was produced in the field. Therefore, the two results were combined to provide a larger population for the backcross analysis. The combined data were designated as B_1 in the case of the backcross to the resistant parent and B_2 in the case of the backcross to the susceptible parent.

Disease induction

Sand-cornmeal-nutrient salt inoculum

This inoculum was described by Ludwig *et al* (38) 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 white silica sand, and the mixture was dispensed into 500 ml. Erlenmeyer flasks at approximately 200 gms. per flask. This was moistened with a nutrient solution based on Czapek's medium* and sterilized by autoclaving for half an hour. Each flask was then inoculated with a heavy spore suspension of *H. sativum* prepared from 7-to 10-day old slant cultures of a 514 isolate, and was incubated for 7- to 8 days at room temperatures.

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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 "
Potassium chloride	Kcl	.50 "
Magnesium sulphate	$MgSo_4.7H_2O$	.50 "
Ferrous sulphate	$FeSo_4.6H_2O$	.01 "

The cornmeal inoculum was then passed through a 1/8-inch sieve to obtain a uniform particle size, and then mixed in a cement-mixer with white silica sand in a 1:14 ratio. This mixture formed the medium for performing the seedling plant tests. The planting medium was placed in plant bands, 3x3x3 inches, 25 bands to a galvanized metal flat. For the tests, 20 seeds of a line were seeded in each plant band. Based on exploratory tests, it was found that greater uniformity of environment was obtained by exposing the plant bands, on at least one side, to sand (Plate 1). The bands were placed on a metal screen, one inch above the bottom of the metal flat, which formed a false bottom. The metal screens were wrapped with plasticized burlap and a thin layer of fibre glass. The excess water leaked through the screen to the space beneath the screen, from which the water was siphoned out through a pipe located in one corner of the flat. The hollow space which contained the excess water, and the sand around the bands, were used to provide uniform moisture throughout the flat. The flats were kept in a growth-cabinet in which the temperature ranged from 58°-64°F., and the humidity was maintained at as high a level as was practically possible.

Disease evaluation in the growth-cabinet test

The plants were grown for a period of 21 days from seeding. The seedlings were removed from the planting medium and washed free of sand and inoculum. Each seedling was placed in one of five reaction classes (x_1 - x_5) according to the amount of stunting and blight of the seedling, and the amount of development and rotting of the root; the lowest class number designated the most resistant type (Plate 2).

Plate 1 - Arrangement of 25 bands in a galvanized metal flat for disease reaction in growth-cabinet test.

Plate 2 - Five reaction classes for disease index.

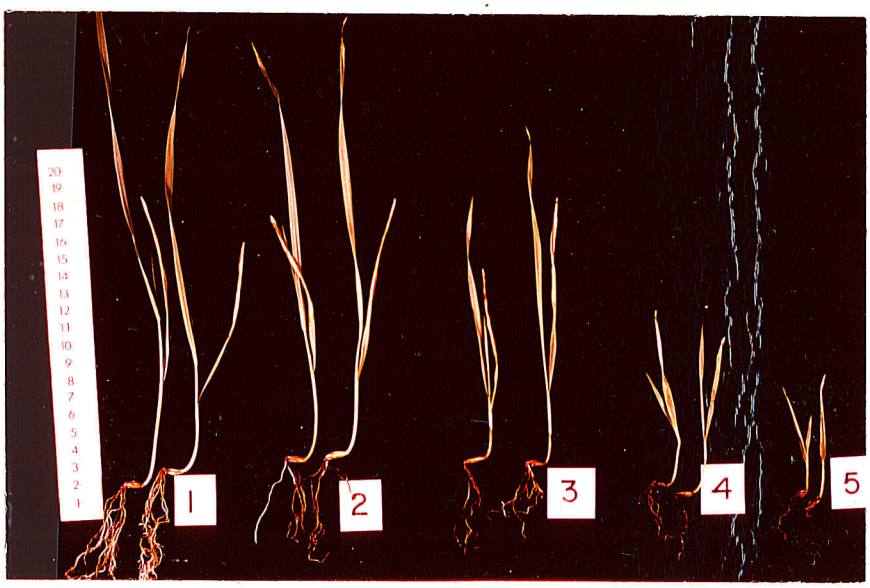
20
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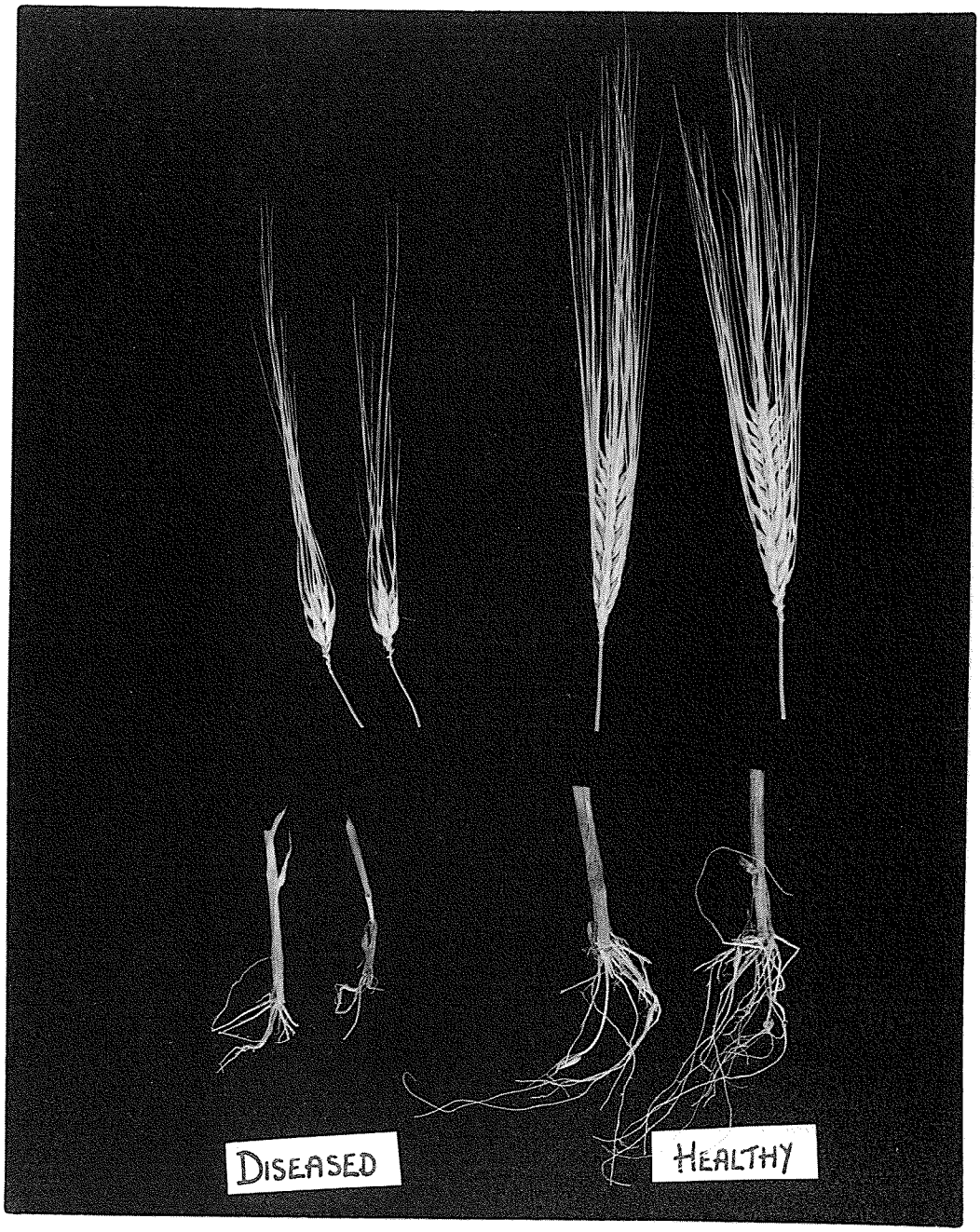
Plate 3 - Roots and heads of plants representing the most susceptible class (5) and the most resistant class (1) from the field test.


DISEASED
HEALTHY

A disease index was calculated from each F_3 family, backcross family or parental lot in percent, according to the following formula:

$\left(\sum_{i=1}^{5} fx / 5 \sum_{i=1}^{5} f\right) \times 100$; in which 'f' represents plant frequency in each class and 'x' the class reaction number.

Disease evaluation in the field

The infection in the field was developed from naturally occurring inoculum at the Canada Department of Agriculture Station at Morden. A hundred seeds of each F_3 line and each of the parents were sown in rows 16 feet long and one foot apart. The experiment extended on three long ranges; each range started and ended with three rows of rye to eliminate border effect. Plants were pulled from the soil in the dough stage. The assessment of reaction was based on the degree of sub-crown internode discoloration, and the amount of stunting, tillering and root development, but mainly on subcrown internode discoloration and lesioning. The plants were each placed in one of five classes as before, with the lowest class designating the resistant type (Plate 3), and the disease index was calculated for each F_3 family or parental lot in percent, by the formula previously described for the growth-cabinet test.

Statistical Analysis

The data on disease index of the crosses involved were subjected to two analytical procedures in an attempt to determine the gene action involved; first by the method proposed by Elston (16), and second, by the partitioning method of genetic analysis developed by Powers (49), and McNeal (43).

Elston's Procedure

A brief outline will be given of a test proposed by Elston to provide information on which to accept or reject the possibility of

one locus being involved in a specific cross between homozygous lines. It requires the determination of a response in at least four of the following six classes: P_1, P_2, F_1, F_2, $P_1 \times F_1$, $P_2 \times F_2$.

Let the two parental lines be P_1 and P_2, with corresponding genotype, if only one locus is involved, of AA and BB (alternative alleles). Let the probability that any given individual will be susceptible to the disease be α_0 for AA genotype, α_1 for AB genotype, and α_2 for BB genotype. Then the genotypic constitution and probability of response, P_i, for the six different classes are as given in Table 1. Suppose that in each class we examine n_i individuals, x_i of which showed susceptibility and $n_i - x_i$ of which did not. Thus, under the null hypothesis that only one locus is involved, the x_i are independent binomially distributed random variables with parameters p_i and n_i, where p_i is the appropriate function of α_0, α_1 and α_2. If we do not examine any individuals in a particular class, then $n_i = x_i = 0$ for that class.

To estimate α_0, α_1 and α_2, the matrices derived from the modified minimum X^2 are used, which are as follows:

$$(16m_0+4m_1+m_3)\alpha_0 + 2(2m_1+m_3)\alpha_1 + m_3\alpha_2 = 4(4z_0+2z_1+z_3)$$

$$2(2m_1+m_3)\alpha_0 + 4(m_1+4m_2+m_3+m_4)\alpha_1 + 2(m_3+2m_4)\alpha_2 = 8(z_1+2z_2+z_3+z_4)$$

$$m_3\alpha_0 + 2(m_3+2m_4)\alpha_1 + (m_3+4m_4+16m_5)\alpha_2 = 4(z_3+2z_4+4z_5)$$

where $z_i = n_i^2/(n_i - x_i)$ and $m_i = n_i z_i / x_i$.

Once we have obtained estimates of α_0, α_1 and α_2 we compare the observed numbers (x_i and $n_i - x_i$) with the expected numbers in each class to perform a X^2 test for goodness of fit; the number of degrees of freedom associated with the total X^2 statistic obtained is the number of classes examined less the number of parameters estimated.

Table 1 - Genotypic constitution and probability of response on a one locus hypothesis, and observed results.

Class	P_1	$P_1 \times F_1$	F_1	F_2	$P_2 \times F_1$	P_2
Genotypic constitution	AA	$\frac{1}{2}$AA+$\frac{1}{2}$AB	AB	$\frac{1}{4}$AA+$\frac{1}{2}$AB+$\frac{1}{4}$BB	$\frac{1}{2}$AB+$\frac{1}{2}$BB	BB
Probability of response, p_i	α_0	$\frac{1}{2}(\alpha_0+\alpha_1)$	α_1	$\frac{1}{4}(\alpha_0+2\alpha_1+\alpha_2)$	$\frac{1}{2}(\alpha_1+\alpha_2)$	α_2
Number examined, n_i	n_0	n_1	n_2	n_3	n_4	n_5
Number responding, x_i	x_0	x_1	x_2	x_3	x_4	x_5

Partitioning method

A part of the 'partitioning method' which concerns the present study will be outlined briefly here (32,49). The disease indices of the F_3 families, backcross families, and parental lots were summarized in the form of frequency distributions, and converted to a percent basis. The obtained frequency distributions of P_1 and P_2, with this theoretical percent (25 percent according to the null hypothesis of one gene difference) were used to estimate the theoretical frequency distributions of the aa and AA genotypes, and by subtracting these from the obtained frequency distribution data of the F_3 families, the frequency distribution of the Aa genotype was obtained. Then the theoretical mean of the Aa genotype was calculated from the frequency distribution of the Aa genotype as follows: Each frequency was multiplied by the class center. The sum was divided by the theoretical percent of the Aa genotype (50 percent according to the null hypothesis). The variance of the Aa genotype was estimated using the formula $y = mx + b$, the m and b were computed from mean and variance of P_1 and P_2. The theoretical values of the Aa genotype were calculated according to Leonard et al (32). The upper class limit was subtracted

from the theoretical mean of the Aa genotype; this value was divided by the theoretical standard error of the Aa genotype. This is the x-value in Pearson's Table II (Pearson, 1930). The probability associated with each x-value was calculated using Table A4 in Steel and Torrie (56). The above calculation gave the theoretical frequency distributions that included the three F_2 genotypes. The theoretical percentage of each genotype was multiplied by the values for that genotypic class. The computed values were accumulated to give the theoretical F_2 frequencies in percentage. A X^2 test for homogeniety was applied between the data of the original observed F_2 frequency distribution, and those of the theoretical F_2 frequency distribution, reconstituted from the three groups of genotypes. Similarly, the partitioning method was applied to the data from the B_1 (backcross of P_1) and the B_2, (backcrosses to P_2) to calculate the theoretical mean, standard error, and frequency (backcross to P_1) distribution of the Aa genotype from B_1, the Aa genotype from B_2, and the theoretical frequency distribution of B_1 and B_2, assuming that the frequency distribution of the P_1 affords the best estimate of a plant of the AA genotype, and the frequency distribution of the P_2 parent affords the best estimate of a plant of the aa genotype.

Frequency distribution of the disease indices

The data for the disease indices of each cross were summarized in frequency distributions using class intervals of five percent. The means, variances and standard errors of single determinations were computed by conventional methods using the class centers rather than the upper class limits. The actual frequency distributions, expressed in numbers, were converted to frequency distributions expressed in percent.

Both the numerical and percent distributions were used in the partitioning method as described by Powers (32,49). The susceptibility categories of the entries, for the purpose of Elston's procedure, were determined in each cross according to the frequency distribution expressed in percent of the susceptible parent Olli (see arrows in the Figures 1-4 for each cross). The arrows point to the mid-classes which obviously mark the reaction boundary of the susceptible parent. Entries to the left of the arrows were considered as resistant, and the entries to the right of the arrows were considered as susceptible. Since differences between resistant and susceptible plants were not clear-cut, as with some other diseases, the disease indices of the entries were based on the average reading of 20 seedlings in the growth-cabinet test, and 100 plants at the dough stage in the field test; thus, in both the "partitioning method" and in Elston's procedure, F_3 and B.C.S-1 families were used to represent the genotype of F_2 and backcrosses, respectively.

Field nursery

To establish a field nursery for studies on root-rot caused by H. sativum, dry spore inoculum of the 514 isolate of H. sativum was prepared. Approximately 110 gms. of barley grain were dispensed into 500 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 514 isolate of H. sativum from a slant culture and incubated for fourteen days at room temperature. 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. Seed of Olli was mixed with dry spores of the 514

isolate of H. sativum, and sown in the field at the University Farm. In the seedling stage, the nursery was roto-tilled, mixing the seedlings with the soil. The same procedure was repeated again.

Determination of kernel weight

Determination of the kernel weights of the parents was made by taking three samples of 1000 kernels each, which were counted by the seed counter and were weighed, and the mean of the three samples was taken as the kernel weight of the variety. For the segregating families, samples of 100 seeds were taken at random from each line for kernel weight determination. The mean for each group of lines was based on a random sample of lines from that group; the number in the sample was shown in tabulating the information. A similar procedure was followed to obtain comparative kernel weights of parents in the same experiment.

Procedure for surface-sterilization of seed

Micro-organisms other than H. sativum, carried on barley seed, may reduce seedling infection because of competition or being antibiotic to H. sativum. To test this, the seed surface was sterilized in a 2 percent solution of chlorine for six minutes and then allowed to dry before planting in cornmeal inoculum. The treated seeds were placed on a nutrient agar (Difco) and showed a negligible amount of contamination compared with the untreated seeds.

A floating method for spore-counting of H. sativum in the soil.

The floatation method, as described by Ledingham et al. (31), was followed in this study to estimate the number of spores in the soil from the nursery, from the University Farm, and from the Morden field in which tests were made. Each sample, amounting to about ten pounds,

was taken from the surface 2-3 in. of soil. Soil for examination was screened and a 20-gm. sample was weighed and mixed with 10 ml. of mineral oil in a watch glass. The mixture was next transferred to a test tube, 25 by 250 mm. size, and 50 ml. of tap water was added. The tube was agitated vigorously for four or five minutes, then placed in a vertical position. In about half an hour, most of the soil had settled and an emulsion had collected at the surface. Approximately twelve millilitres of emulsion were collected, and drops of 1/50 ml. volume were placed on a slide. The spores in ten drops were counted, and the approximate total number of spores in the emulsion, and hence spore numbers per gram of soil, were computed.

RESULTS

Results and discussion of the tests conducted in the growth cabinets.

Test for homogeneity

Obtained and theoretical frequency distributions for disease indices of the three parents used, with X^2 tests for normality, are given in Table 2. The non-segregating populations follow the normal probability integral. The results indicate that the scale on which the data were based appears to be appropriate for quantitative analysis, and that the partitioning method can be applied. However, the experiment was designed on the assumption that the inheritance of resistance to root-rot was qualitative; therefore, two methods were applied to analyse the data - one appropriate for qualitative data and the other for quantitative data. The qualitative method was Elston's procedure, which provided a statistical procedure to determine whether or not the observed differences in susceptibility in the various classes, P_1, B_1, B_2, F_2, P_2 could be accounted for by control at a single locus. Susceptibility and resistance were determined according to the curve of the susceptible parent, as was described previously. The quantitative method was the "partitioning method", in which the two parents, the resistant and the susceptible, were used to determine the genotypes of groups of individuals in the segregating populations.

Analysis of the crosses involving CI.8969 and Olli

Elston's procedure

Elston's procedure was applied to the five available classes, P_1 (CI.8969), B_1, F_2, B_2 P_2 (Olli). The susceptibility of the entries

was determined according to the frequency distribution of Olli, as was described previously in the section on Materials and Methods (see the arrows in Figs. 1-2). The values of "α" were estimated accordingly, and the observed numbers (x_i and n_i-x_i) with the expected numbers in each class, are presented in Tables 3-4. A X^2 test for goodness of fit, under the null hypothesis of a one-locus difference between CI.8969 and Olli, was conducted. The test rejected the null hypothesis (Tables 3-4) due to the large proportion of entries in the F_2 class, which showed susceptibility.

Table 2 - Obtained and theoretical frequency distributions of the parents for disease index, showing the number of entries per population and the goodness of fit test for normality.

Disease Index	CI.8873* O	CI.8873* C	CI.8969 O	CI.8969 C	011i O	011i C
27.5	-		-		-	-
32.5	-		3		-	-
37.5	-		2	11	-	-
42.5	1		2		-	-
47.5	4	6	11	9	-	-
52.5	11	9	13	9	-	-
57.5	8	9	6		-	-
62.5	4		2	12	1	
67.5	2	6	2		2	14
72.5					11	
77.5					14	12
82.5					5	8
87.5					4	4
92.5					1	
97.5						
N	30		41		38	
D.F.	1		1		1	
X^2	.764		2.020		.810	
P	.400-.350		.200-.150		.400-.350	

* Some adjacent classes are combined in order to provide at least 5 individuals in each of the classes.

Table 3 - Probability of response to root-rot, observed and expected, in each class on a one locus hypothesis in the crosses involving CI.8969 and O11i.

	CI.8969	B_1	F_1	F_2	B_2	O11i
Probability of response, P_i	.0301	.03131	.0326	.2620	.49265	.9527
Number examined, n_i	41	40	0	134	34	38
Number responding, x_i	2	.5	0	48	18	35
Number expected	1.2341	1.252	0	35.108	16.75	36.202

Table 4 - Obtained numbers (x_i and n_i-x_i) and expected numbers in each of the five classes, and goodness of fit test on a one-locus hypothesis in the crosses involving CI.8969 and O11i.

Classification	Obtained	Expected
x_i	2.0	1.0
	0.5	1.0
	48.0	35.0
	18.0	17.0
	35.0	36.0
(n_i-x_i)	39.0	40.0
	39.5	39.0
	86.0	99.0
	16.0	17.0
	3.0	2.0
Number	287	
D.F.	2	
X^2	8.463	
P	.025 - .010	

Fig. 1 - Frequency distribution of the disease indices in percent of the parental variety CI.8969 and Olli, and of the F_3 families in the growth cabinet test.

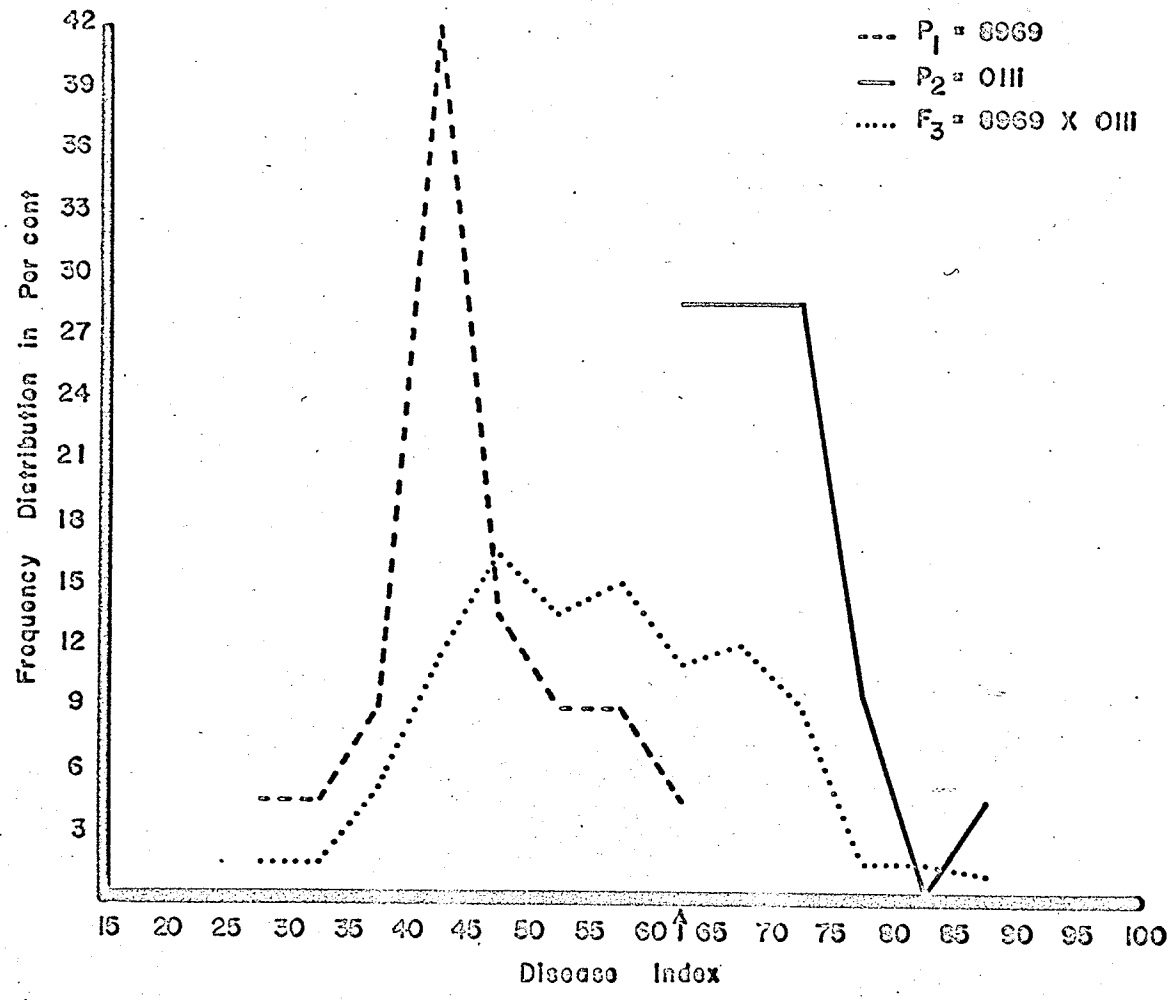
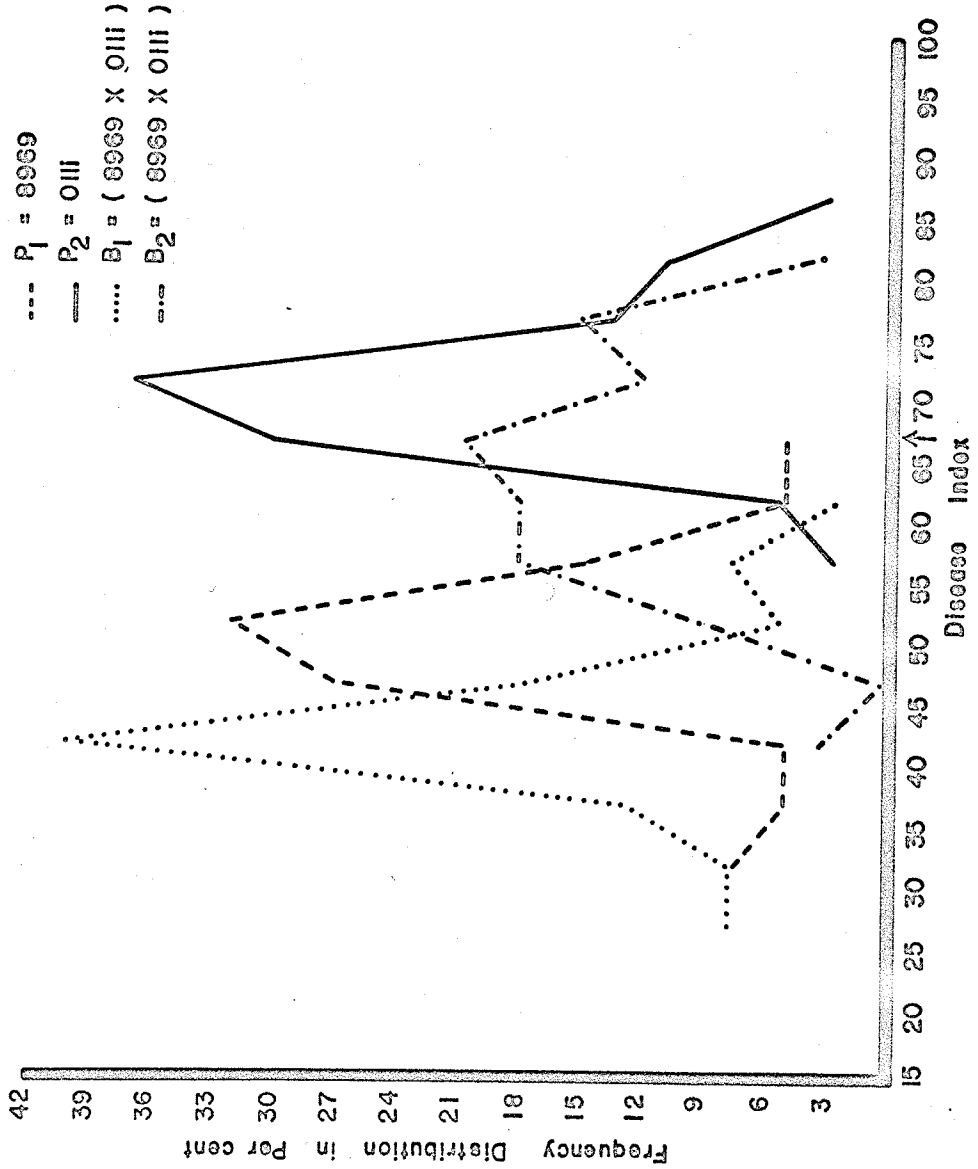

--- P1 = 8969
— P2 = 0111
..... F3 = 8969 X 0111
Frequency Distribution in Per cent
42
39
36
33
30
27
24
21
18
15
12
9
6
3
15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Disease Index

Fig. 2 - Frequency distribution of the disease indices in percent of the parental variety CI.8969 and Olli, and of the backcrosses to each parent in growth cabinet test.


--- P_1 = 8969
—— P_2 = OIII
···· B_1 = (8969 X OIII) X 8969
-·- B_2 = (8969 X OIII) X OIII
Frequency Distribution in Per cent
42
39
36
33
30
27
24
21
18
15
12
9
6
3
15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Disease Index

Partitioning method

The mean and standard error of the disease indices and kernel weights of P_1, F_2 and P_2, and the same for P_1, B_1, B_2 and P_2, are presented in Tables 5 and 6, respectively. It is apparent from the Tables and frequency distributions in Figs. 1-2 that the B_1 shows an indication of heterotic effect, whereas the B_2 and F_2 showed little or no indication of it. Heterotic effect could be due to: Inter-intragenic interaction. Intergenic interaction (Epistasis) is defined by Allard as "Interallelic interaction, whereby manifestation at any locus is affected by genetic phase at any or all loci". Intragenic interaction (over-dominance) is defined by Falconer as "the property shown by two alleles when the heterozygotes lie outside the range of the two homozygotes in genotype value with respect to the character under discussion". If heterotic effect for resistance were due to one major gene which showed intra-allelic interaction or two major genes which showed intra-allelic or interallelic interaction, or both, the following conditions would be expected.

(1) The mean disease index of B_1 would be smaller than that of P_1.

(2) The mean disease index of B_2 would be smaller than half the combined mean indices of both parents $(P_1 + P_2)/2$.

(3) A portion of B_2 would be within the range of B_1 outside the curve of P_1.

The hypothesis of one or two genes was rejected because conditions two and three were not met as far as the B_2 was concerned. The mean disease index of B_2 (65.880) was greater than $(P_1 + P_2)/2$ (61.330), and there was not a single entry of B_2 within the portion of B_1 outside the curve of P_1. The third condition could be used also to reject a

three-gene hypothesis. Without taking the B_1 into consideration, it was more likely that the B_2 and F_2 distributions would lead to acceptance of the null hypothesis of a one-gene difference between CI.8969 and Olli. To investigate this further, the partitioning method was applied to all the segregating populations, B_1, B_2 and F_2, under the null hypothesis of a one-gene difference. The results (Table 7) show that the null hypothesis was accepted by the B_2 and F_2 but rejected by the B_1. From these results it can be said that if inter-intragenic interaction for resistance to H. sativum were taking place in this study, a polygenic situation must have been involved. Since Olli is considered to be universally susceptible to root-rot, it could be assumed that the polygenes for resistance to H. sativum show an over-dominance rather than epistasis. The over-dominance in B_1 brought about the heterotic effect, whereas in B_2 and F_2 the heterotic effect was neutralized by increasing the proportion of the susceptible parent. The fit to the null hypothesis shown by the B_2 and F_2 could be the result of an overall effect of segregation of polygenes, for resistance to isolate 514 of H. sativum.

Table 5 - Disease index and kernel weight of the parents and F_3 families in the cross CI.8969 x Olli.

Population	Disease Index: Number of Plants	Disease Index: Means and S.E.	Disease Index: Range	Kernel Weight: No. of Plants	Kernel Weight: Means and S.E.
CI.8969	22	44.775 ± 1.838	27.5-62.5	25	4.442 ± .0386
Olli	21	69.405 ± 1.400	62.5-87.5	25	3.158 ± .0323
F_3	134	55.635 ± 1.033	27.5-87.5	25	4.029 ± .1450

Table 6 - Disease index and kernel weight of the parents, B_1 and B_2 families in the crosses of CI.8969 and of Olli.

Population	Disease Index: Number of Plants	Disease Index: Means and S.E.	Disease Index: Range	Kernel Weight: No. of Plants	Kernel Weight: Means and S.E.
CI.8969	41	50.425 ± 1.295	32.5-67.5	25	4.442 ± .0386
Olli	38	72.235 ± 1.012	57.5-87.5	25	3.158 ± .0323
B_1	40	43.000 ± 1.276	27.5-62.5	12	5.051 ± .0728
B_2	34	65.880 ± 1.685	42.5-82.5	12	3.491 ± .1420

Table 7 - Obtained and theoretical frequency distributions for disease index and goodness of fit test on a one-locus hypothesis, in the crosses of CI.8969 and Olli.

Disease Index	Frequency Distribution					
	B_1		F_2		B_2	
	O	C	O	C	O	C
27.5	3		2		-	-
32.5	3	16	2	5	-	-
37.5	5		7		-	-
42.5	16	4	15	21	1	
47.5	7		22	16	0	
52.5	2	20	18	19	3	9
57.5	3		20	19	6	
62.5	1		15	21	6	
67.5			16	14	7	13
72.5			12	11	4	
77.5			2		5	
82.5			2	8	1	12
87.5			1		1	
92.5					-	
97.5					-	
D.F.	1		7		1	
X^2	5.634		5.015		.134	
P	.025 - .010		.700 - .600		.750 - .650	

Seed weight effect

The polygenic hypothesis could explain the heterotic effect for resistance in B_1 and the lack of such a response in B_2. However, other hypotheses were also considered. Kaufman et al (1967) showed that seed size in barley affects the seedlings' vigor. Thus, an entry with higher seed weight would be expected to produce more vigorous seedlings. If more vigorous seedlings were to overcome the effect of disease, it would be expected that the seed weight of the segregating material would affect the disease index. Such an hypothesis as seed weight effect could be justified if it would explain the striking differences in the behaviour of B_1 and B_2. Without assuming polygenic interaction for disease reaction, the following would be expected:

(1) The mean seed weight of B_1, exceeds that of P_1.

(2) The mean seed weight of B_2 is less than the mean seed weight of $(P_1 + P_2)/2$.

The mean seed weights (Table 6) show agreement with the above assumptions. The mean seed weight of B_1 is greater by 14.7% than the mean seed weight of CI.8969, whereas the mean seed weight of B_2 (3.491) is less than that of CI.8969 and also less than the mean seed weight of $(P_1 + P_2)/2$ (3.800).

Analysis of the crosses involving CI.8873 and Olli

Elston's procedure

Elston's procedure was applied to the five classes, P_1, B_1, B_2, F_2 and P_2, as previously described. The susceptibility of the entries was determined according to the frequency distribution in percent of Olli, as was described in the Materials and Methods section (see arrows in Figs. 3-4). The α determinations were made accordingly, and the observed

numbers, (x_i and n_i-x_i), with the expected numbers in each class, are presented in Table 8. A X^2 test for goodness of fit for a one-locus difference between CI.8873 and Olli was calculated. The test agreed with the null hypothesis (Table 9). These results are in contrast with those obtained with the crosses involving CI.8969 and Olli, in which there was an excess of susceptibles in the F_2 class. In the F_2 of CI.8873 and Olli, there was close agreement with expectation. The X^2 test was based only on four classes instead of five, the P_1 class being omitted because the expected number was equal to zero. Thus, the X^2 lost one degree of freedom.

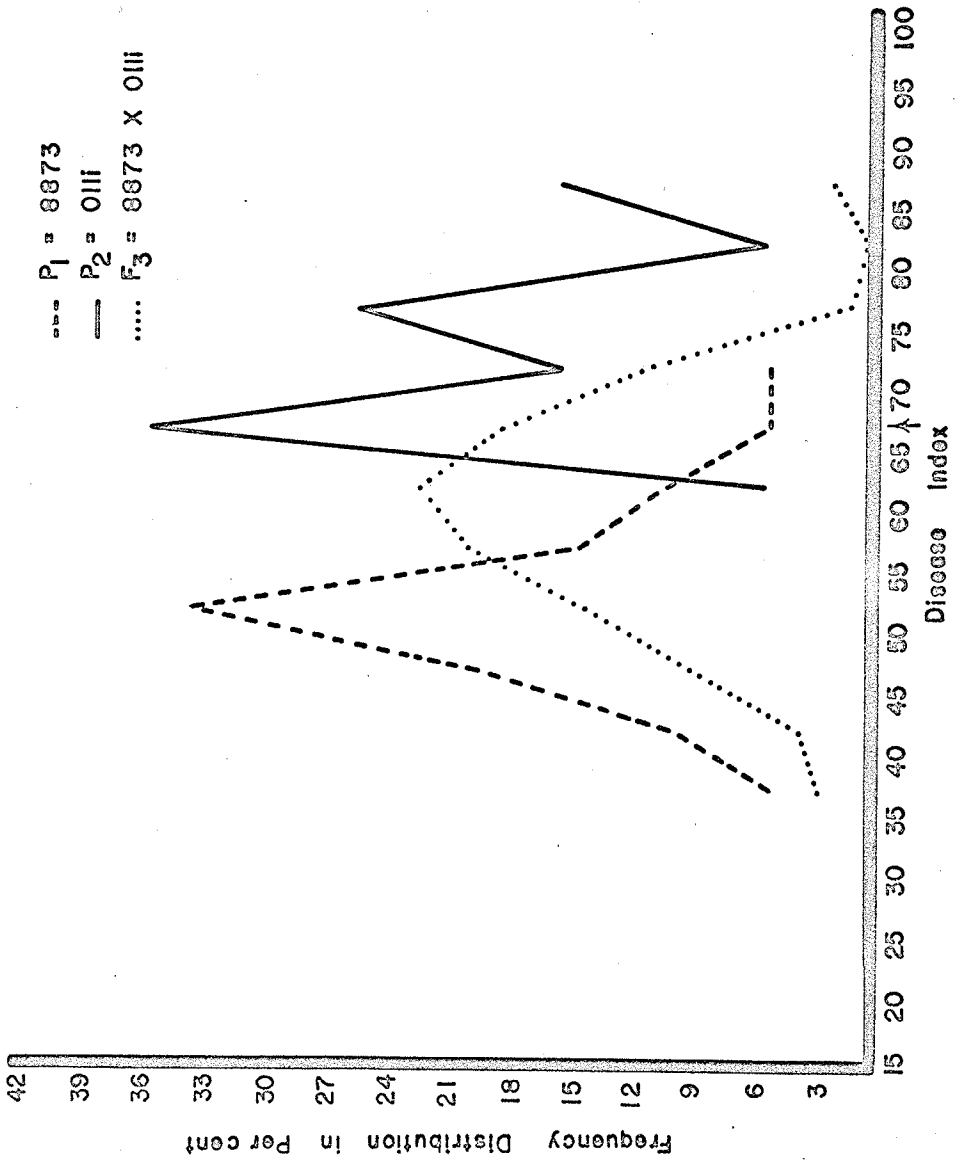
Table i - Probability of response to root-rot, observed and expected, in each class on a one-locus hypothesis in the crosses involving CI.8873 and 011i.

	CI.8873	B_1	F_1	F_2	B_2	011i
Probability of response, P_i	.004	.062	.119	.285	.503	.896
Number examined, n_i	30	36	0	124	36	30
Number responding, x_i	2	2	0	38	16	27
Number expected	0	2	0	35	18	27

Table 9 - Obtained numbers (x_i and n_i-x_i), expected number in each of the four classes, and goodness of fit test on a one-locus hypothesis in the crosses involving CI.8873 and 011i.

Classification	Expected	Obtained
x_i	2	2
	35	38
	18	16
	27	27
(n_i-x_i)	34	34
	89	86
	18	20
	3	3
Number	226	
D.F.	1	
X^2	1.074	
P.	.350-300	

Fig. 3 - Frequency distribution of the disease indices in percent of the parental variety CI.8873 and Olli, and of the F_3 families in the growth cabinet test.


--- P1 = 8873
—— P2 = Olli
..... F3 = 8873 X Olli
Frequency Distribution in Per cent
42
39
36
33
30
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24
21
18
15
12
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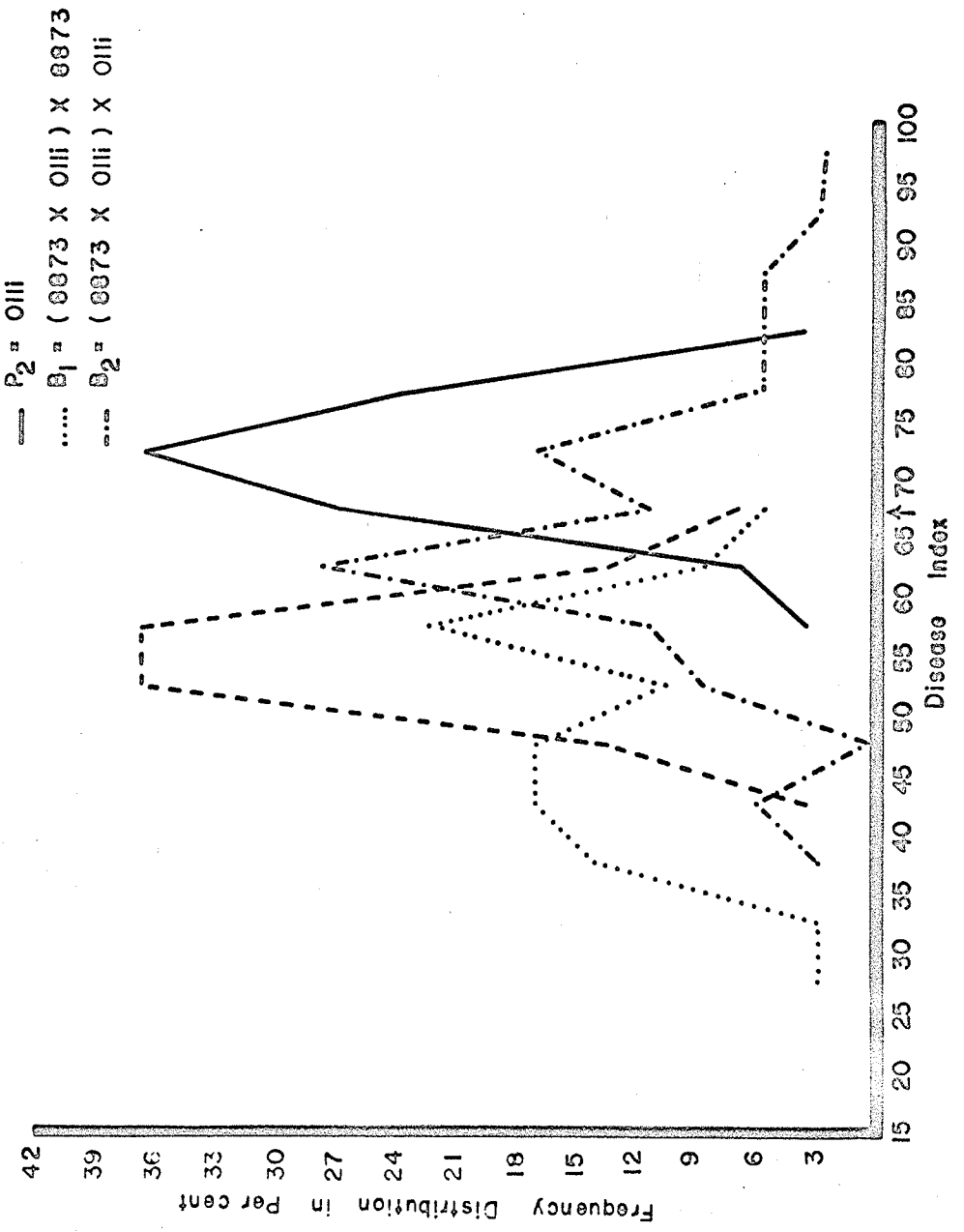
6
3
15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Disease Index

Fig. 4 - Frequency distribution of the disease indices in percent of the parental CI.8873 and Olli, and of the backcrosses to each parent in the growth cabinet test.


--- P_1 = 8873
— P_2 = Olli
.... B_1 = (8873 X Olli) X 8873
-.- B_2 = (8873 X Olli) X Olli
Frequency Distribution in Per cent
42
39
36
33
30
27
24
21
18
15
12
9
6
3
15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Disease Index

Partitioning method

The means and standard errors of the disease indices and the kernel weights of P_1, F_2 and P_2, and the same for P_1, B_2 and P_2, involving CI.8873 and Olli, are presented in Tables 10 and 11. It is apparent, both from the Tables and Figs. 3-4 that the inheritance pattern for disease resistance was similar to that obtained using CI.8969 and Olli as parents. The B_1 showed heterotic effect, but the B_2 and F_2 did not (see Plates 4-5). The mean disease index of the B_1 was less than that of the P_1, whereas the mean disease index of the B_2(65.555) was greater than, or equal to, $P_1 + P_2/2$(63.331). There was no entry of the B_2 within the range of B_1, outside the curve of P_1. Again, the hypothesis of one, two or three genes was rejected, although Elston's procedure had not rejected the one-locus hypothesis. To differentiate between the two results, the partitioning method was applied to the B_1, F_2 and B_2 of each population. The partitioning analysis, Table 12, shows agreement with the null hypothesis for a one-locus difference, although the data for B_1 showed a substantial deviation, as indicated by the small value of P = .150 - .100. The entries of B_1 and B_2 were grown in the same test. If the one-locus hypothesis were valid, it would be expected that the balanced genotype estimated for B_1, using P_1 as the AA genotype, would be the same as the balanced genotype estimated for B_2, using P_2 as the aa genotype. Both estimates would represent the Aa genotype. The theoretical means of the Aa genotype of both estimates are presented in Table 13. A "t" test comparison of the two estimated means showed that they were significantly different at the 1% level. Therefore, the balanced genotypes do not represent the same genotype, as was expected under the null hypothesis that there was a one-locus difference. The above results can be summarized as follows:

(1) The combined data of B_1, F_2 and B_2 accept the null hypothesis of a one-locus difference (Elston's procedure).

(2) The analysis of each population separately accepts the null hypothesis of a one-locus difference (partitioning method).

(3) The means and the frequency distributions of B_1 and B_2 reject the null hypothesis of a one-locus difference.

(4) The balanced genotypes derived from B_1 and B_2 reject the null hypothesis of a one-locus difference.

The above results can be explained under the polygenic hypothesis for resistance to H. sativum. The heterotic effect detected in the B_1 population could occur as the result of polygenic interaction, whereas in B_2 and F_2 the heterotic effect of genes for resistance to H. sativum was neutralized by increasing the proportion of the susceptible parent. The fit to the null hypothesis shown by the B_2 and F_2 could be the result of an overall effect of segregation of polygenes which controlled the resistance to H. sativum.

Plate 4 - Reaction of representative entries in the B_1 population and parental varieties, CI.8873 (2440) and Olli.

Plate 5 - Reaction of representative entries in the B_2 population and parental varieties, CI.8873 (2440) and Olli.


Olli
[2440xOlli]x2440
2440


Olli
[2440xOlli]xOli
2440

Table 10 - Disease index and kernel weight of the parents and F_3 families in the cross CI.8873 and 011i.

Population	Disease Index: Number of Plants	Means and S.E.	Range	Kernel Weight: No. of Plants	Means and S.E.
CI.8873	21	53.215 ± 1.629	37.5-72.5	25	4.322 ± .0364
011i	20	74.250 ± 1.712	62.5-87.5	25	3.158 ± .0323
F_3	124	60.040 ± .939	37.5-87.5	25	4.451 ± .1701

Table 11 - Disease index and kernel weight of the parents, B_1 and B_2 families in the crosses of CI.8873 and 011i.

Population	Disease Index: Number of Plants	Means and S.E.	Range	Kernel Weight: No. of Plants	Means and S.E.
CI.8873	30	55.165 ± 1.100	42.5-67.5	25	4.322 ± .0364
011i	30	71.500 ± 1.000	57.5-82.5	25	3.158 ± .0323
B_1	36	49.445 ± 1.670	27.5-67.5	11	5.072 ± .198
B_2	36	65.555 ± 2.154	37.5-97.5	13	3.661 ± .166

Table 12 - Obtained and theoretical frequency distributions for disease index and goodness of fit test on a one-locus hypothesis, in the crosses of CI.8873 and 011i.

Disease Index	Frequency distribution					
	B_1		F_2		B_2	
	O	C	O	C	O	C
27.5	1		-		-	
32.5	1	12	0		-	
37.5	5		3	8	1	
42.5	6		4		2	
47.5	6	16	11	14	0	10
52.5	4		17	25	3	
57.5	8	22	24	21	4	
62.5	3		27	16	10	6
67.5	2		22	18	4	
72.5			13		6	15
77.5			1	22	2	
82.5			0		0	5
87.5			2		2	
92.5					1	
97.5					1	
D.F.	1		5		2	
X^2	2.278		6.312		2.140	
	.150-.100		.300-.250		.350-.300	

Table 13 - Theoretical means of disease index of the Aa genotype estimated from B_1 and B_2 on a one-locus hypothesis in the crosses involving CI.8873 and Olli.

Genotype	Mean	S^2	S.E.
AA (P_1)	55.165	36.319	5.980
Aa (B_1)	43.165	40.771	6.385
Aa (B_2)	59.525	34.633	5.885
aa (P_1)	71.500	30.000	5.475

Seed weight effect

In addition to the polygenic hypothesis, the seed weight effect hypothesis was again examined to explain the results obtained in the CI.8873 x Olli crosses. The kernel weights of B_1 and B_2 are presented in Table 11. The mean seed weight of B_1 exceeded the mean seed weight of P_1 by 10.4%, which could explain the heterotic effect for disease reaction in B_1. The mean seed weight of B_2(3.661) was less than, or equal to, the mean seed weight of $P_1 + P_2/2$(3.740), and could account for the lack of the heterotic effect for disease reaction in B_2, and explain the discrepancy between B_1 and B_2 as far as a one-locus hypothesis was concerned. To estimate the correlation between seed weight and disease index, the F_3 seed of each entry in the F_2 class was weighed before it was tested for disease reaction. After the disease indices of the entries were obtained, a multiple and partial correlation test was conducted, using three variables, as follows:

(1) Disease indices of the entries (124 in number).

(2) Seed weights of the same entries.

(3) Classification into two-row vs. six-row heads.

The correlation coefficients are presented in Table 14, and the partial correlations in Table 15.

Table 14 - Correlation coefficients between combinations of three variables in the CI.8873 x 011i cross.

Variables*	1	2	3
1	1.0000	-.4966	.3817
2		1.0000	-.6234
3			1.0000

* 1 - Disease index

2 - Seed weight

3 - Head row-number

Table 15 - Partial correlation of the three variables in the CI.8873 x 011i cross.

Variables	Partial Correlation	t value	Significant
12.3	-.3578	4.215	H.S.*
13.2	.1062	1.175	N.S.
23.1	-.5407	7.070	H.S.

* H.S. - highly significant at 1% level.

It is apparent from these values that there is a negative correlation between disease index and seed weight; a negative correlation between seed weight and two- or six-row head type; and no correlation between disease index and two- or six-row head type. Apparently two-row heads have a higher weight than six-row types, and thus have smaller disease

indices, or show better resistance. These results showed also the reason for the high mean seed weight of B_1 in both crosses, CI.8969 and CI.8873 (both two-row types) which, under the seed weight effect hypothesis, brought about the heterotic effect of B_1 for disease reaction in both crosses. On the other hand, B_2 had a lower mean seed weight (Olli is a six-row type), and thus heterotic effect for disease reaction was lacking.

The seed weight effect hypothesis explains the discrepancy between B_1 and B_2 in both sets of crosses as far as a one-gene hypothesis is concerned. It also explains the complicating results obtained when these crosses were analysed by Elston's procedure, where the one-locus hypothesis was not rejected in the CI.8873 x Olli cross, but was rejected in the CI.8969 x Olli cross. The difference in result was due to a large proportion of F_3 entries in the CI.8969 x Olli cross which showed susceptibility (Tables 3-4 and Tables 8-9). Since CI.8969 showed higher resistance than CI.8873 in all tests (in the overall tests the disease index of CI.8873 exceeded the disease index of CI.8969 by 14.0%), the above results were contrary to expectation, since it was expected that the CI.8969 x Olli cross would yield more resistant entries than the CI.8873 x Olli cross. It was found (Tables 5 and 10) that the mean seed weight of the F_3 entries from CI.8969 x Olli was lower, by 9.5%, than the mean seed weight of the F_3 entries from CI.8873 x Olli, which could explain this discrepancy.

Analysis of CI.8969 x CI.8873 cross

The cross between the two resistant parents, CI.8873 and CI.8969, is similar to some extent to the backcrosses of the F_1 to the resistant parents. It was expected, according to the polygenic hypothesis for disease reaction, that the CI.8969 x CI.8873 cross would show heterotic

effect similar to the B_1. The heterotic effect in CI.8969 x CI.8873 is apparent from Fig. 5 and from the ranges and the means of the disease indices (Table 16), which confirmed again the above hypothesis. On the other hand, the seed weight of the F_3 seed in the CI.8969 x CI.8873 cross exceeded the mean seed weight of CI.8969 by 11.8%, and that of CI.8873 by 14.9% (Table 16), and this could be mainly responsible for the heterotic effect for disease reaction in the CI.8969 x CI.8873 cross. It is apparent then, that the CI.8969 x CI.8873 cross behaved the same as B_1 and confirmed that each of the two hypotheses, the polygenic hypothesis for disease reaction, and the seed weight effect hypothesis, can be applied.

Table 17 summarizes the disease index values of the segregating material in terms of percent of the parents, for all the crosses tested in the growth-cabinet, in the seedling stage of barley. The negative correlation between the disease indices and seed weight is apparent; the higher the disease index the smaller the seed weight in percent of the parents.

Table 16 - Disease index and kernel weight of the parents and F_3 families in the cross CI.8873 x CI.8969.

	Disease Index			Kernel Weight	
Population	Number of Plants	Means and S.E.	Range	No. of Plants	Means and S.E.
CI.8873	23	34.675 ± 1.801	22.5-57.5	25	4.322 ± .0364
CI.8969	22	30.230 ± 1.102	22.5-42.5	25	4.442 ± .0386
F_3	186	26.640 ± .508	17.5-57.5	25	4.968 ± .0853

Fig. 5 - Frequency distribution of the disease indices in percent of the parental variety CI.8969 and CI.8873, and of the F_3 families in the growth-cabinet test.

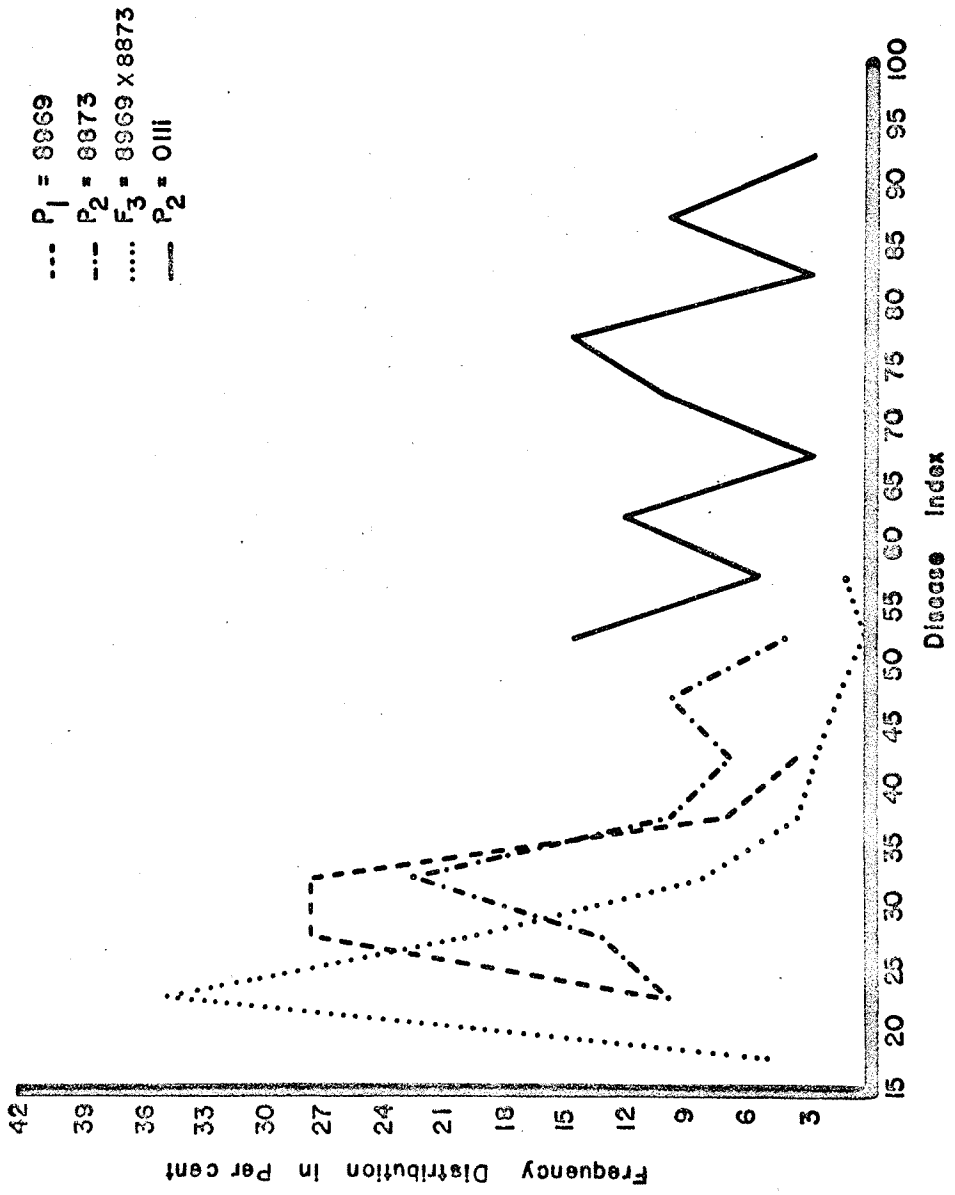

--- P1 = 8869
--- P2 = 8873
..... F3 = 8869 X 8873
—— P2 = OIII
Frequency Distribution in Per cent
42
39
36
33
30
27
24
21
18
15
12
9
6
3
15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Disease Index

Table 17 - Disease indices and the means of kernel weight of the segregants in percent of the parents.

Population	Disease Index		Kernel Weight	
	P_1*	P_2*	P_1*	P_2*
CI.8969 x 011i	124.3	80.2	90.7	127.6
CI.8873 x 011i	112.9	80.9	103.0	140.9
(CI.8969 x 011i) x CI.8969	85.3	59.5	113.7	159.9
(CI.8969 x 011i) x 011i	130.7	91.2	78.6	110.5
(CI.8873 x 011i) x CI.8873	89.6	69.2	117.4	160.6
(CI.8873 x 011i) x 011i	118.8	91.7	84.7	115.9
CI.8873 x CI.8969	88.1	76.8	111.8	114.9
011i**	170.1	149.2	71.1	73.1
CI.8873**	114.1	-	93.3	-

* P_1 - the resistant parent, P_2 the susceptible.

**P_1 - CI.8969; P_2 - CI.8873

The influence of seed weight on disease reaction

Two hypotheses, polygenic control of disease resistance and seed weight effect, had been considered in interpreting the results obtained from the analysis of the segregating populations in the crosses involving CI.8969, CI.8873 and 011i, based on rejection of the null hypothesis of a one-gene difference. Evidence of an inter-relationship between the two factors was considered. If large seeds of the susceptible parent produce seedlings with greater resistance than do small seeds, and equal in resistance to the resistant entries, CI.8873 and CI.8969, this would provide evidence to support the assumption that seed weight was the principal factor responsible for resistance. To test this assumption, seeds of the susceptible parent, 011i, were separated into

large and small sizes. The 1000-kernel weights of CI.8969, CI.8873 and the two samples of Olli are shown in Table 18. It is apparent that the sample of Olli was composed mainly of small-sized kernels, and the seed weight of the large kernels separated out of the Olli sample was still less than the seed weights of CI.8969 and CI.8873, which were both relatively uniform in size. A test was conducted in four replicates in pots, using CI.8873 as a check to measure the disease index of seedlings from small and large seeds of Olli. It is apparent from the results (Table 19 and Plates 6-9) that seedlings from the large seeds of Olli were less variable in response and showed greater resistance than those from the small seeds (at P=.01), or those from an unsized seed, (although statistically non-significant, see Plates 6-9). They are, however, more susceptible than those of the resistant parent,CI.8873. A second test, using CI.8969 as a check was similarly conducted. The results indicated that seedlings from large seeds of Olli approached the resistance of CI.8969 (Table 20 and Plates 10-13). The evidence that the seed size was the only factor determining disease reaction is not conclusive, but it was obviously a major factor, which justified the assumption that the so-called polygenic hypothesis for disease reaction and seed weight effect are inter-related. The seed weights are controlled by polygenes (46) which in turn affect the disease index.

Table 18 - Weights per 1000 kernels of the field-produced seeds of the resistant parents and of the susceptible parent, both sized and unsized.

Entry	Weight per 1000 kernels (gm.)
CI.8969	43.573
CI.8873	41.050
Olli - unsized	31.763
Olli - small seed	26.970
Olli - large seed	39.700

Table 19 - Disease indices of CI.8873 and small, large, and unsized field-produced seeds of the susceptible parent, Olli.

	Disease Index			
Replicates	CI.8873	Olli	Olli L*	Olli S*
1	29.5	73.7	57.0	81.3
2	44.0	56.2	60.0	74.7
3	35.0	58.8	60.0	70.0
4	37.5	70.0	62.0	75.6
$\bar{x}$	36.500^{**}_{a}	64.675_{b}	59.750_{b}	75.400_{c}

* Olli L - Olli, large seed.

* Olli S - Olli, small seed.

** Means designated by different letters are significantly different at P - .05, by Duncan's multiple range test.

Table 20 - Disease indices of CI.8969 and small, large and unsized field-produced seeds of the susceptible parent, O11i.

Replicates	Disease Index			
	CI.8969	O11i	O11i L.	O11i S
1	32.0	82.0	40.0	90.0
2	30.0	84.0	42.0	82.0
3	35.0	75.5	44.0	85.0
4	45.0	70.0	50.0	87.5
$\bar{x}$	35.000^{*}_{a}	77.875_{b}	44.000_{c}	86.125_{d}

* Means designated by different letters are significantly different at P = .05, by Duncan's multiple range.

Table 21 - Disease index of the parents and F_3 families in the cross, CI.8969 x O11i at Morden.

Population	Number of Rows	Mean and S.E.	Ranges
CI.8969	8	64.375 ± 2.099	52.5-72.5
O11i	15	67.165 ± 1.723	52.5-77.5
F_3	125	65.860 ± 1.139	37.5-92.5

Table 22 - Disease index of the parents and F_3 families in the cross, CI.8873 x O11i at Morden.

Population	Number of Rows	Mean and S.E.	Ranges
CI.8873	8	45.000 ± 2.834	32.5-57.5
O11i	15	67.165 ± 1.723	57.5-77.5
F_3	162	56.515 ± 1.093	27.5-87.5

Plate 6 - Reaction of seedlings from field-produced seed of CI.8873 (2440).

Plate 7 - Reaction of seedlings from field-produced seed of Olli unselected for size.


2440. F.

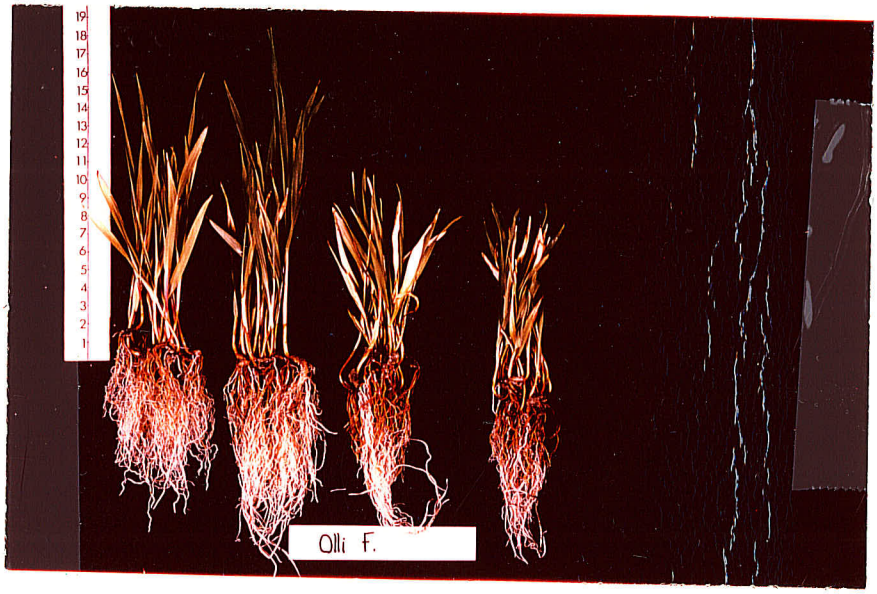

Olli F.

Plate 8 - Reaction of seedlings from field-produced seeds of Olli, selected for small size.

Plate 9 - Reaction of seedlings from field-produced seeds of Olli, selected for large size.


Olli F. S


Olli F. L

Plate 10 - Reaction of seedlings from field-produced seeds of CI.8969 (4958).

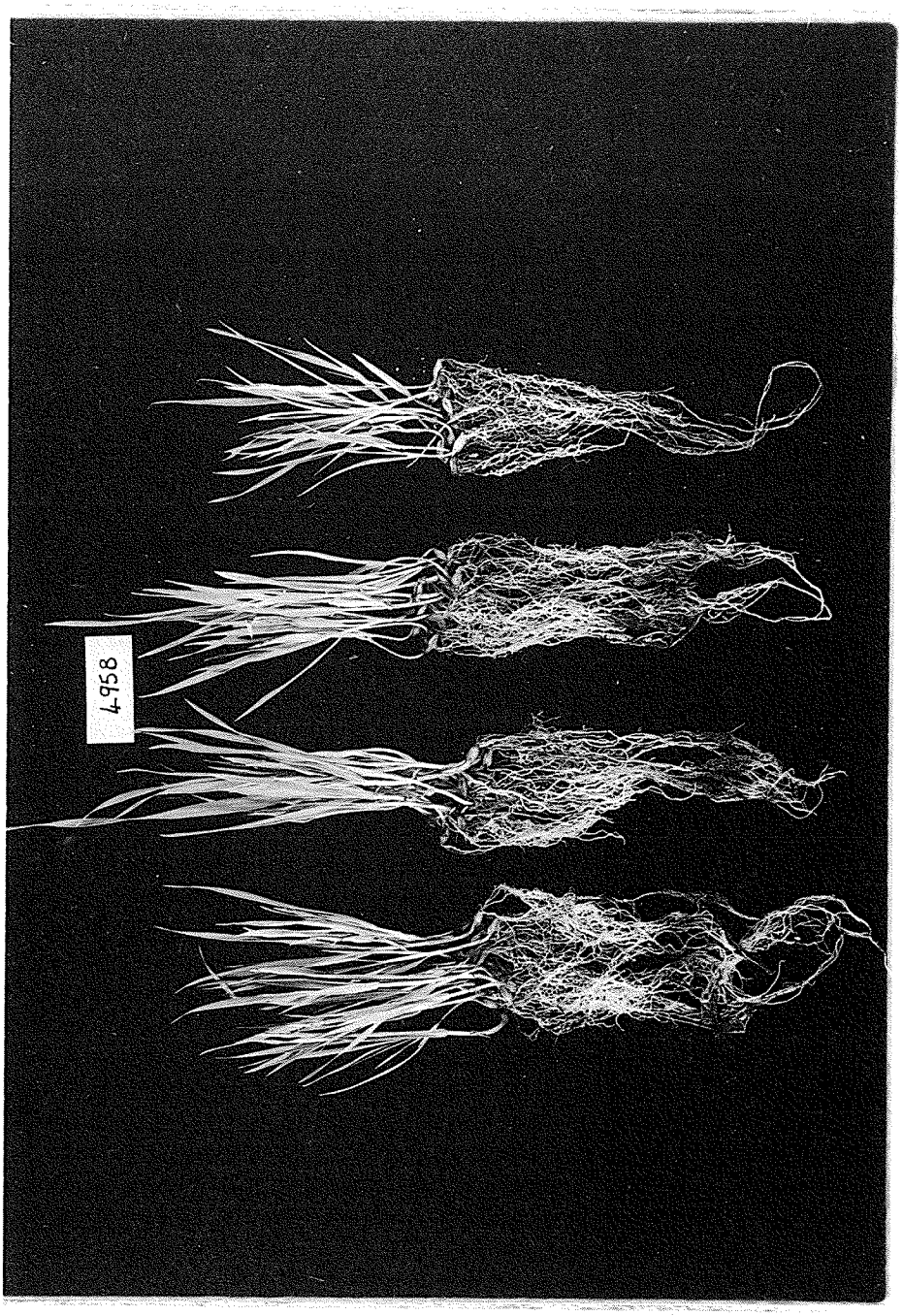

4958

Plate 11 - Reaction of seedlings from field-produced seeds of Olli, selected for large size.

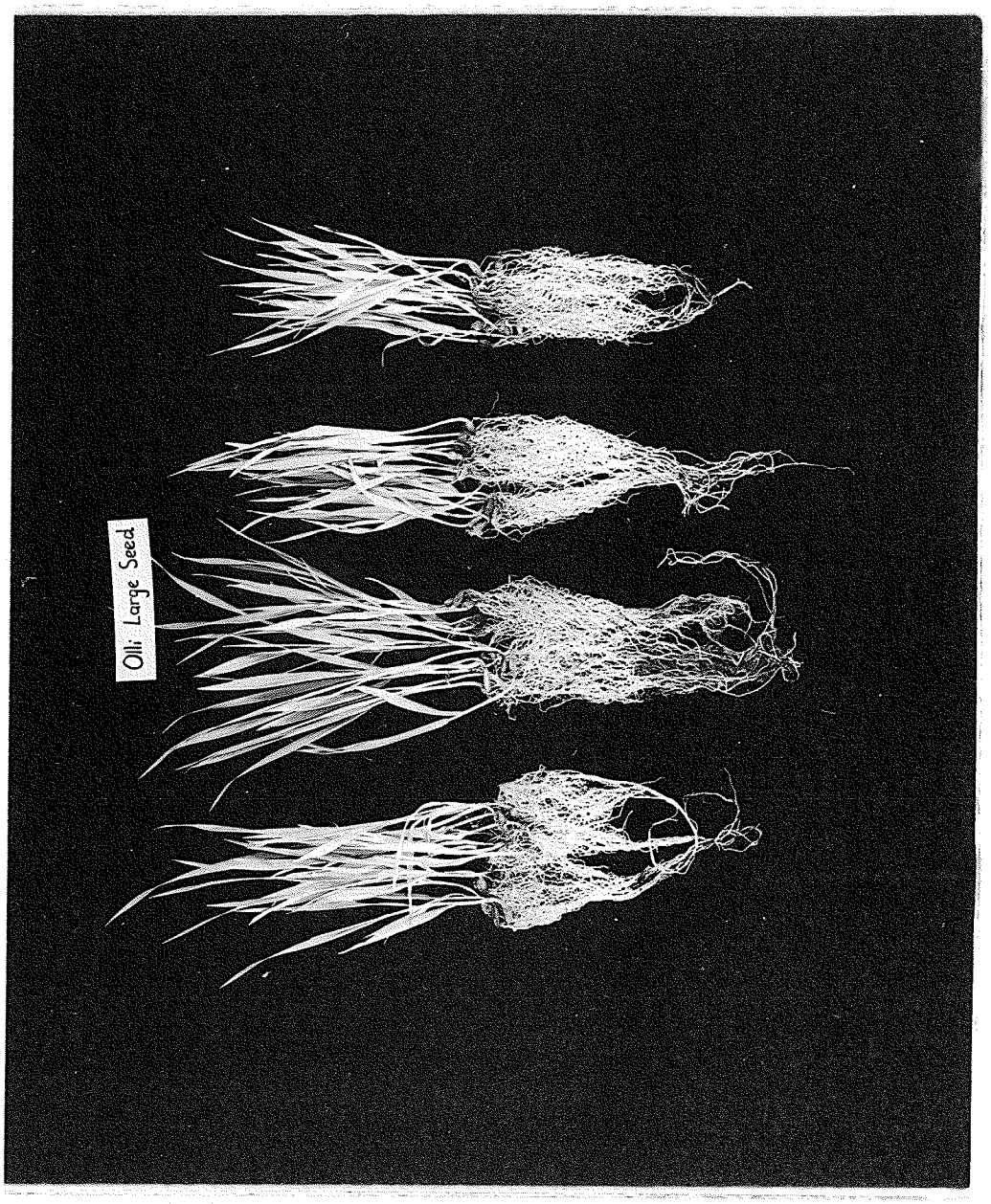

Oli Large Seed

Plate 12 - Reaction of seedlings from field-produced seeds of Olli, unselected for size.

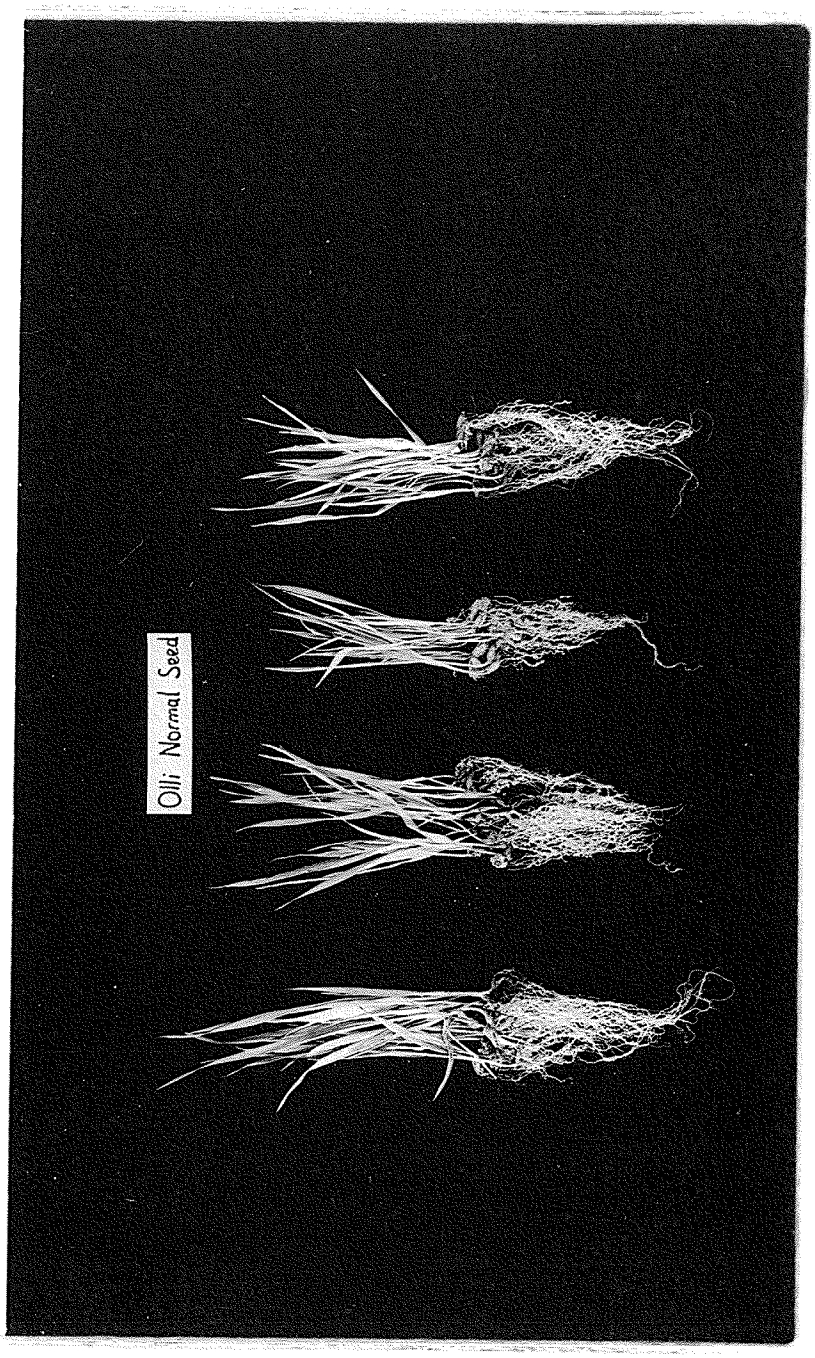
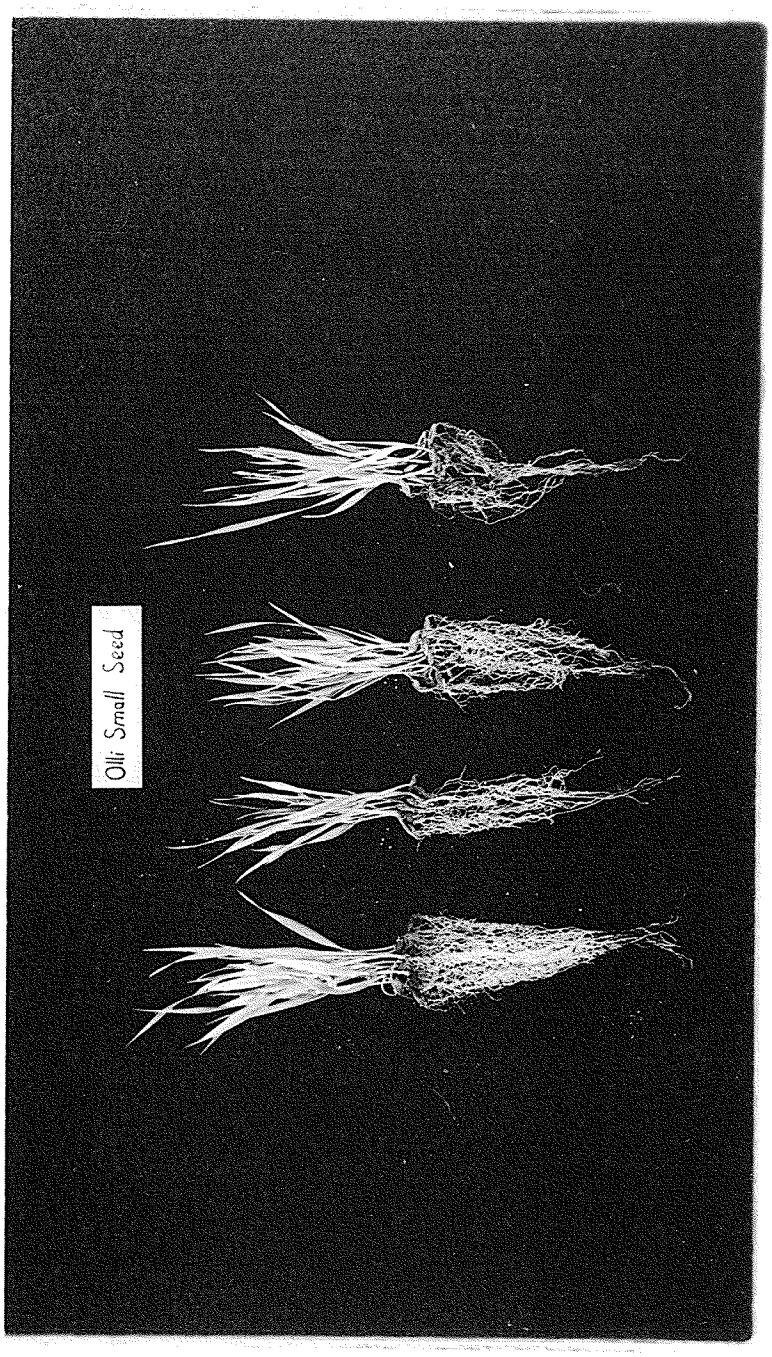

Olli Normal Seed

Plate 13 - Reaction of seedlings from field-produced seeds of Olli, selected for small size.


Olli Small Seed

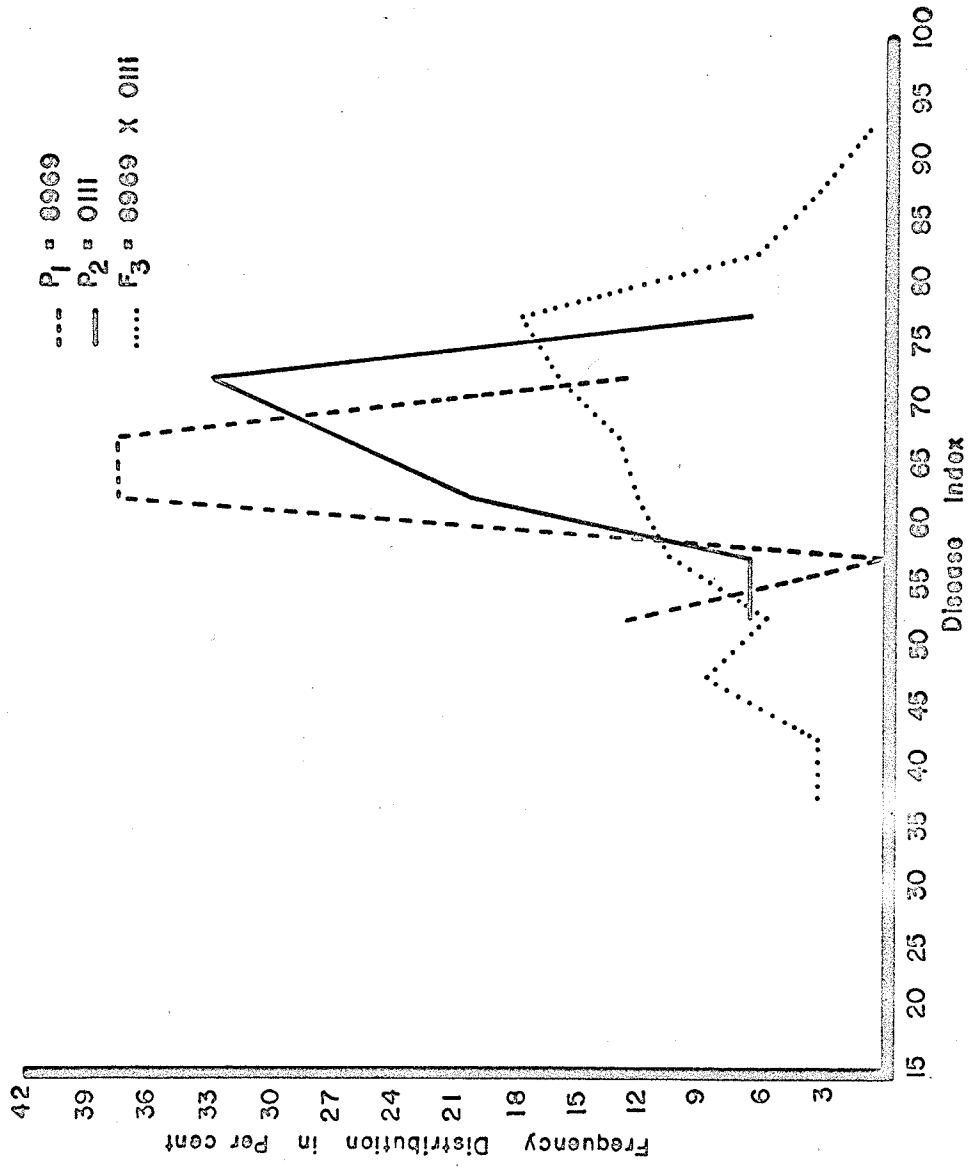
Field tests

It was considered of interest to determine the reaction of the parents and segregating families in field tests, based on readings in the adult stage. Seeds of CI.8969 x 011i and CI.8873 x 011i were grown in the field at Morden, Manitoba, under a natural field nursery disease environment. The disease indices of the entries at the adult stage from the field test were calculated in a manner similar to that used in classifying seedling reaction. The results were as follows:

Field test of CI.8969 X 011i at Morden

The means and standard errors of the parents and the segregating progeny are presented in Table 21. It is apparent from Table 21 and Fig. 6 that there were no differences of consequence between the mean disease indices of the parents involved in the cross. A "t" test that was conducted between the mean disease indices of the parents confirmed this result. The frequency distribution in percent (Fig. 6) shows that 74.4 percent of the segregating entries were within the curves of the parents, while 15.2 percent showed better resistance than either parent, and 10.4 percent showed more susceptibility than either parent. If any major complimentary gene action for resistance to H. sativum was taking place in the segregating entries, it would be expected that a higher percent of the entries would surpass both parents in their resistance to disease infection, and a smaller percent of entries would show more susceptibility than both parents. Since the deviations beyond the range of the parents were 15.2 percent and 10.4 percent on the resistant and susceptible side, respectively, it must be concluded that the deviations were likely due to environment.

Fig. 6 - Frequency distribution of the disease indices in percent of the parental variety, CI.8969 and Olli, and the F_3 families in the field test at Morden.


P1 = 0969
P2 = 0111
F3 = 0969 x 0111
Frequency Distribution in Per cent
Disease Index
42
39
36
33
30
27
24
21
18
15
12
9
6
3
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100

Field test of CI.8873 x 011i at Morden

The means and standard errors of the parents and the segregating progeny are presented in Table 22. It is apparent from Table 22 and Fig. 7 that differences existed between the means of the disease indices of the parents involved in this cross. The "t" test between the means of their disease indices show significant differences at the 1% level. Multiple correlation analyses were carried out between all combinations of four variables, which were as follows:

(1) Disease indices of the entries (108 in number) tested in the growth-cabinet at the seedling stage.

(2) The seed weight of the same entries before testing in the growth-cabinet.

(3) Classification into two-row vs. six-row heads.

(4) Disease indices of the same entries tested in the field at Morden.

The matrix of the correlation coefficients between all combinations of the f our variables is presented in Table 23, and some of the partial correlations concerned are presented in Table 24.

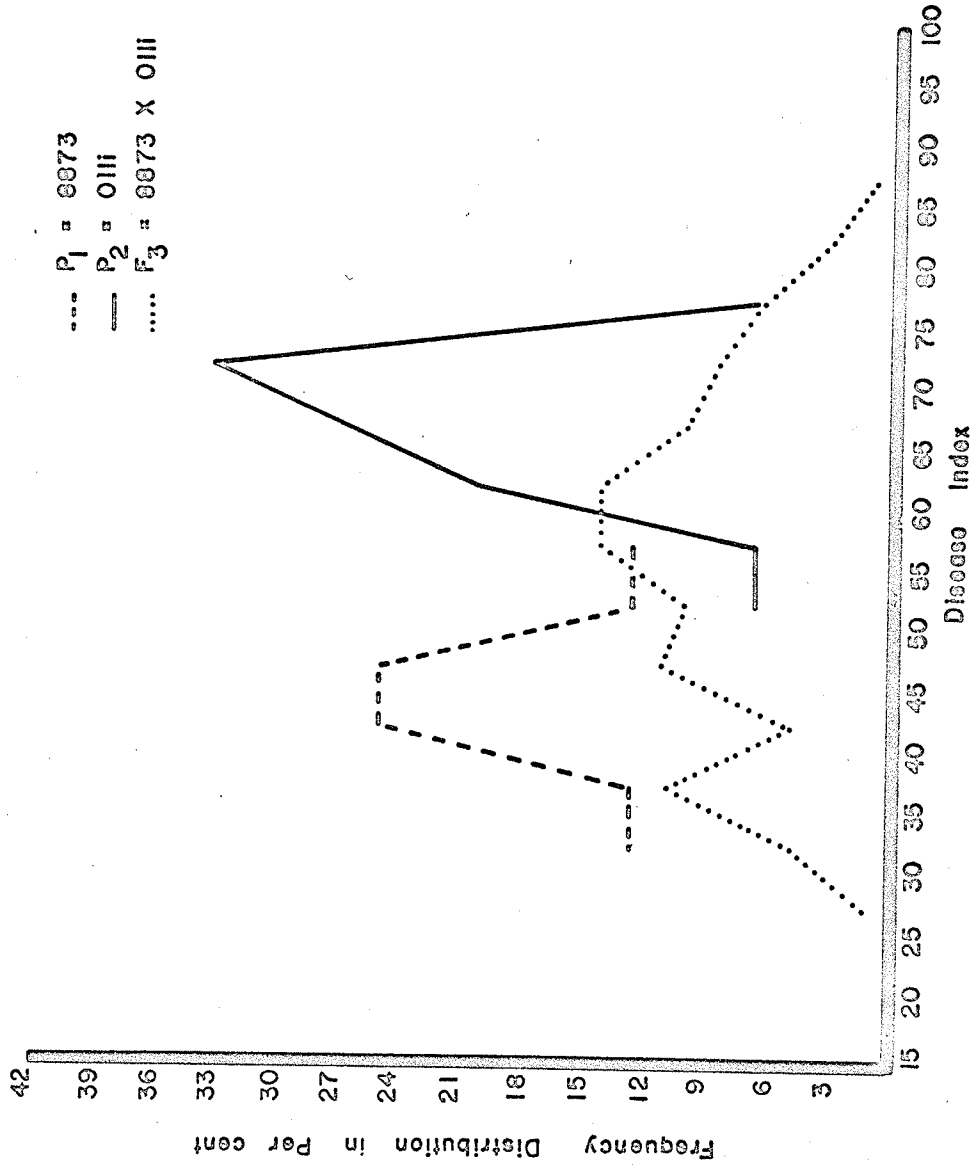
It is apparent from Tables 23-24 that the correlation between the disease indices of the entries obtained in the growth-cabinets and those obtained in the field was actually non-significant. There was a moderate but significant correlation between the disease index of the entries in the field and the seed weight, but it was smaller in magnitude than the correlation between the disease indices of the entries in the seedling stage tested in the growth-cabinets, and the seed weight. It is apparent from Fig. 7 and Table 21 that CI.8873 showed field resistance, whereas CI.8969 did not. The field resistance of CI.8873 was not correlated with the seedling stage resistance tested in the growth cabinet.

An analysis of the nature of the field resistance in CI.8873 was not made. It was apparent from the analysis of the CI.8969 x Olli cross in the field that environment-genotype interaction could take place. Therefore, a suitable design for quantitative analysis would be required to determine the nature of the field resistance of CI.8873. The results of the field experiment can be summarized as follows:

(1) There was no correlation between reaction of the seedlings to root-rot caused by H. sativum tested in the growth-cabinets, and field resistance.

(2) The CI.8873 entry showed field resistance, whereas the CI.8969 entry did not.

Fig. 7 - Frequency distribution of the disease indices in percent of the parental variety CI.8873 and Olli, and the F_3 families in the field test at Morden.


--- P_1 = 8873
—— P_2 = OIII
..... F_3 = 8873 X OIII
Frequency Distribution in Per cent
42
39
36
33
30
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18
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12
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15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Disease Index

Table 23 - Correlation coefficients between combinations of four variables in the CI.8873 x 011i cross.

Variables*	1	2	3	4
1	1.0000	-.5575	.3990	.2849
2		1.0000	-.6580	-.4632
3			1.0000	.4147
4				1.0000

* (1) Disease index of the entries tested in the growth-cabinet.

(2) Seed weight.

(3) Head row number.

(4) Disease index of the entries tested in the field.

Table 24 - Partial correlation of the four variables in the CI.8873 x 011i cross.

Variable	Partial Correlation	t values	Significance*
14.2	.0363	.3719	N.S.
14.3	.1431	1.4816	N.S.
12.3	-.4302	4.8829	H.S.
13.2	.0531	.5448	N.S.
23.1	-.5723	7.1492	H.S.
42.3	-.2777	2.9620	S.
14.23	.0272	.2788	N.S.

N.S. - Not significant.

S. - Significant at 5% level.

H.S. - Significant at 1% level.

Condition of seed production

Seed weight had shown an interaction with resistance, but it was also noted that the conditions under which the seed was produced affected the reaction obtained from a specific seed lot. Investigation of this factor was considered desirable. For this purpose, seeds of CI.8969, CI.8873 and Olli, were produced in the field and in the greenhouse. The seeds of Olli produced under each set of conditions were separated into small and large sizes, and tested for disease reaction in comparison with seeds of CI.8873 grown in the field and in the greenhouse, using four replicates in pot cultures (Table 25 and Plates 14-17). The relative kernel weights of the entries produced in the field are shown in Table 18, and those of the parental seed produced in the greenhouse, in Table 26. The test showed that all entries from seed produced in the greenhouse were susceptible. Seedlings from greenhouse-produced CI.8873 were as susceptible as those from field-produced Olli, but more resistant than Olli produced in the greenhouse. Greenhouse-grown CI.8873 showed susceptibility even though the weights of CI.8873 seeds produced in the greenhouse exceeded the weights of CI.8873 seeds produced in the field by 12.9%. Selected large seed from greenhouse-produced Olli, showed more susceptibility than small seed of Olli produced under the same conditions, as well as more susceptibility than small seed from the field-grown lot. Another experiment was conducted using bands in galvanized metal flats, following the procedure previously described. Eight replicates of each entry from seed produced in the greenhouse and in the field were seeded, using a random arrangement. The results, presented in Table 27, confirmed that CI.8873 from greenhouse-grown seed showed susceptibility, and that greenhouse-produced seed of Olli were

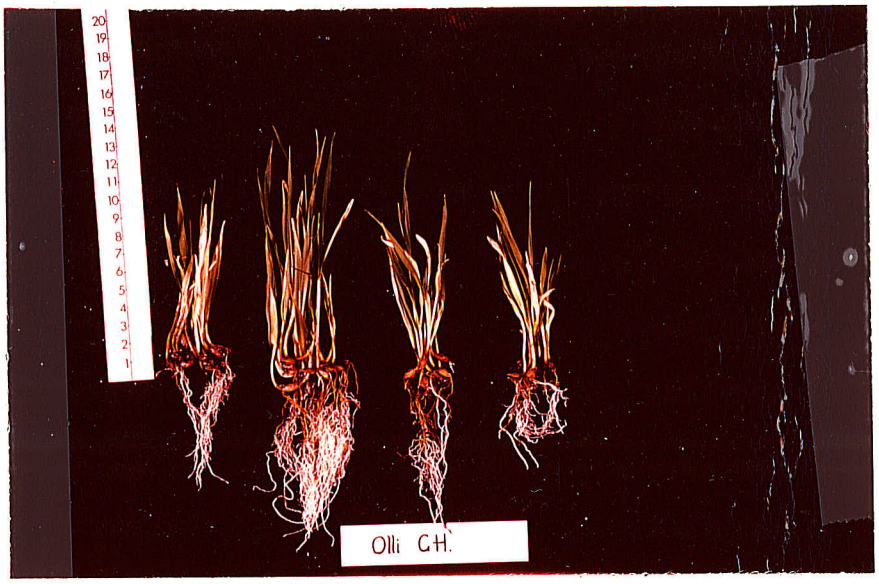
more susceptible than those from field-produced seeds of Olli. Some of the seeds of B.C.S-1 of all crosses were produced in the greenhouse and tested for their disease reaction. The results (Table 28) show the extreme susceptibility of all entries in the test, especially the backcrosses to the susceptible parent. In this test, CI.8873, CI.8969 and Olli from seed produced in the field, were used as a check. The disease indices of these checks (Table 29), showed that the reactions of the checks were typical, indicating that no exceptional factor in the test environment contributed to the unfavourable reaction of the B.C. lines. These results can be summarized as follows:

(1) The resistance of the parental varieties CI.8873 and CI.8969 (evidence from the backcross to CI.8969) broke down when their seeds were produced in the greenhouse.

(2) Interaction of seed weight and disease reaction was sbsent when the seeds of the entries were produced in the greenhouse. From these results, it can be assumed that the absence of seed weight interaction brought about the susceptibility of the resistant entries. If the assumption is correct, it sheds light on the nature of seed weight interaction. It indicates that seed weight interaction is not due to the increase in weight per se, but to the increase of a particular factor which was absent in the seeds produced in the greenhouse. This factor could be either in the seed itself or on the seed surface of those produced in the field. In an attempt to clarify these alternatives, surface-sterilized and non-surface sterilized seeds of Olli produced in the greenhouse and in the field were tested in the growth cabinets for their reaction to H. sativum. The test was conducted in eight replicates in a random arrangement, using bands in galvanized metal flats, following the procedure previously described.

Plate 14 - Reaction of seedlings from greenhouse-produced seed of Olli, unselected for size.

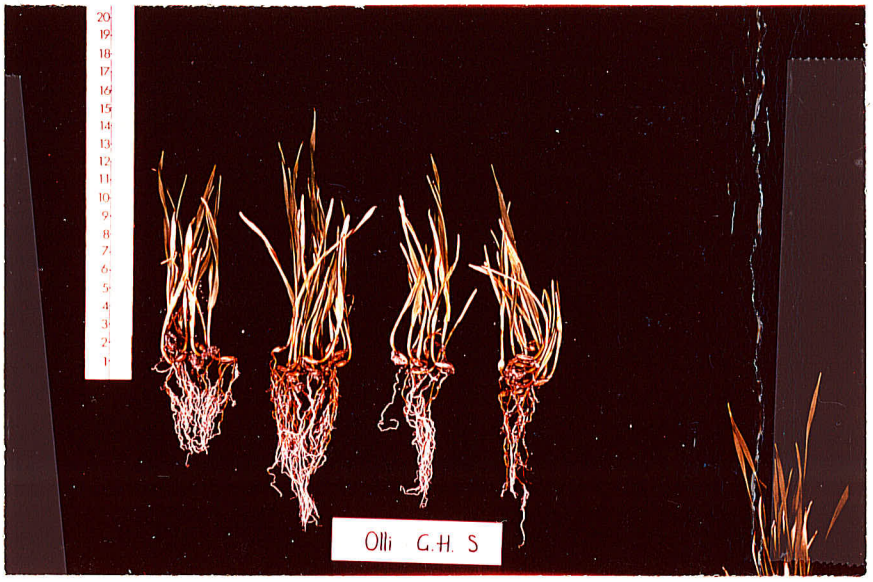
Plate 15 - Reaction of seedlings from greenhouse-produced seed of CI.8873 (2440).


Olli G.H.


2440 G.H

Plate 16 - Reaction of seedlings from greenhouse-produced seed of Olli, selected for small size.

Plate 17 - Reaction of seedlings from greenhouse-produced seed of Olli, selected for large size.


Olli G.H. S


Olli G.H.L.

The results (Table 30) showed that the surface-sterilization had little effect on seed grown either in the field or in the greenhouse. The fact that field-grown seed had a lower disease index than greenhouse-grown seed, and that surface sterilization did not change the result, indicated that the added resistance was not due to organisms present on the surface of the seed.

Table 25 - Disease indices of field- and greenhouse-produced seeds of the parental varieties CI.8873 and O11i.

Replicate	CI.8873 F.	CI.8873 G.H.*	O11i F.	O11i G.H.	O11i F.S.	O11i GHS*	O11i F.L.*	O11i GHL.
1	29.5	65.0	70.0	84.4	81.3	90.7	57.0	86.7
2	44.0	69.2	73.7	77.5	74.7	83.6	60.0	88.6
3	35.0	60.0	56.2	80.0	70.0	80.0	60.0	86.7
4	37.5	66.3	58.8	95.0	75.6	78.3	62.0	90.7
$\bar{+}$	36.500	65.125	64.675_b	84.225	75.400_c	83.150	59.750_d	88.175

* G.H. - Greenhouse-produced seeds unselected for size.

* G.H.S. - Greenhouse-produced seeds selected for small size.

* F.L. - Field-produced seeds selected for large size.

Table 26 - Weights per 1000 kernels of the greenhouse-produced seeds of parental varieties.

Entry	Weight per 1000 kernels (gm.)
CI.8969	34.315
CI.8873	46.363
O11i	29.900

Table 27 - Disease indices of field and greenhouse-produced seeds of the parental varieties CI.8873 and Olli.

Replicate	Disease Index			
	CI.8873 F*	CI.8873 G.H.	Olli F	Olli G.H.
1	44.4	80.0	54.0	86.1
2	60.0	60.0	70.0	74.2
3	40.0	78.8	50.0	82.5
4	45.5	91.8	56.2	88.6
5	40.0	82.2	63.7	90.0
6	63.1	95.7	61.4	95.7
7	45.3	66.6	67.7	96.8
8	34.4	89.4	67.3	96.4
x	46.588	80.563	61.288	88.788

* F = Field-produced seeds.

G.H. Greenhouse-produced seed.

Table 28 - Disease index of greenhouse-produced seeds of B.C.S-1 lines.

Entry No.	Disease Indices of lines from crosses (CI.8873 x O11i) x O11i	(CI.8873 x O11i) x CI.8873	(CI.8969 x O11i) x O11i	(CI.8969 x O11i) x CI.8969
1	100.0	94.0	100.0	76.6
2	82.0	90.0	100.0	67.7
3	92.5	100.0	100.0	90.6
4	96.4	90.0	100.0	66.2
5	100.0	84.7	92.7	77.8
6	92.0	85.7	100.0	69.2
7	88.0	97.0	100.0	94.3
8	84.3	85.0	100.0	79.0
9	100.0	82.7	95.6	85.3
10	88.3	94.1	92.8	97.6
11	100.0	87.4	100.0	93.3
12	100.0	84.6	82.5	70.0
13	100.0	81.1	100.0	84.3
14	83.2	77.1	94.5	78.8
15	94.5	94.4	92.7	83.3
16	97.6	86.2	100.0	74.1
17	90.0	73.0		91.1
18		84.0		53.7
19				57.3
20				64.2
$\bar{x}$	93.412	87.278	97.375	77.720

Table 29 - Disease index of field-produced seed of the parental varieties.

Replicates	Disease Index		
	CI.8969	CI.8873	Olli
1	47.8	35.3	81.3
2	43.5	44.2	73.0
3	42.2	43.2	63.3
4	38.8	38.7	58.6
5	23.5	42.1	63.5
6	37.8	43.3	74.1
7	32.2	48.7	78.8
8	44.4	52.5	70.5
9	43.2	43.3	78.7
10	39.0	41.2	67.1
11	34.7	51.1	72.2
$\bar{x}$	38.827	43.964	71.009

Table 30 - The effect on disease index of surface sterilization on field- and greenhouse-produced seeds of Olli.

Replicates	Disease Index			
	Olli F.*	Olli F.St.	Olli G.H.	Olli G.H.St.
1	51.7	48.0	87.1	89.2
2	53.7	48.2	86.0	52.9
3	53.7	52.5	82.7	84.6
4	57.3	62.1	60.0	86.7
5	56.5	70.0	76.8	73.3
6	50.5	49.3	87.7	80.0
7	33.3	75.6	95.7	78.7
8	66.3	53.7	83.3	64.0
$\bar{x}$	52.8750	57.4250	82.4125	76.1750

* F. - Field-produced seed.

F.St. - Surface sterilized field-produced seed.

G.H. - Greenhouse-produced seed.

G.H.St.- Surface sterilized greenhouse-produced seed.

Spore populations in the field

An attempt was made to establish a nursery for studies on root-rot caused by H. sativum. The soil of the nursery was inoculated by viable dry spores during the summer of 1964, as was described in the Materials and Methods section. In the summer of 1965, CI.8873 and O11i were sown at the nursery and at Morden, where disease infection occurs naturally. Disease indices of both entries at both locations are shown in Table 31. Only a trace of disease infection was detected on the O11i and the CI.8873 at the nursery, whereas substantial disease infection was observed on the same entries at Morden. Samples of soil were taken from the nursery, from the rest of the farm at the University, and from Morden. From each sample, ten counts were taken, a total of 25 samples was analysed for the number of spores in the soil. The results in Table 32 indicated that the difference in natural infection with root-rot caused by H. sativum, in the University farm, and in the nursery, compared with that at Morden, was due to the soil environment rather than the lack of inoculum.

Table 31 - Disease index of CI.8873 and O11i at the field nursery and at Morden.

Entry	Location	
	Nursery	Morden
O11i	28.0	65.5
CI.8873	17.3	41.8

Table 32 - Spore population per gram of soil at the nursery, University farm, and Morden.

Location	No. of Samples	Spores per gram of soil
Morden	10	138
University Farm	10	91
Nursery	5	248

DISCUSSION

The two varieties CI.8873 and CI.8969, which showed considerable resistance to root-rot caused by H. sativum, were crossed with the susceptible parent Olli and with one another, to study the inheritance of resistance to H. sativum. Two procedures were applied in the analysis of the various crosses and backcrosses involved. The partitioning method and Elston's procedure. Even though a majority of the segregating materials did not reject the hypothesis of monogenic control of resistance to H. sativum, it was not considered applicable, due to disagreement between the observed reaction means, and frequency distribution in B_1 and B_2, and those expected on a monogenic basis. Moreover, under this hypothesis, the F_2 genotype of CI.8873 x CI.8969 would show either no segregation, if the resistant genes of the parents were allelic, or segregation for two genes if they were not allelic, but neither case applied. An explanation based on polygenic action for resistance to H. sativum could account for the discrepancy between the observed and the expected B_1 and B_2 distributions, and the heterotic effect in the B_1 and in the F_3 families of the CI.8873 x CI.8969 cross. But the correlation of seedling weights and disease indices puts the problem of analysis into new dimensions. Reports in the literature have shown that seed weight is polygenically controlled (46). Hagberg reported (19) that "in F_1 of crosses between two-rowed and six-rowed barley, the weight of 1000 seeds is about 20% superior to the two-rowed parent, which has heavier seeds than the six-rowed one". Large seeds of Olli, when tested in the present study for root-rot reaction showed, in one

experiment, resistance to H. sativum approaching the CI.8969 resistance. From these results, it was assumed that the polygenic hypothesis for resistance to H. sativum and the seed weight reaction hypothesis were inter-related.

H. sativum attacks a large range of hosts in various phases of the disease. It produces seedling blight, foot- and root-rot, head blight, and leaf spot of cereals and grasses (13-14). The nutritional theory of disease resistance, particularly when applied to facultative parasites like H. sativum, is far from satisfying. This organism is non-specific in its in vitro nutritional requirements, and will undergo some growth at very low levels of nutrition (38). Further, it produces a toxin capable of killing plant cells and providing a nutritional substrate of dead cells in which it can continue growth (36). The same toxin also shows physiological activity, similar to that of gibberellic acid, on the leaf sheaths of rice seedlings and in relation to the synthesis of amylase in the alevurone layer of the endosperms of rice and barley seeds. Therefore, an alternative explanation of resistance should be considered, probably in terms of a chemical which would inhibit growth of the fungus through fungicidal action in the host tissues. Such a fungicidal material was found by Ludwig et al (37) in the young coleoptiles (up to five days old) of barley seedlings, but the same author demonstrated the presence of inhibitors to the anti-fungal factor in barley coleoptiles which were six days old or more.

In the present study, seedlings from the greenhouse-produced seeds of the resistant entries showed susceptibility, and seedlings of Olli from greenhouse-produced seeds were more susceptible than the ones from field-produced seeds. The seed weight effect was absent in the reaction

of the seedlings from the greenhouse-produced seeds. From these results and discussion, it is evident that there was a principle, or active agent, which could be either an antibiotic or an antitoxin, either on the surface or on the inside of barley seeds produced in the field, but not on those produced in the greenhouse. This factor would appear to be found in both susceptible and resistant varieties, under specific seed production conditions, and its quantity or activity was correlated with seed size. This correlation brought about the inter-relation between the polygenic hypothesis for resistance to H. sativum and the seed weight interaction hypothesis. This could also be the reason for the lack of correlation of the disease indices in the seedling stage and the adult stage of the plants under test. Reports in the literature reveal that natural surface flora, mainly bacterial, on wheat seed are antagonistic or antibiotic to H. sativum (30,55). According to these reports, seedlings from surface-sterilized seed showed an increase in disease reaction due to removal of surface organisms. In the present study seed surface sterilization did not show an increase in disease reaction. Hamilton et al (20), in their study of barley reaction to H. sativum, using the same type of test, stated "No differences were observed in a series of experiments comparing surface sterilized and non-surface-sterilized seed". Ark et al (2) reported that seeds of both barley and wheat contain an antibiotic principle, active against fungi and gram-positive bacteria when tested in vitro. Therefore, it is most logical to assume that the active principle was present inside the seed of barley, but it is also possible that, though the sterilized seed surface was free from living bacteria antagonistic to H. sativum, it was not free from a product of these bacteria antibiotic to H. sativum.

A method described by Ledingham et al (30) may have a bearing on this point. In their study, mature wheat plants grown in the greenhouse were artifically weathered. This was done by placing the plants in a moist chamber equipped with a fog-nozzle, which was allowed to operate intermittently. It was shown that artificial weathering was very effective in reducing the amount of disease caused by H. sativum, as it increased the bacterial population on the seed surface. Till further study gives final answers to the above two alternatives, all that can be said is that either the seed of barley contains an antibiotic principle, or that the bacteria on the seed surface, or their produce, are antagonistic to H. sativum, their function being proportional to seed weight.

Loiselle studied the inheritance of resistance to H. sativum in the seedling stage of barley (35), and reported that Anoidium and Br.3962-4 each possess duplicate genes at two loci for resistance in crosses involving the susceptible parent Olli. The F_3 families of the hybrid between the two resistant entries, Anoidium and Br.3962-4, were also tested for root-rot caused by H. sativum. It was shown that all the entries were within the range of the two parents, and it was concluded that the genes for root-rot and seedling blight resistance in Anoidium were allelic or closely linked to those in Br.3962-4. Since weight per 1000 kernels (gm.) of Anoidium and Br.3962-4 are greater than that of Olli by 32.9 percent and 11.1 percent respectively, in one set of seed samples (although relative seed weight can vary with environment), it can be assumed that Loiselle's results were also affected by seed weight differences.

In the present study, it was apparent that seed weight and seed growing conditions were affecting the reaction of the entries to H. sativum in the seedling stage. Further, it was shown that no

correlation existed between disease indices of the entries tested in the field in the adult stage, and the same entries tested in the growth-cabinet in the seedling stage. Taking into consideration the complexity of this organism and its unspecific toxin, it seems that selection for resistance to H. sativum in the seedling stage in the growth-cabinet is less promising than that in the adult stage in the field.

The method described by Sallans et al (51) for selection of resistance in wheat to H. sativum in the adult stage in the field may have a bearing on this point. In this selection program, the pedigree method of breeding was used, in which the resistance of a plant was measured by the disease index of the progeny in the next generation, tested in several locations. As the variety CI.8873 showed field resistance to root-rot, this method would be appropriate for selecting for field resistance to H. sativum in crosses involving this variety. It should have general application for selection against root-rot in barley.

Old (48) reported on the presence of fungistatic effects of soil bacteria on root-rot caused by H. sativum. This could account for the lack of root-rot in the field nursery inoculated with isolate 514 of H. sativum at the University. Investigation of these microflora may provide a way to control root-rot caused by H. sativum. This could have application in at least two ways, through the direct procedure of adding the antibiotics to the soil or to the seed, or through the less direct method of modifying the microflora by cropping or other treatments to promote an increase in the desired organism (63).

SUMMARY AND CONCLUSIONS

The inheritance of seedling reaction to root-rot caused by the 514 isolate of Helminthosporium sativum was studied in crosses and backcrosses among the barley varieties CI.8969 and CI.8873, which show resistance, and the variety Olli, which is susceptible. The study was carried out under a controlled environment in growth-cabinets. The data from reaction tests of F_3 and backcross families were analysed by Elston's procedure for testing a one-locus hypothesis, and by Powers' partitioning method. A majority of the segregating materials did not reject the hypothesis of monogenic control of resistance to H. sativum, yet it was not considered applicable due to disagreement between the observed and the expected B_1 and B_2 distribution, and the heterotic effect in the B_1 and in the F_3 families of the CI.8873 x CI.8969. Therefore, the polygenic hypothesis was adopted to explain the inheritance for the seedling reaction to root-rot caused by H. sativum. A high correlation of seed weight with resistance, and the reaction of selected large seed of Olli, which approached that of CI.8969 in one experiment, led to the assumption that the seed weight, which is known to be polygenically controlled, could account for the polygenic inheritance of seedling reaction. Seedlings of all the greenhouse-produced seed were extremely susceptible, irrespective of genotype or seed weight. Therefore, it was assumed that resistance was related to a principle, or active agent, which could be either an antibiotic or an antitoxin, either on the surface or on the inside of field-produced seeds of barley. This factor would appear to be found in both

susceptible and resistant varieties, under specific seed production conditions, and its quantity or activity was correlated with seed weight. Whether there is a genotype influence on the quantity of the resistance principle aside from that effecting seed weight, was not established conclusively. It was apparent that seed weight and seed growing conditions were affecting the reaction of the entries to H. sativum. Further, as a field study based on natural infection showed that no correlation existed between disease indices of the entries tested in the field in the adult stage, and the same entries tested in the growth cabinet, in the seedling stage, it seems that selection for resistance to H. sativum in the seedling stage in the growth-cabinet is less promising than that in the adult stage in the field. CI.8873 showed field resistance, whereas CI.8969 did not. Based on the field reactions of lines derived from the cross of CI.8873 x Olli, selection for field resistance to H. sativum in barley would seem quite feasible. In this regard, the method of selection for root-rot resistance in wheat described by Sallans et al (51) would appear to be worthy of investigation for its application to barley.

An attempt to establish a nursery for root-rot study at the University Farm failed, not due to the absence of inoculum in the nursery soil, but due to soil environment. Study of this factor may provide ways to control root-rot caused by H. sativum.

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