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GENE DOSAGE EFFECTS ON MONOGENIC CHLOROTIC LESION RESISTANCE TO NORTHERN CORN LEAF BLIGHT

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ABSTRACT

GENE DOSAGE EFFECTS ON MONOGENIC CHLOROTIC LESION RESISTANCE TO NORTHERN CORN LEAF BLIGHT

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TED NAMM

The Ht gene conditions chlorotic lesion resistance to northern corn leaf blight. The causal agent of this disease is Helminthosporium turcicum Pass.

The purpose of this study was to evaluate gene dosage effects of the Ht and ht alleles. Monoploid, diploid, triploid, and tetraploid seedlings were obtained for both alleles. These seedlings were inoculated at the three or four leaf stage with H. turcicum in a cool, humid chamber. Disease reaction was evaluated by first measuring the area of the third or fourth leaf, then measuring the area of the lesions and calculating percent infection. The null hypothesis of no difference between treatment means was accepted or rejected on the basis of Satterthwaite's value of "F". Duncan's multiple range analysis was used to test for differences among treatment means.

There was no difference in resistance between monoploids and homozygous diploids containing one and two doses, respectively, of the Ht allele. There was also no difference between triploids and tetraploids containing three and four

doses, respectively, of Ht. However, three and four doses of the Ht allele conferred a higher level of resistance on seedlings than did one or two doses. Heterozygous diploid (Ht ht) seedlings always showed the least resistance of any of the levels of Ht in this study.

Diploid, triploid, and tetraploid seedlings containing two, three, and four doses, respectively, of the ht allele did not differ in their degree of susceptibility. Monoploid (ht) seedlings were much more susceptible than seedlings of the other three dosage levels.

SECTION I

INTRODUCTION

The highest incidence of northern corn leaf blight in the United States occurs in the corn belt and extends predominantly southeastward to the Atlantic coast. The degree of infection depends upon the weather conditions, with the most severe infection being manifested in areas having cool temperature and high humidity. The incidence of this disease may be high in the New England area when these weather conditions are prevalent.

The causal agent of the disease is Helminthosporium turcicum Pass. It may infect as early as the three-leaf seedling stage. Leaf lesions initially appear black to tan in color and soon coalesce into necrotic, wilt-type lesions which typify susceptibility. Incidence of the disease may reduce yield considerably when temperature and humidity favor seedling infection. Early infection may predispose the corn to stalk rot (76).

Inbred lines of corn exhibit two types of resistance to H. turcicum, both of which are heritable. The most common type is characterized by a significant reduction in the size and number of lesions. Wilt-type lesions are confined to the lower leaves on highly resistant plants of this nature. This type of resistance is multigenically inherited.

Certain other inbred lines of corn exhibit a second type of resistance which is conditioned by a single, domi-

nant gene. Resistance in these lines is characterized by reduced sporulation of the fungus and yellow, chlorotic lesions which do not wilt.

The major objective of this study was to evaluate, quantitatively, the effects of different gene dosage levels on the expression of chlorotic lesion resistance. The dosage levels analyzed were monoploid, diploid, triploid, and tetraploid. Susceptible and monogenic resistant plants were tested on each of the four levels. The heterozygote between diploid resistant and susceptible was also included in the analysis. The term "gene dosage" was applied with the full realization that the gene being studied was tested on different chromosome levels. All other genes in the chromosome complement were added or detracted according to the change in whole chromosome sets.

Uniform environmental conditions were imperative for accurate quantitative evaluations. Field inoculations were impractical because of fluctuations in the weather. Monogenic resistance in corn is expressed as early as the four-leaf seedling stage, which made this plant material quite amenable to artificial inoculation techniques. All data were therefore obtained from seedling inoculations in a cool, humid chamber.

SECTION II

LITERATURE REVIEW

INHERITANCE OF RESISTANCE TO NORTHERN CORN LEAF BLIGHT

A serious epidemic of northern corn leaf blight inflicted heavy damage on the midwestern corn crop in 1942. Susceptibility to the disease was characterized by large, necrotic lesions on the leaves. Highly susceptible plants turned completely brown by the end of the growing season.

Elliott and Jenkins (17) found that the disease spreads quite rapidly during cool, rainy seasons. Spread of the disease was slow when the weather was hot and dry. By means of artificially induced infection, the workers were able to obtain some lines of corn which were considerably more resistant than others. The degree of infection was graded on a scale of 0.5-5.0 with the lower numbers representing the least infection. The plant was designated as resistant when wilt-type lesions were confined to the lower leaves.

Robles (60) demonstrated that different races of the pathogen, Helminthosporium turcicum Pass., showed differences in host specificity. For example, the fungus was more virulent on sweet corn than on sudangrass or johnsongrass. He also stated that there are at least two parasitic races of H. turcicum. Ullstrup (76) reported that isolates from a single corn host showed no differential pathogenicity on

hosts within the same line.

Robert and Findley (58) reported that diseased leaf refuse scattered near corn plants in the field resulted in heavier infection than did direct inoculation with pulverized leaf inoculum. They showed that mycelia in diseased leaves remained alive for more than a year in conditions of uncontrolled temperatures. The workers concluded that infected leaf refuse which has overwintered in the soil provides a good source of inoculum.

Multigenic inheritance.

Elliott and Jenkins (17) found that resistance to northern corn leaf blight was transmitted from generation to generation. Jenkins and Robert (37) evaluated mature plants which had been infected artificially, and noted that individual resistant lines differed in their effective degree of resistance. The susceptible lines also showed different grades of infection. A wide range of disease expression was noted in F_2 populations of resistant x susceptible lines. This led the workers to conclude that a large number of genes condition the resistant phenotype.

Andrew et al (3) used pulverized, diseased leaves for inoculation of seedlings. Plants were exposed to high humidity for four hours before treatment. They found that a heavy concentration of inoculum at mild temperatures resulted in the best infection. Another part of the same study revealed that there was no correlation between field inoculations and those performed in the greenhouse.

Jenkins and Robert (38) located some of the factors for resistance by means of chromosomal translocations. The chromosomes carrying genes for susceptibility were also identified by this technique. Findley and Leffel (20) used marker gene stocks to identify factors conditioning resistance. Their data indicated that factors concerned with this type of resistance were situated on at least 12 chromosome arms.

Robert and Sprague (59) reported a tendency of isolates from a particular line to be more virulent on plants of that line than of other lines. This was true for both resistant and susceptible plants. Fleming and Kozelnicky (21) tested the same multigenically resistant stock in six different geographical areas and found significant differences from area to area. These variations were heritable, and probably arose as a result of mutation and/or residual segregation.

Monogenic inheritance.

A new type of resistance to northern corn leaf blight was reported in 1961 by Hooker (31). Lines GE440 and Ladyfinger popcorn exhibited small, chlorotic spots on the leaves when exposed to infection. These spots did not develop into necrotic lesions as they did in susceptible and multigenically resistant plants. Sporulation was delayed and significantly reduced. These lesions eventually developed a tan, necrotic center, surrounded by a yellow-green "halo." Wilting was not

observed in these lesions. Segregation tests indicated that this type of resistance is conditioned by a single, dominant gene.

Hooker (32) reported that monogenic resistance is expressed as early as the four-leaf seedling stage. Necrotic lesions did appear on the lower leaves in seedling inoculations, but isolates from these lesions could not produce the susceptible phenotype on monogenic resistant plants.

Hooker (33) reported that the genes for resistance in GE440 and Ladyfinger popcorn are either identical, allelic, or closely linked. He suggested that this gene be given the symbol Ht. Linkage studies in GE440 by Patterson et al (48) indicated that Ht is in the long arm of chromosome two, between v_4 (virescent) and Ch (chocolate pericarp).

Hooker (34) reported ratios of 1:2:1 in the F_2 progeny of resistant x susceptible lines. Other ratios were 0:1:1 when the F_1 hybrid was backcrossed to a susceptible line and 1:1:0 when the F_1 hybrid was backcrossed to GE440. It was presumed that the highly resistant phenotype was conditioned by Ht Ht and the resistant phenotype by Ht ht. The susceptible plants were ht ht. These data indicated that the Ht gene is not completely dominant. Saxena and Hooker (65) reported that the Australian inbred, NN-14, contains two independent genes for chlorotic lesion resistance. When NN-14 was crossed to a susceptible line and selfed to the F_2, a ratio of 15:1 was obtained.

Hilu and Hooker (28) showed that the age of the seedling was not a limiting factor in disease expression. In the

same study, sections of leaves with susceptible, multigenic resistant, and monogenic resistant lesions, were exposed to high humidity. Sections from susceptible and multigenic resistant leaves showed profuse mycelial growth, while those from monogenic resistant leaves showed little or no mycelial growth.

Hooker et al (36) pointed out that the Ht gene conditions resistance against a wide range of H. turcicum isolates. They explained that selection pressure favoring certain isolates of a pathogen will increase as more and more sources of resistance are found. It is then possible that certain isolates of H. turcicum may produce wilt-type lesions on plants carrying dominant Ht genes. Sharma and Aujla (67) reported the occurrence of wilt-type lesions on Ladyfinger popcorn in the Kulu Valley in India. The workers suggested the use of Ladyfinger popcorn to help in the classification of new types of virulence.

Hooker et al (35) reported several new sources of monogenic resistance originating from many geographical areas throughout the world. Hilu and Hooker (27) reported chlorotic lesion resistance on Hastings Prolific dent corn. Ullstrup (75) reported that line PI 217407 maintained a high level of chlorotic lesion resistance during severe epidemics of leaf blight in Kenya, East Africa.

Host-pathogen interaction.

Hilu and Hooker (29, 30) examined the growth habit of

the fungus in susceptible, multigenic resistant, and monogenic resistant plants. The initial stages of infection were the same in all three types. The infection peg penetrated directly through the surface of the leaf and subsequent hyphal growth advanced intracellularly through the mesophyll. In a susceptible host the hyphae continued through the mesophyll and clogged the xylem and tracheids. Water conduction was stopped in that area causing the tissue to wilt. Further spread of the fungus occurred when hyphae left the xylem and penetrated the surrounding healthy tissue. These hyphae invaded the vascular bundles and grew very rapidly in adjacent cells.

Hyphae also became established in the xylem and the tracheids in a multigenic resistant host. Hyphal growth was very much curtailed and did not severely clog the xylem. Spread of the fungus occurred when the hyphae advanced through the mesophyll tissue. The type of lesion was the same as that on a susceptible host, but lesions usually appeared on lower leaves only.

In a monogenic resistant host hyphae became established very sparsely in the xylem. Hyphal growth proceeded quite slowly through the mesophyll, and rapid killing of the cells was not apparent. Spreading and coalescence of lesions was rarely observed.

Hilu and Hooker (30) described a response to the pathogen which was unique to the monogenic resistant host. The xylem walls became much thicker than normal when the

infection was initiated near a vein.

Cultural variability.

Hilu (26) showed that both virulence of the pathogen and conidial production increased after it was passed through a susceptible host and reisolated. The fungus lost virulence after many subcultures without passage through the susceptible host. Such avirulent isolates sometimes caused chlorotic lesions on susceptible plants. Hilu also demonstrated a direct relationship between the number of conidia and the degree of disease development.

Rodriguez and Ullstrup (61) studied Trichometasphaeria turcica Luttrell, the perfect stage of H. turcicum. They showed that monoascosporic progenies of the fungus showed great variation in their attack on susceptible plants. Some of these progeny of T. turcica attacked corn but not sudangrass; some attacked corn but not sorghum, etc. Virulence among progenies derived from in vitro matings of monoconidial isolates was thus quite variable.

Luttrell (41) studied the morphology of T. turcica. The number of ascospores within any given ascus was normally quite variable. Genetic study of this fungus would therefore be quite difficult.

GENE DOSAGE STUDIES

The effects of gene dosage have been studied in both plants and animals. Stern (73) studied the expression of the ci gene in Drosophila melanogaster. The recessive ci produces a gap in the cubitus vein of the wing. Stern showed that an increase in the dosage of this allele reduced the size of the gap. He postulated that ci acted in the same manner as ci^+ but less efficiently. Additional doses of ci increased the efficiency of the gene and produced an expression resembling the dominant phenotype. He called this phenomenon "hypomorphic gene action."

Mangelsdorf and Fraps (43) demonstrated a direct linear relationship between the amount of vitamin A and the dosage of the Y gene in corn. Randolph and Hand (52) doubled the number of chromosomes in a strain of pure yellow (Y Y Y) corn and produced six doses of Y in the endosperm. The result was a 40 percent increase in the carotenoid content of the kernel. They also doubled the chromosome number in pure white (y y y) corn. This resulted in a 19 percent decrease in the amount of carotenoid in the kernel.

Rhoades (54) showed that the dosage effect of the a_1 gene was additive for the production of dots in corn aleurone when in a Dt Dt dt background. Rhoades (56) compared the effects of different doses of Dt on the rate of mutation at the A locus. He found that Dt dt dt produced 7.2 mutations per seed; Dt Dt dt produced 22.2 mutations per seed; and

Dt Dt Dt produced 121.9 mutations per seed. The rate of mutation in these experiments was determined by the number of dots produced in kernels which were originally of the genotype a_1 a_1 a_1.

Most of the recent work on gene dosage in corn was performed on factors which alter the carbohydrate content of the endosperm. Ellis et al (18) compared the chemical compositions of diploid and tetraploid corn. They found that doubling the chromosome number increased the amount of proteins and carbohydrates in the kernel.

Extensive work has been done with the gene for waxy endosperm, wx. Kernels with the wx wx wx genotype possess endosperm starch in the form of amylopectin. Wx Wx Wx kernels possess starch which is 75 percent amylopectin and 25 percent amylose. Wx starch stains blue-black with Lugol's solution while wx starch stains red-brown. Sprague et al (69) studied the effects of wx at different dosage levels. They found a small cumulative effect of the wx gene in the endosperm. They also found that the Wx gene was not completely dominant. Greenblatt (23) reported that the sporophyte tissue of a monoploid containing one wx gene stained blue-black. This reaction was indistinguishable from that of diploid sporophytes which were homozygous Wx. The sporophyte tissue of tetraploid plants (wx wx wx wx) stained red-brown as did the sporophyte of diploids (wx wx). Tsai (74) correlated the activity of certain enzymes with the dosage of Wx. By means of the appropriate crosses, he was

able to obtain seeds with zero, one, two, and three doses of Wx in the endosperm of diploids and zero, two, four, and six doses in the endosperm of tetraploids. The increase in amylose starch and protein content, and the activity of ADP-glucose transferase were almost directly proportional to the number of Wx genes. Nelson (46) worked with stocks containing the gene complement wx wx wx ae ae ae. The phenotype of these seeds was distinguishable from those containing Wx wx wx ae ae ae and Wx wx wx Ae ae ae.

The gene for sugary endosperm, su_1, also produces a high amylose content in the kernel. Dunn et al (16) investigated dosage effects of the su_1 and su_2 genes. These were also studied in combination with the gene for dull endosperm, du. The amount of amylose remained the same with increasing doses of su_1 and su_2 regardless of the dosage of the du gene.

Haunold and Lindsey (25) studied the effects of dosage on the amylose extender gene, ae. They found that the kernels with Ae Ae embryos had a low amylose content and those with ae ae embryos had a high amylose content. The kernels with Ae ae embryos had an amylose content intermediate between the other two. Crane (15) obtained kernels with five doses of ae in the cells of the endosperm. These lines, designated $3n + 2^V$, had triploid endosperm cells homozygous for ae as well as two extra fifth chromosomes, each carrying the recessive ae gene. The $3n + 2^V$ lines had lower amylose levels than did the 3n lines (ae ae ae), but the difference was not statistically significant. He concluded

that the *ae* gene may be hypomorphic. Fergason *et al* (19) analyzed the dosage effects of the four possible levels of the *ae* gene in the endosperm. No difference was found in percent amylose between the nulliplex and the simplex. The duplex had a significantly higher percentage than either of the first two. The amylose percentage of the triplex was almost double that of the duplex.

Additional work has been done with many other genes which exhibit dosage effect. Rhodes and Myers (57) studied the knotted gene, *Kn*. *Kn Kn* plants had many knots, resulting in severely dwarfed plants with short internodes. *Kn kn* plants had fewer knots and longer internodes. Plants which were *kn kn* exhibited the normal phenotype.

The effects of gene dosage on disease resistance in plants have not been studied to any great extent. Sears (66) transferred a gene for leaf rust resistance from a chromosome of *Aegilops umbellulata* to a chromosome of wheat. He suggested that the effectiveness of resistance could be increased if the same gene could be introduced at two or more other loci.

No data on the effects of gene dosage on monogenic resistance could be found in the literature at this time.

MONOPLOIDY IN MAIZE

Kimber and Riley (39) reviewed the important work on the occurrence of haploidy in angiosperms. They distinguished between monoploids and polyhaploids. Monoploids are sporophytes containing a single genome, while polyhaploids contain half the chromosome number of plants which are ordinarily polyploid. Another good review of haploidy was presented by Magoon and Khanna (42).

Monoploids occur naturally in maize, but infrequently. Chase (7, 8) stated that both the pollen parent and the seed parent play a role in determining the frequency of maize monoploids. He found that proper selection of the parents increased the frequency. Chase (9) estimated the frequency to be one monoploid in every 1000 seeds. He noted that spontaneous doubling of the chromosome number occurred in a mature monoploid. Selfed seed was produced when this doubling occurred in the anthers and ear shoot of the same plant. In this study Chase concluded that many years of breeding and selection could be saved if inbred lines were produced by doubling monoploids instead of by conventional inbreeding procedures.

Chase (10) showed that monoploids were generally smaller than diploids, having longer, thinner leaves. He compared the various morphological characters of monoploids and diploids with respect to the number of plant parts, linear dimensions, area, and volume. It was expected that the ratio of n/2n would be 0.5 since the nuclear volume of

a monoploid is approximately half that of a diploid. The actual ratio for such comparisons was less than 0.5. The monoploids in this study had fewer plant parts than their diploid sibs.

Chase and Nanda (11) found no difference in variation between new monoploids and long term monoploids. The newly developed homozygous diploids showed less genetic variation than conventionally developed inbreds. Sprague et al (70) studied the mutation rate in the selfed progeny of monoploids with respect to quantitative characters such as yield, leaf area and volume, maturity, and tassel and ear features. These were studied from the S_3 through the S_6 generations. All characters showed significant differences from one selfed generation to the next. It was assumed that these differences were due to mutations. The workers estimated the rate of mutation to be 4.5 per attribute per 100 gametes tested.

Detection of maize monoploids.

In earlier work Chase (6) isolated maternal monoploids by using pollen from a line with the genotype A B Pl. This set of factors produced purple aleurone and purple seedling roots on kernels carrying the recessive complement. Seeds obtained from the cross a b pl x A B Pl were germinated and those seedlings having purple aleurone and non-purple roots were classified as putative monoploids. These seedlings were presumed to have monoploid embryos and triploid endosperm. Ultimate confirmation of monoploids was based upon

chromosome counts.

More recently Chase and Nanda (12) and Nanda and Chase (45) developed a more simplified procedure for the detection of putative monoploids. This technique involved the use of a line called "purple embryo marker" (PEM). The genotype of PEM is b pl A C $R^{nj:Cudu}$ pr P^{wr}. When PEM was used as the pollen parent in a cross, the hybrid seeds developed a purple pigment in the embryo and a red to purple pigment in the endosperm. The seeds with endosperm pigment and colorless embryo were classified as putative monoploids. Positive confirmation of monoploids was still based upon chromosome counts as the colorless embryo may have been due to maternal diploidy or mutated color genes. Mass germination of seeds was eliminated since the marker was visible on the dormant kernel.

Greenblatt and Bock (24) described the "R-navajo" allele in PEM. They stated that any line of corn could be screened for maternal monoploids with this set of markers as long as the plants are not homozygous R. Ghidoni (22) suggested incorporating purple scutellum markers into PEM for further clarification of putative monoploids. Such a system would require at least one of the dominant S alleles.

Coe (13) reported a frequency of 3.23 percent monoploids in a line of corn designated "stock 6." He attributed the high frequency to the abnormally large pollen grains. To test this hypothesis he used pollen from many different lines (including stock 6) on maternal parents which were homozygous

for $\underline{gl}_1$. Putative monoploids had glossy endosperm. He isolated 2.41 percent monoploids from lines crossed to the stock 6 parent. Crosses to other pollen parents yielded much lower frequencies.

Coe and Sarkar (14) detected putative monoploids by using stocks homozygous for $\underline{C}^I$ (color inhibitor). The maternal parents were homozygous $\underline{C}$ (colored endosperm and scutellum). Putative monoploids had colored scutellum and colorless endosperm. Most agronomically desirable stocks do not carry the necessary genes for detection of putative monoploids by the $\underline{C}^I\underline{C}^I$ system. The use of this technique is thus limited to special cases.

Sarkar and Coe (62) confirmed the fact that both parents influence the development of monoploid kernels. They also demonstrated the presence of a triploid endosperm in kernels with monoploid embryos. The fate of the sperm nucleus which fails to fertilize the egg of a monoploid was not traced.

TETRAPLOIDY IN MAIZE

Polyploid plants offer a suitable means for studying the dosage of genetic factors. Stebbins (71) summarized the evolutionary and breeding significance of polyploids.

Randolph (49) outlined the differences in morphology and cytogenetics between diploid and tetraploid maize. The tetraploid plants resembled their diploid sibs in growth habit, but the tetraploids had broader, thicker leaves, larger tassels, thicker stalks, larger pollen grains, and greater cell size. The fertility of tetraploids was up to 20 percent less than that of diploids. The selfed progeny of tetraploids had chromosome numbers which varied between 37 and 43. More extreme variations were sometimes evident. Randolph (51) pointed out that tetraploid corn plants did not exhibit the "gigas" characters typical of many other plant species. Randolph et al (53) compared shoot apex development in diploid and tetraploid maize. The shoot apex had the same number of cells during development of the tenth leaf, but these cells were larger in tetraploid shoot apices.

Levings and Alexander (40) tested the effects of inbreeding and crossbreeding in tetraploid maize. Hybrid vigor was quite evident. Heterosis increased as the inbreeding coefficient increased within the inbred lines used in the single crosses.

Induction of tetraploidy in maize.

The direct treatment of corn seeds with colchicine

as a means of inducing tetraploidy has met with limited success. Randolph (50) treated ears with high heat during the first divisions of the proembryo and some tetraploid kernels were produced. Alexander (1) induced tetraploidy by using plants homozygous for el (elongate). These plants produce a large number of unreduced megaspores. This technique involved a series of outcrosses and backcrosses to the original parent. The main advantage of this method was that a diploid species was made tetraploid and it still retained the original genetic diversity. The breeding procedures were quite laborious.

Shaver (68) developed a successful colchicine method for induction of tetraploidy. Corn seeds were germinated until the primary root was about one inch long. The root tip was cut off and only the cut root was immersed in a dilute solution of colchicine. Shaver had the most success with two 24-hour treatments in colchicine and an interim treatment in distilled water. Survival of seedlings was quite low but the frequency of tetraploidy was fairly high among the survivors.

TRIPLOIDY IN MAIZE

Triploid corn kernels are normally obtained from crosses between a tetraploid and a diploid. Cavanah and Alexander (5) showed that pollen from a diploid plant had a competitive advantage over pollen from a tetraploid on both types of silks. The workers applied diploid pollen to a diploid silk and haploid pollen to the same silk about three and one-half hours later. Most of the triploid kernels appeared on the proximal end of the ear. This indicated that the haploid pollen tubes "overtook" those of the diploid and reached the embryo sac first in the longest silks.

Bauman (4) reported a frequency of 0.58-5.23 unreduced eggs per thousand in one line of corn. Fertilization of these eggs by haploid pollen produced triploid kernels. Rhoades (55) cited a case of triploidy where the diploid complement was donated by the pollen parent.

Triploid corn kernels are usually shriveled and have poor viability. Alexander and Beckett (2) crossed a tetraploid, as female, to Euchlaena perennis and obtained triploid kernels which were round, plump, and had excellent viability.

McClintock (44) showed that 2n x 3n crosses can be made when the triploids produce fertile gametes. Selection was usually against pollen carrying the extra chromosomes.

SECTION III

MATERIALS AND METHODS

Description of inbred lines.

The inbred lines of corn used in this study were obtained from Dr. Albert L. Hooker, Professor of Plant Pathology and Genetics at the University of Illinois in Urbana.

Inbred R223 is chlorotic lesion resistant to infection by H. turcicum. W153R is a highly inbred susceptible line. R223 is W153R crossed to Ladyfinger popcorn, backcrossed two generations to W153R, and selfed until homozygous for the Ht gene. The two inbred lines have similar genetic backgrounds but are not isogenic as evidenced by maturity differences and morphological dissimilarities.

Isogenic lines, both with and without the Ht gene, were obtained from Dr. Hooker in order to equalize differences in disease reaction which might reflect genetic background. Line 65-225-1 is chlorotic lesion resistant to H. turcicum. This line is W153R crossed to GE440, backcrossed six generations to W153R, and selfed until homozygous for the Ht gene. Lines 65-225-1 and W153R are identical in maturity and morphology.

Purple embryo marker.

Seeds of purple embryo marker (PEM) were obtained from Dr. Sherrett S. Chase presently on appointment at Harvard University.

In the summers of 1965, 1966, and 1967 PEM was crossed, as pollen parent, to the resistant and susceptible lines. The hybrid seeds developed a purple pigment in the embryo and a red to purple pigment in the endosperm. Full maturity of the kernels was very important. Complete development of these pigment systems required at least 45 days from the time of pollination to the time of harvest.

The flowering period of PEM was about the same as that of R223 but PEM flowered much later than 65-225-1 and W153R. The two isogenic lines were planted 10 to 12 days later than PEM to insure coincidence of flowering periods.

Isolation of monoploids.

The kernels with red to purple pigment in the endosperm and purple pigment in the embryo were discarded as being diploids. Those kernels with endosperm pigment but with colorless embryos were classified as putative monoploids.

Putative monoploids were germinated and the seedlings with purple roots were discarded. Seedlings with white roots were examined cytologically and those with 20 chromosomes in the cells were discarded. Seedlings with 10 chromosomes in the root tip cells were monoploids.

Monoploids were always verified by counting the chromosome number in root tip cells. Only the confirmed monoploids were used in dosage tests.

Induction of tetraploidy.

In the summer of 1965 an attempt was made to induce

tetraploidy using the technique developed by Randolph (50). Developing ears were wrapped in a heating pad 18 hours after pollination. The ear shoots were heated to 40°C and kept at this temperature for one hour. This treatment did not produce tetraploid kernels in our lines.

Tetraploids of R223 were obtained by first soaking the diploid seeds in 0.02 percent colchicine for 96 hours. About 2000 seeds underwent this treatment but only 12 survived. Eleven of these seedlings reached the two-leaf stage and died. The remaining seedling continued to grow quite well. Subsequent chromosome counts in the developing pollen indicated that the plant was tetraploid. Self-pollination of this plant yielded 163 kernels. These kernels were germinated and examined cytologically. Some of the seedlings were used in dosage tests and some were grown to maturity and self-pollinated.

Subsequent treatment of seeds using this technique failed to produce tetraploids. In most cases the seeds germinated in the colchicine and produced primary roots up to one inch long. Root tips and embryos soon swelled and no further growth took place. One octoploid seedling was detected following this treatment. This seedling died after reaching the four-leaf stage.

Diploid seeds of 65-225-1 and W153R were subjected to the colchicine treatment discussed by Shaver (68). Seeds were germinated and one half inch of the primary root was cut off under distilled water exposing the empty xylem

vessels of the differentiated portion of the root. The "decapitated" seedlings were then placed on a wire grid in such a manner that the cut root protruded through and below the grid. The grid was then placed on a dish containing a 0.02 percent solution of colchicine. Thus the cut root was the only part of the seedling immersed in the solution. The seedling was now forced to absorb some of the colchicine. This treatment was applied for 24 hours.

An average of one seed in 200 survived this treatment and one seed out of five survivors was a confirmed tetraploid. The chromosome number was determined by cytological analysis of the developing pollen.

A method of increasing the survival rate of the treated seedlings was tried. It seemed that the desiccation of secondary root growth played a part in keeping the survival rate of seedlings at a low level. Secondary roots initiated during the treatment period grew straight upward because of the position of the seed on the grid. A beaker lined with wet blotting paper was inverted over the treatment assembly. Thus a "moist chamber" was constructed around the treated seedlings. The average survival rate in our lines increased from one in 200 to one in 175 with this modification of Shaver's technique.

Preparation of root tips for chromosome counts.

The meristematic cells of the root tip presented many problems when ordinary fixation procedures were used. Many layers of cells were often the major problem and the

individual cells were often clumped together.

Root tip smears were prepared according to the technique of Satyanarayana and Kermicle (64). Seeds were germinated and actively growing root tips were treated in a solution of 0.002M 8-hydroxyquinoline. Root tips were then fixed in 3:1 acetoalcohol for eight hours. Fixed material was hydrolyzed in N HCl for two minutes at 54°C. The root tips were transferred to 5 percent pectinase and incubated at room temperature for three hours. The treated material was then placed on a slide and teased apart with fine needles. Teasing was easy at this point because the tissue was quite malleable from the pectinase treatment. A drop of aceto-orcein was applied and the tissue was squashed gently under a cover glass. The cells were well-separated from one another in favorable preparations. The chromosome arms were spread in the "X" pattern typical of arrested metaphase. The best slides were stored in the refrigerator after sealing the cover glass with vaseline or paraffin.

Growth of seedlings for inoculation.

Plants grown for inoculation were raised in the greenhouse under conditions of warm days and cool nights. Supplementary light was used to extend the daylength in the fall and winter.

Most experiments contained some plants which required chromosome counts and others which did not. The diploids and tetraploids were direct-seeded. Putative monoploids and triploids were germinated two days later. This system allowed

certain material to be analyzed cytologically and still remain at the same stage of growth as those which were direct-seeded since seeds normally germinate 40-50 hours faster in Petri dishes than in soil.

Culturing the pathogen for use in inoculation.

The agar medium used to culture isolates of H. turcicum had the following composition:

Dextrose	20.0 grams
L-Asparagine	1.0 "
$MgSO_4 \cdot 7H_2O$	0.5 "
KH_2PO_4	1.0 "
KCl	0.5 "
$Ca(NO_3)_2$	0.1 "
Agar	15.0 "
Distilled water	1000 ml.

The use of this medium was suggested by Dr. Hooker in a private communication.

The mixture was heated until it became fairly homogeneous and then autoclaved for 20 minutes at 15 pounds of pressure and 120°C.

Leaf lesion isolates of H. turcicum were obtained periodically from Dr. Hooker. Cultures used in inoculation tests were initiated from leaf lesions occurring on susceptible plants. The lesions were cut into small squares and surface-sterilized in a 10 percent solution of commercial "Clorox." The sections were constantly agitated in the solution until the edges became transparent. Sections were then dried on absorbent blotting paper. Dry sections were transferred aseptically to sterile Petri dishes containing 20 ml. of the agar medium. The dishes were incubated at room tem-

perature. Mycelial growth was usually observed on this medium one to two days after the cultures were initiated. Mycelial growth continued for about two weeks. Spores started to form during the third week of growth and many spores were present at three to four weeks of age. Cultures 21-25 days old were optimal for inoculation although cultures up to five weeks of age showed satisfactory virulence. Spores dried out and appeared shriveled after the cultures were six weeks of age. These cultures showed little virulence.

Preparation of the inoculum.

Petri dish cultures were examined microscopically to insure that spores were plump and abundant. Crosswalls were observed on spores under a high power lens.

Satisfactory cultures were mixed with water for two minutes in a "Waring Blendor." Two Petri dish cultures were used for every 100 seedlings to be inoculated and 100 ml. of water were added for every culture. The spore suspension was then transferred to a "Black Flag" insect sprayer.

Inoculation of seedlings.

Corn seedlings three to four weeks old were used in inoculation tests. The seedlings had to be in one of the following stages of growth to be suitable for inoculation and subsequent quantitative evaluation:

(1) Three-leaf stage with the fourth leaf about one inch long.

(2) Four-leaf stage.

(3) Four-leaf stage with the fifth leaf about one inch long.

The chamber used for inoculation of seedlings was located at the Agronomy Field Station in Durham. The room consisted of two benches, each three feet wide by eight feet long. One bench was sealed with heavy plastic sheets and contained a humidifier. The other bench was not sealed and was thus subject to inoculation temperature but not humidity. A heater was placed on the floor of the room and an overhead light remained on during treatment of seedlings.

The heater was turned on the day before inoculations were to be made. The inoculation bench was left open with the humidifier off. This allowed the inside of the inoculation bench to come to the optimum inoculation temperature of 66-70°F.

The following morning seedlings were placed on the open bench for predisposition to inoculation temperature. The inoculation bench was then sealed and the humidifier was turned on. When the humidity of the inoculation bench reached 100 percent, the plants were placed on this bench and the humidifier was turned off.

Inoculum was sprayed on the plants from above until all the leaves were covered with fine droplets of water. When all the plants were sprayed, the chamber was resealed and the humidifier turned on again.

The humidifier was attached to a clock which allowed

it to operate for 25 minutes out of each hour. The seedlings were left in the inoculation chamber for 18 hours.

Chlorotic lesions usually took seven to nine days to develop. These lesions were yellow when they were evaluated but the center of the lesion eventually turned tan with the retention of a yellow "halo" around the necrotic center. Lesions on susceptible plants were fully developed within seven days after inoculation.

Quantitative evaluation of disease reaction.

A single leaf from each seedling was evaluated for disease reaction. The choice of leaf depended upon the stage of growth of the seedling. Measurements were taken either on the fourth leaf or on the leaf which was emerging from the whorl at the time of inoculation.

The area of the leaf was measured with a transparent grid containing 144 square inches in a 12 x 12 pattern. Each square inch contained 25 evenly spaced dots. The leaf was placed under the grid and the number of dots covering the leaf was counted. This count was performed at five randomly selected positions under the grid and an average of the five readings was taken. The area of the leaf was derived from the following proportion:

$$\frac{1}{25} = \frac{x}{\text{average number of dots counted}}$$

In the above proportion "x" is equal to the area of the leaf in square inches.

The area of the lesions was then measured. Lesions occurring on susceptible plants were usually easy to measure

because smaller lesions coalesced into one or more larger lesions. These were measured by direct counting of dots. Lesions on resistant plants appeared as small spots or blotches which did not usually coalesce. These lesions were outlined on very light tracing paper by placing the upper left part of the paper over the first lesion and tracing it with a sharp pencil. The next lesion was traced next to the first. All lesions were traced flush with one another forming a single area on the tracing paper. The traced area was then placed over a grid containing 100 squares per square inch. The number of squares covering the traced area was counted and the area of the lesions was determined on the basis of 100 squares = one square inch.

The percent infection of the leaf was determined in the following manner:

$$\text{percent infection} = \frac{\text{Area of the lesions on the leaf}}{\text{Area of the leaf}} \times 100$$

Measurements with the grid were compared with planimeter measurements and both gave the same reading $\pm$ 0.5. The grid measurements were obtained very much faster than were those with the planimeter.

Analysis of variance.

The inoculation tests always involved an unequal number of plants within pots and of pots within treatments. A completely randomized design with subsampling was used to analyze the data since environmental conditions within the inoculation chamber were uniform with respect to temperature and humidity. The experimental units were the individual pots.

The data as percentages represented scores for the individual plants and were normally distributed. Thus conversion to arcsine angles was not made and the percentages were used directly in the analysis of variance. The sources of variation, degrees of freedom, and expected mean squares were as follows:

Source	d.f.	E.M.S.
Dosage	t-1	$\sigma_\omega^2 + k_2\sigma_E^2 + k_3\sigma_T^2$
Among Pots	p-1	———
Pots/Dosage	(p-1) - (t-1)	$\sigma_\omega^2 + k_1\sigma_E^2$
Plants/Pots	(n-1) - (p-1)	σ_ω^2
Total	n-1	

The coefficients for σ_E^2 (k_1 and k_2) were not equal because of the unequal frequencies at the different levels of sampling and subsampling. Thus an exact test of the null hypothesis was not possible. Satterthwaite (63) proposed an approximate test procedure. This procedure was outlined by Ostle (47).

The data were analyzed by Satterthwaite's approximate test procedure and by the analysis of variance. The value of "F" obtained by Satterthwaite's test was used to test the null hypothesis.

Duncan's multiple range test as outlined by Steel and Torrey (72) was used to test for differences among the mean percent infections.

SECTION IV

RESULTS AND DISCUSSION

Artificial induction of northern corn leaf blight.

Resistant and susceptible seedlings containing one, two, three, and four doses, respectively, of the Ht and ht alleles were inoculated with H. turcicum and evaluated for disease reaction. Figure 1 illustrates the stage of growth at which the seedlings were most amenable to artificial inoculation.

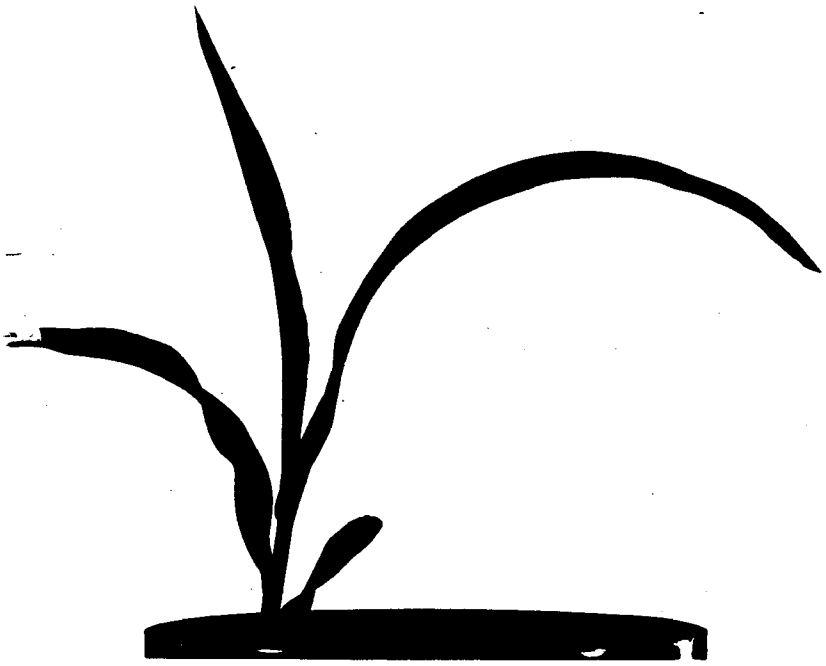

Figure 1. Seedling at the proper stage of growth for artificial inoculation with H. turcicum.

The fungus cultures used in seedling inoculations were about three weeks of age. Cultures with no spores (Figure 2) or old cultures with shriveled spores (Figure 3)

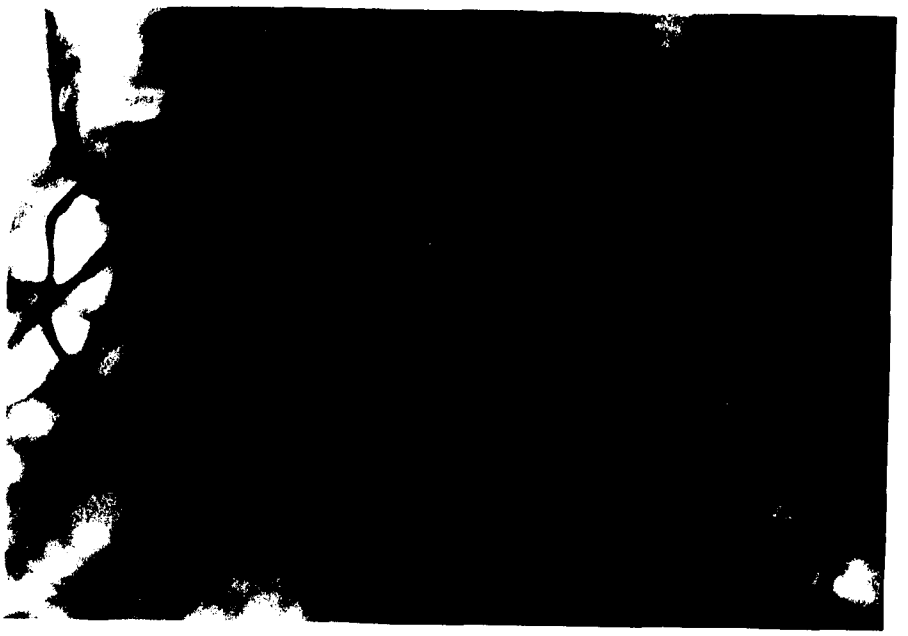

Figure 2. Seven-day old culture of H. turcicum in which spore formation has not started. (x 100)

Figure 3. Six-week old culture of H. turcicum in which the spores are shriveled. (x 100)

were not usually virulent. Only cultures with plump, well-developed spores (Figure 4) were used to inoculate seedlings.

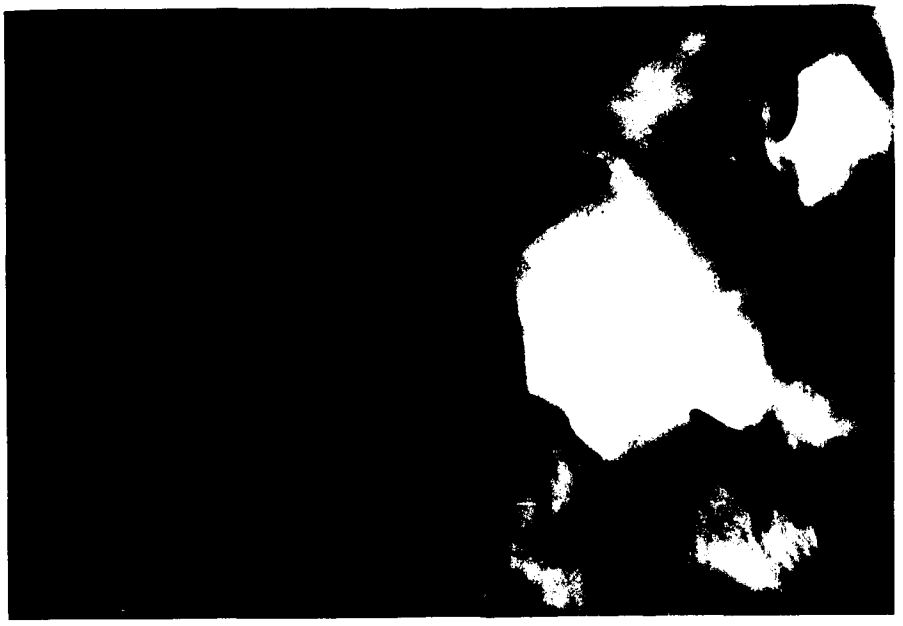

Figure 4. Three-week old culture of H. turcicum in which the spores are well-developed. (x 100)

Figure 5 illustrates susceptibility and chlorotic lesion resistance to the disease. Note that chlorotic lesions did not usually coalesce or cause wilting of the leaf. Necrotic lesions coalesced and caused severe wilting.

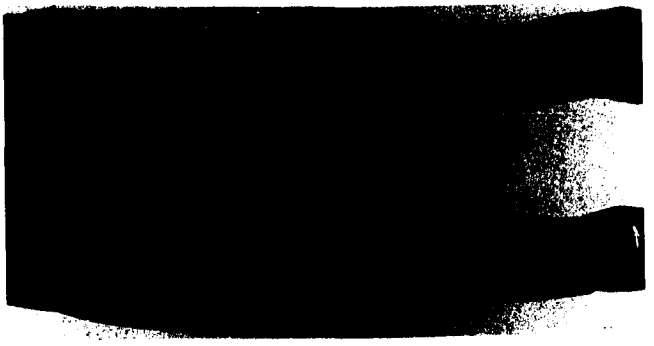

Figure 5. Susceptibility and monogenic resistance to northern corn leaf blight. The top leaf is susceptible; the bottom leaf is monogenic resistant.

Isolation of monoploids.

Monoploids were isolated from the progeny of inbred lines crossed to purple embryo marker. Putative monoploids were germinated and the chromosome number was verified by counts on growing root tips. Figure 6 illustrates the types of kernels found on the ear of a plant crossed to purple embryo marker.

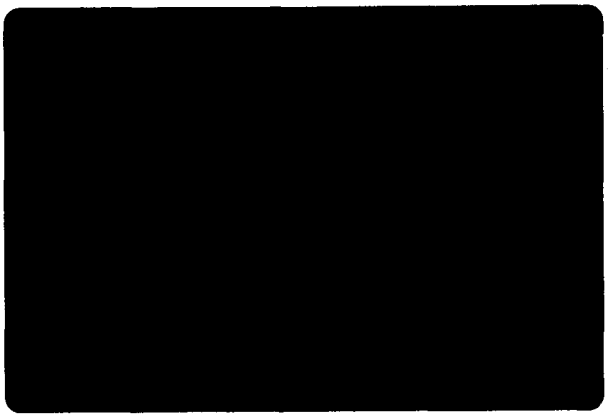

Figure 6. The three types of kernels on the ear of a plant crossed to purple embryo marker.

The kernel at the top of Figure 6 is a putative monoploid with purple endosperm and colorless embryo. The four seeds in the middle are hybrid diploids. The bottom kernel is assumed to have received pollen from a source other than purple embryo marker because it shows neither the endosperm nor embryo pigments.

Figure 7 shows the three types of kernels viewed from the opposite side. Note that putative monoploids and hybrid diploids cannot be distinguished from one another, but contaminant kernels can be readily recognized.

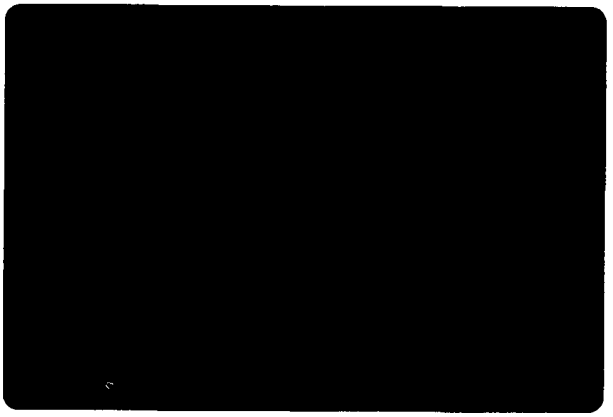

Figure 7. Back view of the three types of kernels on the ear of a plant crossed to purple embryo marker.

Appendix table 1 illustrates the frequency of monoploidy in the resistant and susceptible lines used in this study.

Triploids and tetraploids.

The morphology of diploid, triploid, and tetraploid kernels was one criterion used to distinguish these three dosage levels. Figure 8 shows kernels of these three chromosome types of R223. Tetraploid kernels were usually larger than diploid kernels. Triploid kernels were about the same size as diploid kernels, but they appeared wrinkled or shriveled at maturity.

Figure 9 shows diploid and tetraploid plants of R223. The tetraploids were larger than their diploid sibs, having broader leaves and thicker stalks. Tetraploid plants had larger tassels and produced more pollen than diploids. Seed set on tetraploids was usually reduced following selfing.

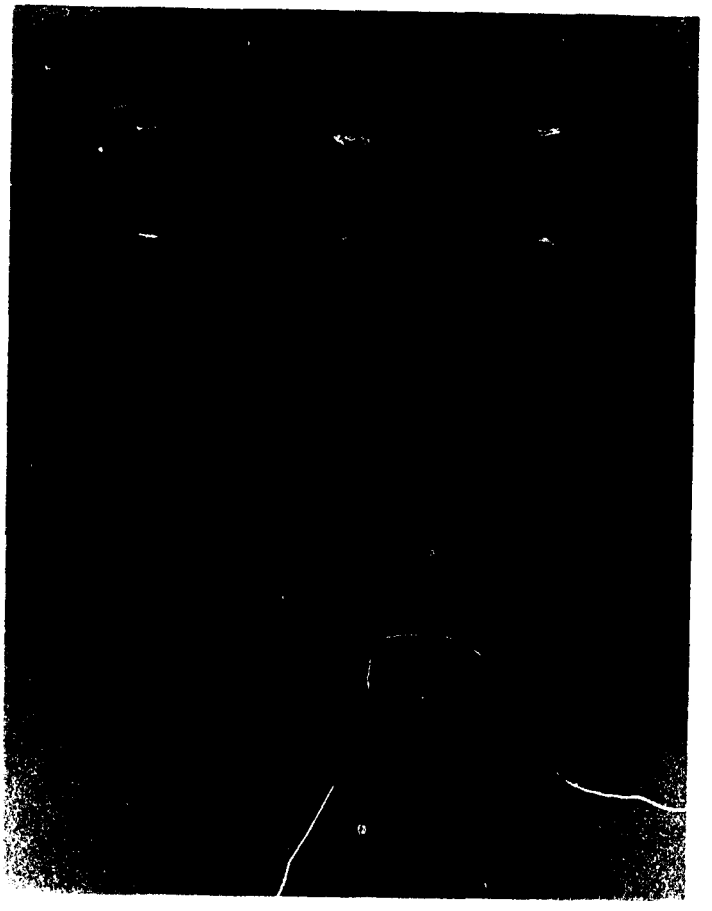

Figure 8. Kernels of R223 with three dosage levels. Tetraploid kernels in the top row; diploid kernels in the middle row; triploid kernels in the bottom row.

Figure 9. Diploid and tetraploid plants of H223 at eight weeks of age. Diploid plants on the left; tetraploid plants on the right.

The shriveled appearance of triploid kernels was the main criterion for their identification, especially in crosses where haploid and diploid pollen was applied to the silks of tetraploid plants. In some cases the chromosome number was counted for verification. Figure 10 shows the chromosome complement from the root tip of a triploid.

Figure 10. The 30 chromosomes from the root tip of a triploid seedling.

Dosage studies of the Ht allele.

Most of the data on dosage of the Ht allele were obtained from R223. Some information was obtained from 65-225-1.

The data for experiment 1, shown in tables 1-4, compare percent infection of heterozygous diploids, homozygous diploids, and tetraploids of R223. These were planted July 20, 1966, inoculated August 6, 1966, and evaluated August 16, 1966.

Table 1. Percent infection on heterozygous diploid (*Ht ht*) seedlings of R223 x W153R following inoculation with *H. turcicum*.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
1	7.26	1.18	16.25
	6.83	1.30	19.03
	6.65	0.98	14.74
	6.77	0.90	13.29
2	4.09	0.91	22.25
	7.04	1.91	27.13
	5.50	1.07	19.45
	5.36	0.76	14.18
3	3.98	0.50	12.56
	6.40	0.64	10.00
	7.27	1.63	22.42
	5.54	1.53	27.62
4	6.38	1.33	20.85
	5.94	1.58	26.60
	5.62	1.39	24.73
	3.95	0.53	13.42
5	4.42	0.88	19.91
	4.99	1.12	22.44
6	7.16	1.61	22.49
7	7.41	1.72	23.21
	6.12	1.56	25.49
	5.34	0.43	8.05
	6.78	0.76	11.21
8	5.00	0.82	16.40
	6.45	1.07	16.59
	6.12	1.81	29.58
9	4.35	0.78	17.93
	4.76	0.92	19.33
	6.76	1.24	18.34
10	7.08	1.03	14.55
	4.27	0.97	22.72
	6.92	1.11	16.04
11	5.34	0.72	13.48
	3.79	0.83	21.90
12	4.69	1.03	21.96
	6.40	1.74	27.19
	6.21	1.15	18.52
	6.53	1.16	17.76

Table 1. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
13	5.33	1.06	19.89
	4.51	1.19	26.38
	4.83	0.84	17.35
	3.99	1.18	29.57
14	4.83	1.02	21.12
	6.01	0.96	15.97
15	4.10	0.75	18.29
	7.44	1.92	25.81
	4.19	0.89	21.24

Treatment total for 47 heterozygotes = 925.23
Mean percent infection = 19.69

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* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 2. Percent infection on homozygous diploid (Ht Ht) seedlings of R223 following inoculation with H. turcicum.

Pot No.	Leaf Area *	Infected Area **	Percent Infection
16	5.30	0.70	13.21
	4.71	0.78	16.56
	5.13	0.76	14.81
	5.48	1.01	18.43
17	4.25	0.70	13.21
	3.22	0.56	17.95
	7.60	1.18	15.53
	5.88	1.33	22.62
18	4.55	0.55	12.09
	4.93	0.70	14.20
	4.43	0.81	18.28
	6.85	1.17	17.08
19	5.17	0.86	16.63
	5.23	0.89	17.02
	4.69	0.80	17.06
20	3.29	0.56	17.02
	3.52	0.43	12.22
	5.13	0.68	13.26
	4.75	0.67	14.11
21	4.82	0.55	11.41
	5.64	1.00	17.73
	3.49	0.97	27.80
22	4.37	0.41	9.38
	6.09	1.12	18.40
	6.21	1.11	17.87
	7.20	0.83	11.53
23	5.38	0.68	12.64
	5.80	0.87	15.00
	4.73	1.04	21.99
	7.81	0.63	8.07
24	3.99	0.65	16.29
	4.54	0.76	16.74
	4.72	0.63	13.35
25	6.95	1.48	21.29
	6.67	1.83	27.44
	7.38	1.76	23.85
	5.11	0.54	10.57

Table 2. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
26	3.24	0.38	11.73
	4.45	0.92	20.67
	3.86	0.47	12.18
	3.02	0.51	16.89
27	3.42	0.46	13.45
	3.72	0.50	13.44
	4.39	0.64	14.58
	5.21	0.94	18.04
28	4.55	0.75	16.48
	4.00	0.32	8.00
	4.19	0.53	12.65
29	3.97	0.61	15.37
	5.28	0.74	14.02
	6.01	1.03	17.14
	4.46	0.85	19.06
30	4.91	0.71	14.46
	3.23	0.69	21.36
	3.23	0.22	6.81
	3.81	0.45	11.81

Treatment total for 56 homozygotes = 884.04
Mean percent infection = 15.79

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* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 3. Percent infection on tetraploid (Ht Ht Ht Ht) seedlings of R223 following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
31	4.81	0.75	15.59
	3.99	0.48	12.03
	5.57	0.66	11.85
	5.36	0.61	11.38
32	5.53	0.69	12.48
	5.49	0.65	11.84
	5.94	0.39	6.57
	4.71	0.94	19.96
33	4.83	0.40	8.28
	7.67	1.13	14.73
	3.68	0.60	16.30
	7.25	0.78	10.76
34	7.10	0.78	10.99
	6.26	0.65	10.38
	7.20	0.80	11.11
	7.12	0.86	12.08
35	7.47	0.94	12.58
	7.48	0.82	10.96
	5.02	0.45	8.96
	4.74	0.40	8.44
36	4.54	0.62	13.66
	4.86	0.80	16.46
	3.31	0.42	12.69
	3.21	0.32	9.97
37	7.01	0.58	8.27
	6.42	0.73	11.37
	7.56	0.41	5.42
38	4.93	0.69	13.99
	5.33	0.31	5.82
	6.85	0.66	9.64

Treatment total for 30 tetraploids = 344.56
Mean percent infection = 11.49

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 4. Analysis of variance, Satterthwaite's "F", and multiple range analysis of mean percent infections for experiment 1.

Source of Variation	d.f.	M.S.	F
Dosage	2	623.74	39.75**
Among Pots	37		
Among Pots/Dosage	35	15.69	
Among Plants/Pots	95		
Total	132		

** = Significant at 0.01 level.

Satterthwaite's "F" = 38.79**

Multiple Range Analysis

Genotype =	Ht ht	Ht Ht	Ht Ht Ht Ht
Mean =	19.69	15.79	11.49

(Means not underscored by the same line are significantly different).

The tetraploids showed a higher level of resistance than did the homozygous diploids, although the difference was not significant in this case. Heterozygous diploids were less resistant than either the tetraploids or homozygous diploids. The highly significant value of "F" reflects the extreme difference between the tetraploids and heterozygous diploids.

The data for experiment 2, shown in tables 5-9, compare percent infection of monoploids, heterozygous diploids, homozygous diploids, and tetraploids of R223. These were planted October 10, 1966, inoculated October 29, 1966, and evaluated November 5, 1966.

Table 5. Percent infection on monoploid (Ht) seedlings of R223 following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
17	4.79	0.77	16.08
	3.10	0.58	18.71
	3.41	0.53	15.54
	4.70	0.90	19.15
18	5.32	0.92	17.29
	5.53	1.18	21.34
	6.88	1.40	20.35
19	4.25	0.62	14.29
	3.69	0.36	9.76
	5.92	1.18	19.93
20	5.97	1.32	22.11
	4.80	1.19	24.79
	5.36	0.73	13.62
21	4.23	0.72	17.02
	5.14	0.95	18.48
	3.04	0.53	17.43
Treatment total for 16 monoploids =			286.19
Mean percent infection =			17.89

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 6. Percent infection on heterozygous diploid (Ht ht) seedlings of R223 x W153R following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
8	3.80	0.99	26.05
	4.61	0.93	20.17
	4.26	0.70	16.43
	3.33	0.93	27.93
9	3.54	0.76	21.47
	4.48	1.26	28.13
	4.92	1.12	22.76
10	5.00	1.03	20.60
	4.11	0.96	23.36
	3.27	0.54	16.51
	4.03	0.83	20.60
11	3.69	1.12	30.35
	3.51	0.92	26.21
	3.35	0.87	25.97
	3.44	0.72	20.93
12	4.42	0.99	22.40
	4.11	1.15	27.98
	4.73	1.26	26.64
	3.72	0.85	22.85
13	3.21	0.81	25.23
	3.33	0.88	26.43
	3.88	0.62	15.98
14	3.72	1.01	27.15
	4.69	1.28	27.29
	3.16	0.84	26.58
	4.57	1.01	22.10
15	3.25	0.73	22.46
	4.40	0.89	20.23
	4.31	0.73	16.94
16	4.83	0.73	13.25
	4.68	1.28	27.35
	4.96	1.22	24.60

Treatment total for 32 heterozygotes = 742.93
Mean percent infection = 23.22

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 7. Percent infection on homozygous diploid (*Ht Ht*) seedlings of R223 following inoculation with *H. turcicum*.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
1	4.80	0.77	16.04
	3.44	0.63	18.31
	3.98	0.57	14.32
	4.11	0.51	12.41
2	4.32	0.58	13.43
	4.76	0.95	19.96
	5.83	1.12	19.21
3	5.69	0.98	17.22
	5.47	0.86	15.72
	5.01	0.82	16.37
	4.14	0.77	18.60
4	3.66	0.49	13.39
	5.33	1.04	19.51
	4.81	0.77	16.01
	4.24	0.60	14.15
5	4.15	0.52	12.53
	4.43	0.77	17.38
	3.88	0.75	19.33
	5.39	0.61	11.32
6	6.50	1.21	18.62
	4.67	0.76	16.27
	5.25	0.49	9.33
	3.14	0.69	21.97
7	4.71	0.79	16.77
	5.96	0.84	14.09

Treatment total for 25 homozygotes = 402.26
Mean percent infection = 16.09

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 8. Percent infection on tetraploid (Ht Ht Ht Ht) seedlings of R223 following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
22	4.30	0.39	9.07
	5.14	0.37	7.20
	5.45	0.29	5.32
	3.53	0.23	6.52
23	5.93	0.31	5.23
	4.68	0.32	6.84
	6.00	0.66	11.00
	3.86	0.25	6.48
24	3.79	0.32	8.44
	3.01	0.21	6.98
	3.15	0.22	6.98
25	4.02	0.20	4.98
	4.28	0.44	10.28
	5.37	0.38	7.08
26	4.94	0.29	5.87
	4.94	0.42	8.50
	4.71	0.33	7.01

Treatment total for 17 tetraploids = 123.78
Mean percent infection = 7.28

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 9. Analysis of variance, Satterthwaite's "F", and multiple range analysis of mean percent infections for experiment 2.

Source of Variation	d.f.	M.S.	F
Dosage	3	956.21	95.33**
Among Pots	25		
Among Pots/Dosage	22	10.03	
Among Plants/Pots	64		
Total	89		

** = Significant at 0.01 level.

Satterthwaite's "F" = 97.77**

Multiple Range Analysis

Genotype =	Ht ht	Ht	Ht Ht	Ht Ht Ht Ht
Mean =	23.22	17.89	16.09	7.28

(Means not underscored by the same line are significantly different).

Monoploids and homozygous diploids showed about the same level of resistance. Tetraploids were significantly more resistant than either the monoploids or homozygous diploids. The heterozygotes again were the least resistant. The latter were significantly less resistant than seedlings of the other dosage levels.

The data for experiment 3, shown in tables 10-15, compare percent infection of the five dosage levels of the Ht allele from monoploid through tetraploid in R223. These were planted June 20, 1967, inoculated July 3, 1967, and evaluated July 11, 1967.

Table 10. Percent infection on monoploid (Ht) seedlings of R223 following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
22	3.20	0.61	19.06
	3.10	0.69	22.26
	3.38	0.54	15.98
23	4.46	0.88	19.73
	4.33	0.94	21.71
24	3.29	0.62	18.84
	3.57	0.84	23.53
	4.51	0.92	20.40

Treatment total for 8 monoploids = 161.51
Mean percent infection = 20.18

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 11. Percent infection on heterozygous diploid (*Ht ht*) seedlings of R223 x W153R following inoculation with *H. turcicum*.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
25	4.30	0.96	22.33
	5.78	1.57	27.16
	4.68	1.16	24.79
	4.62	1.08	23.38
26	3.43	1.02	29.74
	4.18	1.18	28.23
	5.75	1.51	26.26
	5.91	1.26	21.32
27	6.52	1.55	23.77
	4.56	1.34	29.39
	3.34	0.64	19.16
	5.81	1.61	27.71
28	4.87	1.50	30.80
	4.65	0.93	20.00
	3.19	0.58	18.18
	5.73	1.19	20.77
29	6.48	1.37	21.14
	5.41	1.26	23.29
	5.32	1.12	21.05
	6.26	0.79	12.62
30	4.14	0.82	19.81
	3.03	0.57	18.81
	4.02	0.85	21.14
	4.01	0.70	17.46
31	4.85	1.33	27.42
	4.16	0.81	19.47
	5.68	1.78	31.34
	4.58	1.23	26.86
32	4.37	1.01	23.11
	4.13	1.01	24.46
	4.58	1.35	29.48
	4.24	0.84	19.81
33	6.99	1.51	21.60
	3.90	1.00	25.64
	4.70	1.17	24.89
	4.71	1.07	22.72

Table 11. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
34	5.10	0.93	18.24
	6.30	1.39	22.06
	4.28	0.81	18.93
	6.13	1.31	21.37

Treatment total for 40 heterozygotes = 925.71
Mean percent infection = 23.14

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* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 12. Percent infection on homozygous diploid (*Ht Ht*) seedlings of R223 following inoculation with *H. turcicum*.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
1	4.09	0.79	19.32
	4.22	0.87	20.62
	4.38	0.81	18.49
	3.63	0.60	16.53
2	3.46	0.78	22.54
	4.59	1.31	28.54
	3.44	0.93	27.03
	4.34	0.60	13.82
3	2.75	0.45	16.36
	2.93	0.64	21.84
	4.48	1.08	24.11
	4.31	0.73	16.94
4	3.86	0.70	18.13
	3.89	0.65	16.71
	2.14	0.42	19.63
	4.67	1.00	21.41
5	4.97	0.99	19.92
	4.87	1.03	21.15
	3.18	0.62	19.50
	3.46	0.69	19.94
6	2.01	0.31	15.42
	2.05	0.36	17.56
	5.39	0.88	16.33
	4.64	0.72	15.52
7	4.17	0.93	22.30
	3.73	0.86	23.06
	4.88	0.67	13.73
	4.46	0.82	18.39
8	3.33	0.66	19.82
	4.99	1.09	21.84
	3.27	0.89	27.22
	4.10	0.95	23.17
9	4.30	1.05	24.42
	3.88	0.80	20.62
	4.27	0.79	18.50

Table 12. Continued.

Pot No.	Leaf Area *	Infected Area**	Percent Infection
10	3.27	0.53	16.21
	3.49	0.52	14.90
	3.27	0.83	25.38

Treatment total for 38 diploids = 756.92
Mean percent infection = 19.92

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* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 13. Percent infection on triploid (Ht Ht Ht) seedlings of R223 following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
35	3.12	0.22	7.05
	3.31	0.41	12.39
	3.37	0.61	18.10
36	3.52	0.23	6.53
	4.72	0.38	8.05
37	2.98	0.27	9.06
38	3.64	0.36	9.89
	3.65	0.30	8.22
	3.18	0.72	22.64
39	3.47	0.42	12.10
	4.23	0.43	10.17
	4.82	0.59	12.24
	3.31	0.55	16.62
40	2.39	0.25	10.46
	4.98	0.62	12.45
41	3.28	0.54	16.46
	3.18	0.49	15.41

Treatment total for 17 triploids = 207.84
Mean percent infection = 12.22

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 14. Percent infection on tetraploid (Ht Ht Ht Ht) seedlings of R223 following inoculation with H. turcicum.

Pot No.	Leaf Area *	Infected Area**	Percent Infection
11	5.01	0.40	7.98
	5.23	0.35	6.69
	6.67	0.83	12.44
	5.29	0.78	14.74
12	5.86	0.82	13.99
	5.52	0.90	16.30
	4.12	0.71	17.23
	5.34	0.49	9.18
13	5.25	0.55	10.48
	5.35	0.54	10.09
	4.88	0.51	10.45
	5.13	0.70	13.65
14	6.07	0.76	12.52
	6.17	0.74	11.99
	4.99	0.56	11.22
	4.81	0.76	15.80
15	3.92	0.58	14.80
	3.91	0.73	18.67
	5.60	1.07	19.11
	5.70	0.53	9.30
16	5.32	0.54	10.15
	3.83	0.32	8.36
	4.81	0.37	7.69
	4.47	0.53	11.86
17	5.03	0.32	6.36
	5.11	0.49	9.59
	6.61	0.85	12.86
18	5.34	0.71	13.29
	5.38	0.89	16.54
	5.59	0.51	9.12
	5.49	0.66	12.02
19	6.53	0.74	11.33
	4.67	0.64	10.06
	4.59	0.43	9.37

Table 14. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
20	6.38	0.48	7.52
	5.84	0.64	10.96
	5.82	0.57	9.79
	4.96	0.58	11.69
21	6.09	0.74	12.15
	5.23	0.44	8.41

Treatment total for 40 tetraploids = 465.75
Mean percent infection = 11.64

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* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 15. Analysis of variance, Satterthwaite's "F", and multiple range analysis of mean percent infections for experiment 3.

Source of Variation	d.f.	M.S.	F
Dosage	4	849.23	46.36**
Among Pots	40		
Among Pots/Dosage	36	18.32	
Among Plants/Pots	102		
Total	142		

** = Significant at 0.01 level.

Satterthwaite's "F" = 48.14**

Multiple Range Analysis

Genotype =	Ht ht	Ht	Ht Ht	Ht Ht Ht	Ht Ht Ht Ht
Mean =	23.14	20.18	19.92	12.22	11.64

(Means not underscored by the same line are significantly different).

The monoploids and homozygous diploids showed about the same level of resistance. The triploids and tetraploids also showed the same level of resistance. The triploid and tetraploid seedlings were significantly more resistant than the monoploid and homozygous diploid seedlings. The heterozygous diploid seedlings were the least resistant, although only significantly less resistant than the triploids and the tetraploids.

The data for experiment 4, shown in tables 16-21, compare percent infection of the five dosage levels of the Ht allele from monoploid through tetraploid in R223. These were planted October 21, 1966, inoculated November 13, 1966, and evaluated November 23, 1966.

Table 16. Percent infection on monoploid (Ht) seedlings of R223 following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
10	4.38	0.57	13.01
	5.13	0.88	17.15
	6.61	1.40	21.18
	6.45	0.97	15.04
11	4.17	0.58	13.91
	5.67	0.68	11.99
	5.01	0.47	9.38
12	6.42	1.05	16.36
	5.31	0.77	14.50
13	6.79	1.04	15.32
	5.54	1.07	19.31
	6.46	1.09	16.87
	6.43	1.11	17.26

Treatment total for 13 monoploids = 201.28
Mean percent infection = 15.48

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 17. Percent infection on heterozygous diploid (Ht ht) seedlings of R223 x W153R following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
4	6.52	1.44	22.09
	6.61	1.27	19.21
5	6.38	1.04	16.30
	5.12	0.89	17.38
	5.05	0.66	13.07
6	6.38	1.40	21.94
	6.46	1.76	27.24
	5.08	0.98	19.29
	6.58	1.53	23.25
7	4.84	0.88	18.18
	4.91	0.79	16.09
	6.19	1.19	19.22
	3.45	0.74	21.45
8	5.60	1.51	26.96
	4.36	0.83	19.04
	3.08	0.66	21.43
9	5.42	0.99	18.27
	6.96	1.54	22.13
	5.82	0.94	16.15

Treatment total for 19 heterozygotes = 378.69
Mean percent infection = 19.93

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 18. Percent infection on homozygous diploid (*Ht Ht*) seedlings of R223 following inoculation with *H. turcicum*.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
14	3.08	0.44	14.29
	4.40	0.82	18.64
	4.12	0.68	16.50
	4.61	0.58	12.58
15	4.39	0.85	19.36
	5.83	0.78	13.38
	4.96	0.86	17.34
16	5.75	1.00	17.39
	5.27	0.91	17.27
	5.27	0.97	18.41
	3.28	0.41	12.50
17	4.56	0.98	21.49
	3.74	0.35	9.36
	4.92	0.81	16.46
18	3.31	0.45	13.60
	3.53	0.80	22.66
	5.72	0.88	15.38
	4.80	0.74	15.42

Treatment total for 18 diploids = 292.03
Mean percent infection = 16.22

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 19. Percent infection on triploid (Ht Ht Ht) seedlings of R223 following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
1	6.10	0.56	9.18
	4.79	0.50	10.44
	4.25	0.41	9.65
2	5.33	0.69	12.95
	4.68	0.41	8.76
3	4.85	0.49	10.10
	4.47	0.39	8.72
		Treatment total for 7 triploids =	69.80
		Mean percent infection =	9.97

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 20. Percent infection on tetraploid (*Ht Ht Ht Ht*) seedlings of R223 following inoculation with *H. turcicum*.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
19	5.35	0.77	14.39
	5.95	0.48	8.07
	4.65	0.39	8.39
20	4.44	0.27	6.08
	4.26	0.18	4.23
	4.28	0.41	9.58
	3.50	0.41	11.71
21	5.00	0.63	12.60
	3.80	0.28	7.37
	4.61	0.33	7.16
	4.91	0.47	9.57
22	4.32	0.52	12.04
	5.87	0.60	10.22
	4.84	0.51	10.54
		Treatment total for 14 tetraploids =	131.95
		Mean percent infection =	9.43

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 21. Analysis of variance, Satterthwaite's "F", and multiple range analysis of mean percent infections for experiment 4.

Source of Variation	d.f.	M.S.	F
Dosage	4	275.76	22.73**
Among Pots	21		
Among Pots/Dosage	17	12.13	
Among Plants/Pots	49		
Total	70		

** = Significant at 0.01 level.

Satterthwaite's "F" = 22.62**

Multiple Range Analysis

Genotype =	Ht ht	Ht Ht	Ht	Ht Ht Ht	Ht Ht Ht Ht
Mean =	19.93	16.22	15.48	9.97	9.43

(Means not underscored by the same line are significantly different).

These results were the same as those for experiment 3. The monoploids and homozygous diploids showed about the same level of resistance. The triploids and tetraploids also showed about the same level of resistance. Again, the triploids and tetraploids were significantly more resistant than the monoploids and homozygous diploids. The heterozygotes were less resistant than seedlings from the other dosage levels, although only significantly less resistant than the triploids and tetraploids.

The data for experiment 5, shown in tables 22-27, compare percent infection of the five dosage levels of the Ht allele in line 65-225-1, which is isogenic with the susceptible variety, W153R. These were planted February 15, 1968, inoculated March 5, 1968, and evaluated March 15 and 16, 1968.

Table 22. Percent infection on monoploid (Ht) seedlings of 65-225-1 following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
54	5.10	0.41	8.04
	5.81	0.71	12.22
	6.21	0.57	9.18
	5.21	0.77	14.78
	5.73	0.63	10.99
55	2.57	0.16	6.23
	4.99	0.37	7.41
	5.01	0.18	3.59
	2.35	0.30	12.77
	4.88	0.64	13.11
56	6.32	0.74	11.71
	5.42	0.78	14.39
	4.21	0.46	10.93
	2.73	0.27	9.89
	6.16	1.15	18.67
57	5.96	0.46	7.72
	3.24	0.27	8.33
	4.43	0.36	8.13
	4.48	0.28	6.25
58	6.51	0.84	12.90
	5.92	0.71	11.99
	2.35	0.36	15.32
	4.83	0.62	12.84
	4.81	0.46	9.56
59	4.74	0.50	10.55
	3.24	0.32	9.88
	2.13	0.13	6.10
	5.47	0.87	15.90
60	5.37	0.68	12.66
	6.19	0.80	12.92
	5.90	0.67	11.36
	5.02	0.71	14.14
61	3.20	0.18	5.63
	5.11	0.48	9.39
	5.75	0.51	8.87

Table 22. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
62	4.72	0.62	13.14
	5.33	0.79	14.82
	5.84	0.74	12.67
	6.26	0.77	12.30
	6.59	0.93	14.11
63	5.48	0.74	13.50
	4.45	0.46	10.34
	4.32	0.46	10.68
64	5.77	0.64	11.09
	3.93	0.41	10.43
	6.88	0.92	13.37
	6.58	0.64	9.73
65	4.05	0.47	11.60
	3.62	0.39	10.77
	2.33	0.24	10.30
	2.22	0.27	12.16
66	3.58	0.67	18.72
	4.14	0.58	14.01
	2.41	0.25	10.37
67	4.37	0.43	9.84
	4.85	0.36	7.42
	5.93	0.45	7.59
	6.72	0.72	10.71
	2.21	0.27	12.22
68	4.89	0.60	12.27
	3.67	0.46	12.53
	3.57	0.47	13.17
	3.15	0.40	12.70
	3.70	0.39	10.54
69	6.02	0.49	8.14
	5.18	0.48	9.27
	3.36	0.39	11.61
70	3.78	0.49	12.96
	4.25	0.44	10.35
	4.55	0.39	8.57
	5.45	0.72	13.21

Table 22. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
71	6.39	0.94	14.71
	4.62	0.42	9.09
	5.11	0.35	6.85
	2.93	0.18	6.14
72	2.87	0.22	7.67
	2.81	0.34	12.10
73	5.25	0.64	12.19
	4.08	0.18	4.41
	6.42	0.79	12.31
	4.54	0.51	11.23
	3.63	0.40	11.02
74	2.76	0.35	12.68
	2.79	0.33	11.83
75	4.19	0.41	9.79
	4.69	0.37	7.89
	3.40	0.25	7.35

Treatment total for 87 monoploids = 948.82
Mean percent infection = 10.91

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 23. Percent infection on heterozygous diploid (Ht ht) seedlings of 65-225-1 x W153R following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
101	6.39	1.15	18.00
	5.31	0.99	18.64
	4.21	0.68	16.15
102	4.82	0.61	12.66
	4.13	0.59	14.29
	4.68	0.62	13.25
	3.78	0.49	12.96
103	3.95	0.38	9.62
	5.84	0.70	11.99
	4.29	0.70	16.32
	3.11	0.23	7.40
	3.42	0.57	16.67
104	2.18	0.38	17.43
	5.91	0.54	9.43
	4.02	0.64	15.92
	4.02	0.63	15.67
	6.35	0.52	8.19
105	3.75	0.25	6.67
	3.65	0.69	18.90
	3.23	0.56	17.34
	5.58	0.39	6.99
	2.32	0.42	18.10
30	4.81	0.88	18.30
	3.87	0.20	5.17
	3.89	1.04	26.74
31	4.22	0.80	18.96
	3.61	0.54	14.96
	3.18	0.14	4.40
	2.58	0.42	16.28
32	5.01	0.82	16.37
	4.33	0.87	20.09
	3.38	0.66	19.53
	6.05	1.06	17.52
33	3.14	0.33	10.51
	3.33	0.34	10.21
	3.98	0.47	11.81
	5.92	0.58	9.80
	6.21	1.15	18.52

Table 23. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
34	4.12	0.71	17.23
	3.73	0.64	17.16
	3.70	0.30	8.11
	5.25	0.81	15.43
	6.66	1.00	15.02
35	3.80	0.48	12.63
	4.21	0.21	4.99
	3.31	0.49	14.80
	4.93	0.73	14.81
	4.98	0.61	12.25
36	5.20	0.84	16.15
	4.72	0.25	5.30
	4.37	0.95	21.74
	4.29	0.68	15.85
37	4.63	0.76	16.41
	3.28	0.57	17.38
	3.35	0.36	10.75
	3.91	0.26	6.39
	3.32	0.46	13.86
38	3.18	0.42	13.21
	3.26	0.46	14.11
	4.71	0.37	7.86
	3.17	0.41	12.93
39	3.89	0.49	12.60
	6.08	1.09	17.93
	5.28	0.85	16.10
40	3.31	0.18	5.44
	3.52	0.43	12.22
	5.12	0.64	12.50
	3.22	0.31	9.63
	4.61	0.47	10.20
41	4.85	0.54	11.13
42	4.85	0.57	11.75
	3.73	0.63	16.89
	3.95	0.32	8.10
43	5.36	0.82	15.30
	3.81	0.54	14.17
	3.49	0.62	17.77
	5.23	0.93	17.78
	4.78	0.94	19.67

Table 23. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
44	3.98	0.78	19.60
	2.62	0.25	9.54
45	3.61	0.48	13.30
	3.17	0.49	15.46
	5.39	0.77	14.29
	4.83	0.37	7.66
46	3.81	0.41	10.76
47	5.15	0.62	12.04
	4.23	0.47	11.11
	4.33	0.29	6.70
	3.48	0.58	16.67
	3.42	0.58	16.96
48	3.34	0.54	16.17
	5.64	0.43	7.62
	4.95	0.72	14.55
49	3.75	0.46	12.27
	4.70	0.48	10.21
	3.15	0.58	18.41
	4.31	0.24	5.57
	5.81	0.94	16.18
50	5.87	1.13	19.25
	4.49	0.81	18.04
	3.20	0.24	7.50
	3.22	0.39	12.11
	3.25	0.43	13.23
51	4.18	0.58	13.88
	3.07	0.42	13.68
	3.11	0.32	10.29
52	5.22	0.27	5.17
	6.98	0.70	10.03
	2.99	0.35	11.71
	2.36	0.28	11.86
53	4.86	0.72	14.81
	3.52	0.32	9.09
	3.31	0.63	19.03

Treatment total for 113 heterozygotes = 1519.77
Mean percent infection = 13.45

* = Total measured area of the leaf (square inches).
** = Total measured area of lesions on the leaf (square inches).

Table 24. Percent infection on homozygous diploid (*Ht Ht*) seedlings of 65-225-1 following inoculation with *H. turcicum*.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
106	4.91	0.45	9.16
	3.31	0.27	8.16
	3.73	0.46	12.33
	4.25	0.61	14.35
	5.39	0.58	10.76
107	4.06	0.45	11.08
	4.62	0.54	11.69
	3.52	0.28	7.95
	4.34	0.31	7.14
	4.73	0.43	9.09
108	2.98	0.37	12.42
	3.18	0.46	14.47
	4.27	0.46	10.77
	4.81	0.46	9.56
	3.87	0.29	7.49
109	5.52	0.42	7.61
	5.38	0.59	10.97
	4.45	0.54	12.13
	4.03	0.53	13.15
	4.27	0.43	10.07
110	3.79	0.33	8.71
	3.98	0.32	8.04
	4.92	0.61	12.40
	4.11	0.44	10.71
	3.33	0.39	11.71
111	5.85	0.77	13.16
	3.55	0.29	8.17
	4.56	0.34	7.46
	4.41	0.55	12.47
	5.67	0.54	9.52
112	4.69	0.35	7.46
	3.73	0.19	5.09
	3.20	0.46	14.38
	2.70	0.28	10.37
	3.21	0.39	12.15
113	3.70	0.86	23.24
	4.00	0.55	13.75
	2.04	0.26	12.75
	4.33	0.47	10.85
	4.40	0.36	8.18

Table 24. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
114	3.52	0.36	10.23
	3.57	0.42	11.76
	3.19	0.35	10.97
115	4.98	0.56	11.24
	2.64	0.31	11.74
	4.23	0.64	15.13
	4.32	0.45	10.42
	4.82	0.62	12.86
116	4.82	0.53	10.99
	5.27	0.70	13.28
	4.49	0.41	9.13
	4.41	0.36	8.16
	2.51	0.36	14.34
117	4.92	0.95	19.31
	5.93	1.02	17.20
	5.74	0.29	5.05
	5.89	0.28	4.75
118	4.67	0.38	8.14
	3.23	0.64	19.81
	3.98	0.33	8.29
	2.37	0.30	12.66
	5.32	0.55	10.34
119	6.41	0.98	15.29
	4.55	0.46	10.11
	2.65	0.39	14.72
	2.83	0.45	15.90
	3.36	0.40	11.90
120	5.82	0.74	12.71
	4.47	0.49	10.96
121	4.96	0.40	8.06
	4.63	0.47	10.15
	5.12	0.61	11.91
	4.20	0.47	11.19
	5.58	0.63	11.29
122	4.12	0.48	11.65
	5.77	0.87	15.08
	3.99	0.42	10.53
	3.28	0.43	13.11
	3.32	0.37	11.14

Table 24. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
123	4.86	0.64	13.17
	4.44	0.41	9.23
	3.42	0.28	8.19
	4.32	0.61	14.12
124	3.70	0.71	19.19
	4.91	0.84	17.11
	2.92	0.15	5.14
	2.92	0.14	4.79
125	4.25	0.35	8.24
	3.37	0.67	19.88
	3.66	0.30	8.20
126	4.17	0.52	12.47
	4.73	0.49	10.36
	4.24	0.65	15.33
	4.82	0.49	10.17
	3.52	0.52	14.77
127	3.51	0.55	15.67
	2.38	0.28	11.76
	3.29	0.33	10.03
128	3.30	0.51	15.45
	3.51	0.53	15.20
	3.42	0.55	16.08
129	4.52	0.63	13.94
	3.01	0.38	12.62
	3.62	0.45	12.43
	5.72	0.72	12.59

Treatment total for 105 diploids = 1218.55
Mean percent infection = 11.61

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 25. Percent infection on triploid (Ht Ht Ht) seedlings of 65-225-1 following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
1	5.98	0.42	7.02
	3.37	0.23	6.82
	4.48	0.17	3.79
2	4.48	0.19	4.24
	3.87	0.32	8.27
	3.29	0.30	9.12
3	3.29	0.29	8.81
	4.67	0.34	7.28
	5.68	0.56	9.86
	4.77	0.37	7.76
4	3.18	0.27	8.49
	3.47	0.25	7.20
	4.49	0.56	12.47
5	2.30	0.29	12.61
	4.82	0.52	10.79
	6.84	0.69	10.09
	6.27	0.75	11.96
6	2.77	0.09	3.25
	3.76	0.48	12.77
	3.96	0.25	6.31
	5.57	0.32	5.75
	4.33	0.38	8.78
7	3.81	0.17	4.46
	5.26	0.37	7.03
	4.35	0.39	8.97
8	4.82	0.16	3.32
	3.71	0.12	3.23
9	3.93	0.21	5.34
	3.82	0.17	4.45
	3.36	0.24	7.14
	3.33	0.25	7.51
10	4.68	0.38	8.12
	3.28	0.27	8.23
	4.24	0.54	12.74
	4.10	0.53	12.93

Table 25. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
11	5.71	0.52	9.11
	5.98	0.63	10.54
	3.23	0.35	10.84
	5.29	0.53	12.93
	5.12	0.42	8.20
12	3.09	0.24	7.77
	3.01	0.27	8.97
13	4.21	0.30	7.13
	4.26	0.26	6.10
	3.33	0.14	4.20
	4.88	0.21	4.30
14	3.45	0.13	3.77
	6.43	0.22	3.42
	3.55	0.06	1.69
	3.54	0.20	5.65
	4.08	0.24	5.88
15	3.72	0.45	12.10
	4.77	0.59	12.37
	4.79	0.52	10.86
16	2.83	0.32	11.31
	2.21	0.26	11.76
	3.12	0.22	7.05
17	4.72	0.46	9.75
	3.35	0.27	8.06
	4.90	0.45	9.18
18	3.90	0.27	6.92
	4.41	0.23	5.22
	4.63	0.22	4.75
	3.18	0.13	4.09
19	4.52	0.32	7.08
	5.80	0.38	6.55
	5.21	0.42	8.06
	4.25	0.33	7.76
	4.35	0.34	7.82
20	3.87	0.31	8.01
	3.57	0.22	6.16
	6.76	0.49	7.25
	4.96	0.37	7.46

Table 25. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
21	3.12	0.17	5.45
	3.20	0.18	5.63
	5.21	0.25	4.80
	4.26	0.18	4.23
22	3.38	0.09	2.66
	3.28	0.41	12.50
	4.41	0.56	12.70
	4.33	0.52	12.01
23	5.90	0.67	11.36
	4.74	0.80	16.88
	3.62	0.33	9.12
	3.86	0.30	7.77
24	4.28	0.93	21.73
	4.73	0.19	4.02
	3.19	0.14	4.39
25	4.82	0.16	3.32
	3.43	0.30	8.75
	4.85	0.34	7.01
26	4.16	0.30	7.21
	3.22	0.19	5.90
	5.00	0.32	6.40
	5.88	0.59	10.03
27	4.22	0.43	10.19
	4.13	0.39	9.44
	6.54	0.84	12.84
	3.46	0.29	8.38
	3.92	0.30	7.65
28	2.35	0.12	5.11
	6.78	0.87	12.83
	2.38	0.16	6.72
	3.49	0.43	12.32
29	4.35	0.34	7.82
	4.36	0.35	8.03
	5.40	0.47	8.70

Treatment total for 107 triploids = 857.97
Mean percent infection = 8.01

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 26. Percent infection on tetraploid (Ht Ht Ht Ht) seedlings of 65-225-1 following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
76	3.02	0.15	4.97
	3.13	0.22	7.03
	6.00	0.75	12.50
	5.82	0.43	7.39
	4.54	0.31	6.83
77	4.70	0.32	6.81
	4.91	0.26	5.30
	3.19	0.15	4.70
	5.33	0.51	9.57
	4.68	0.35	7.48
78	3.26	0.21	6.44
	4.37	0.36	8.24
	2.87	0.25	8.71
	3.43	0.10	2.92
	5.34	0.30	5.62
79	3.34	0.14	4.19
	5.73	0.69	12.04
	2.93	0.33	11.26
	6.76	0.87	12.87
	2.36	0.25	10.59
80	3.68	0.41	11.14
	3.61	0.44	12.19
	5.27	0.46	8.73
	4.49	0.22	4.90
	4.34	0.30	6.91
81	3.52	0.45	12.78
	3.06	0.29	9.48
	3.18	0.23	7.23
	4.38	0.32	7.31
	4.02	0.27	6.72
82	4.81	0.49	10.19
	5.04	0.52	10.32
	4.25	0.42	9.88
	4.33	0.49	11.32
	3.68	0.48	13.04

Table 26. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
83	5.16	0.32	6.20
	4.25	0.29	6.82
	4.83	0.37	7.66
	3.88	0.34	8.76
	5.54	0.27	4.87
84	5.03	0.28	5.57
	3.08	0.17	5.52
	3.18	0.14	4.40
	2.71	0.14	5.17
85	2.32	0.13	5.60
	3.21	0.23	8.19
	2.61	0.17	8.23
	2.66	0.19	7.14
86	5.33	0.36	6.75
	2.81	0.23	8.19
	6.32	0.52	8.23
	4.36	0.33	7.57
	3.48	0.32	9.20
87	6.11	0.60	9.82
	6.33	0.51	8.06
	2.38	0.20	8.40
	5.88	0.23	3.91
	3.52	0.19	5.40
88	2.47	0.09	3.64
	5.47	0.21	3.84
	4.31	0.44	10.21
89	3.38	0.26	7.69
	3.83	0.24	6.27
	3.24	0.39	12.04
	3.24	0.35	10.80
	3.15	0.54	17.14
90	5.03	0.80	15.90
	3.14	0.30	9.55
	4.87	0.47	9.65
	4.87	0.42	8.62
91	3.79	0.34	8.97
	5.36	0.44	8.21
	3.51	0.33	9.40
	5.52	0.51	9.24
	5.08	0.60	11.81

Table 26. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
92	4.26	0.48	11.27
	2.31	0.23	9.96
	2.62	0.26	9.92
	2.92	0.23	7.88
	3.73	0.46	12.33
93	3.18	0.41	12.89
	2.85	0.16	5.61
	5.25	0.28	5.33
	4.13	0.26	6.30
	3.63	0.26	7.16
94	3.82	0.35	9.16
	4.31	0.37	8.58
	5.27	0.68	12.90
	4.97	0.50	10.06
	3.99	0.51	12.78
95	2.88	0.29	10.07
	2.48	0.22	8.87
	2.38	0.17	7.14
96	3.25	0.18	5.54
	3.18	0.19	5.97
	3.04	0.26	8.55
	4.67	0.57	12.21
	4.69	0.43	9.17
97	2.23	0.27	12.11
	3.87	0.31	8.01
	5.39	0.58	10.76
	5.91	0.52	8.80
98	5.47	0.34	6.22
	3.28	0.33	10.06
	2.04	0.25	12.25
	5.12	0.43	8.40
99	4.87	0.39	8.01
	3.07	0.18	5.86
	5.21	0.36	6.91
	4.11	0.28	6.81
	4.33	0.32	7.39

Table 26. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
100	3.78	0.36	9.52
	3.98	0.18	4.52
	3.05	0.16	5.25
	3.58	0.12	3.35
	5.67	0.48	8.47
		Treatment total for 116 tetraploids =	969.85
		Mean percent infection =	8.36

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 27. Analysis of variance, Satterthwaite's "F", and multiple range analysis of mean percent infections for experiment 5.

Source of Variation	d.f.	M.S.	F
Dosage	4	578.43	40.76**
Among Pots	128		
Among Pots/Dosage	124	14.19	
Among Plants/Pots	399		
Total	527		

** = Significant at 0.01 level.

Satterthwaite's "F" = 40.14**

Multiple Range Analysis

Genotype =	Ht ht	Ht Ht	Ht	Ht Ht Ht Ht	Ht Ht Ht
Mean =	13.45	11.61	10.91	8.36	8.01

(Means not underscored by the same line are significantly different).

The monoploids and homozygous diploids showed about the same level of resistance. The triploids and tetraploids also showed about the same level of resistance. The triploids and tetraploids were more resistant than the monoploids and homozygous diploids, although the difference was not statistically significant in this experiment. The trends of disease reaction were the same in 65-225-1 as they were in R223. The heterozygotes were the least resistant as in all previous experiments. The highly significant value of "F" reflects the extreme difference between the triploids and heterozygous diploids.

Figure 11 shows seedling leaves of R223 on the five dosage levels following inoculation with H. turcicum. Note that lesions do coalesce to some degree on the heterozygous diploids, but not on the others.

Figure 11. Infected seedling leaves of the five Ht dosage levels of R223 following inoculation with H. turcicum. Shown from left to right: monoploid, diploid, triploid, tetraploid, heterozygous diploid.

Dosage studies of the ht allele.

The information on dosage of the ht allele was obtained from the susceptible line, W153R.

The data for experiment 6, shown in tables 28-30, compare percent infection of monoploids and diploids of W153R. These were planted July 19, 1966, inoculated August 5, 1966, and evaluated August 12, 1966.

Table 28. Percent infection on monoploid (ht) seedlings of W153R following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
1	4.51	0.97	21.51
	3.24	0.80	24.69
	2.48	0.92	37.10
	4.81	0.93	19.33
2	4.00	2.45	61.25
	2.73	0.73	26.74
	5.02	1.77	35.26
3	3.11	1.22	39.23
	2.49	0.86	34.54
	4.66	0.91	19.53
	3.18	0.79	24.84

Treatment total for 11 monoploids = 344.02
Mean percent infection = 31.27

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 29. Percent infection on diploid (ht ht) seedlings of W153R following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
4	5.68	0.34	5.98
	6.09	1.86	30.54
	6.11	1.34	21.93
	4.27	0.46	10.77
5	3.88	0.83	21.39
	3.60	0.99	27.50
	3.11	0.37	11.90
6	4.66	0.49	10.52
	5.00	0.47	9.40
	5.29	0.27	5.10
	3.19	0.29	9.09
7	3.48	0.92	26.44
	2.99	0.66	22.07
	5.09	0.40	7.86
8	4.73	0.32	6.76
	4.38	0.51	11.64
	3.80	0.27	7.11
9	4.73	0.46	9.73
	4.67	0.41	8.78
	3.94	0.39	9.90
	4.18	0.71	16.99

Treatment total for 21 diploids = 291.40
Mean percent infection = 13.88

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 30. Analysis of variance and Satterthwaite's "F" for mean percent infections for experiment 6.

Source of Variation	d.f.	M.S.	F
Dosage	1	2185.13	29.98**
Among Pots	8		
Among Pots/Dosage	7	104.13	
Among Plants/Pots	23		
Total	31		

** = Significant at 0.01 level.

Satterthwaite's "F" = 31.07**

N.B. Duncan's multiple range analysis was not performed on these data because the experiment involved only two treatments.

The monoploid seedlings were significantly more susceptible than the diploids. Most of the monoploids did not survive after infection.

The data for experiment 7, shown in tables 31-34, compare percent infection of monoploids, diploids, and tetraploids of W153R. These were planted March 8, 1967, inoculated March 28, 1967, and evaluated April 4, 1967.

Table 31. Percent infection on monoploid (ht) seedlings of W153R following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
1	5.56	1.75	31.47
	3.97	1.29	32.49
	3.15	1.09	34.60
2	3.21	0.84	26.17
	5.83	1.84	31.56
	4.64	0.89	19.18
3	6.41	1.47	22.93
	5.38	1.04	19.33
4	4.75	1.46	30.74
	4.24	0.87	20.52
	3.53	1.07	30.31
	3.16	0.85	26.90
5	4.42	0.94	21.27
	3.01	0.87	28.90
		Treatment total for 14 monoploids =	376.37
		Mean percent infection =	26.88

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 32. Percent infection on diploid (*ht ht*) seedlings of W153R following inoculation with *H. turcicum*.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
11	3.13	0.35	11.18
	4.81	0.54	11.22
	3.62	0.78	21.55
	5.33	1.72	32.27
12	3.08	0.96	31.17
	6.44	2.18	33.85
	5.95	1.12	18.82
	5.72	0.93	16.26
13	4.21	0.61	14.49
	3.56	0.51	14.33
	3.59	0.60	16.71
	4.37	0.78	17.85
14	5.82	0.77	13.23
	5.78	1.23	21.28
	4.23	1.31	30.97
	4.94	0.58	11.74
15	3.46	0.95	27.46
	4.06	0.88	21.67
	3.06	0.61	19.93
Treatment total for 19 diploids =			385.98
Mean percent infection =			20.31

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 33. Percent infection on tetraploid (ht ht ht ht) seedlings of W153R following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
6	3.86	0.71	18.39
	5.22	0.88	16.86
	5.12	1.14	22.27
7	4.61	0.99	21.48
	3.54	0.36	10.17
8	3.33	0.92	27.63
	3.96	0.77	19.44
9	4.48	1.62	36.16
	3.25	0.42	12.92
10	5.21	1.13	21.69
	3.10	0.62	20.00
		Treatment total for 11 tetraploids =	227.01
		Mean percent infection =	20.64

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 34. Analysis of variance, Satterthwaite's "F", and multiple range analysis of mean percent infections for experiment 7.

Source of Variation	d.f.	M.S.	F
Dosage	2	198.95	4.19*
Among Pots	14		
Among Pots/Dosage	12	40.47	
Among Plants/Pots	29		
Total	43		

* = Significant at 0.05 level.

Satterthwaite's "F" = 4.97*

Multiple Range Analysis

Genotype =	ht	ht ht ht ht	ht ht
Mean =	26.88	20.64	20.31

(Means not underscored by the same line are significantly different).

The diploids and tetraploids showed about the same level of susceptibility. The monoploids were significantly more susceptible than either the diploids or tetraploids.

The data for experiment 8, shown in tables 35-39, compare percent infection of monoploids, diploids, triploids, and tetraploids of W153R. These were planted January 12, 1968, inoculated January 29, 1968, and evaluated February 4, 1968.

Table 35. Percent infection on monoploid (ht) seedlings of W153R following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
15	6.01	2.51	41.76
	6.12	1.90	31.05
	5.77	2.43	42.11
16	4.36	1.34	30.73
	4.88	1.80	36.89
17	3.22	1.09	33.85
	6.45	2.57	39.84
	5.53	2.52	45.57
	5.30	2.62	47.55
	Treatment total for 9 monoploids =		349.35
	Mean percent infection =		38.82

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 36. Percent infection on diploid (*ht ht*) seedlings of W153R following inoculation with *H. turcicum*.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
18	3.33	0.70	21.02
	3.81	0.89	23.36
	4.11	1.30	31.63
	3.42	0.76	22.22
19	3.58	1.00	27.93
	3.66	1.51	41.26
	4.29	0.98	22.84
	4.12	0.78	18.93
	3.71	0.65	17.52
20	5.75	2.22	38.61
	5.97	1.88	31.49
	4.83	1.16	24.02
	3.59	0.76	21.17
	3.44	1.11	32.27
21	2.34	0.47	20.09
	4.70	1.47	31.28
	4.72	1.45	30.72
22	5.27	0.82	15.56
23	3.64	0.91	25.00
	3.64	1.14	31.32
	3.63	1.13	31.13
24	5.30	1.16	21.89
	3.60	0.98	27.22
	4.91	1.35	27.49
	4.25	0.84	19.76
	5.14	0.87	16.93
25	6.63	1.77	26.70
	6.08	1.16	19.08
	4.06	0.86	21.18
	4.48	1.00	22.32
	3.31	0.64	19.34
26	4.29	0.53	12.35
	4.63	0.99	21.38
	6.74	2.73	40.50
27	4.97	1.91	38.43
	4.12	1.32	32.04
	6.81	1.43	21.00
	4.83	1.89	39.13

Table 36. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
28	3.59	1.14	31.75
	3.39	0.66	19.47
	5.29	0.88	16.64
29	5.18	1.06	20.46
	3.58	0.82	22.91
	4.59	0.82	17.86
30	4.30	0.77	17.91
	4.32	0.83	19.21
	4.22	1.14	27.01
	4.84	1.68	34.71
	4.94	0.74	14.98
31	4.75	1.05	22.11
	4.15	0.96	23.13

Treatment total for 51 diploids = 1274.26
Mean percent infection = 24.99

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 37. Percent infection on triploid (ht ht ht) seedlings of W153R following inoculation with H. turcicum.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
32	3.00	0.36	12.00
	3.38	1.05	31.07
	5.36	1.06	19.78
	5.37	1.23	22.91
33	6.57	0.97	14.76
	4.57	0.75	16.41
	4.98	1.13	22.69
	6.89	1.75	25.40
34	5.89	1.62	27.50
	5.69	2.25	39.54
	5.69	1.66	29.17
35	4.53	0.82	18.10
	3.71	0.88	23.72
	3.72	0.90	24.19
	2.62	0.94	35.88
	2.87	0.37	12.89
36	2.07	0.62	29.95
	3.05	0.45	14.75
	2.14	0.49	22.90
37	5.14	1.44	28.02
	5.23	1.26	24.09
	6.34	1.93	30.44
38	3.82	0.54	14.14
	2.52	0.58	23.02
	2.30	0.49	21.30
39	3.23	0.58	17.96
	4.11	0.94	22.87
	4.11	1.16	28.22
	6.59	1.78	27.01
40	5.46	0.91	16.67
	5.22	0.75	14.37
41	3.76	0.63	16.76
	3.33	0.48	14.41
	2.58	0.67	25.97
	2.47	0.48	19.43
	3.49	0.64	18.34
	Treatment total for 36 triploids = 806.63		
	Mean percent infection = 22.41		

* = Total measured area of the leaf (square inches).
** = Total measured area of lesions on the leaf (square inches).

Table 38. Percent infection on tetraploid (*ht ht ht ht*) seedlings of W153R following inoculation with *H. turcicum*.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
1	3.89	1.24	31.88
	4.16	1.32	31.73
	3.28	0.64	19.51
	5.98	0.78	13.04
2	2.49	0.69	27.71
	3.55	1.36	38.31
	2.30	0.35	15.22
	3.80	0.94	24.74
	2.41	0.81	33.61
3	5.13	1.10	21.44
	6.77	1.39	20.53
	2.72	0.60	22.06
	4.90	1.34	27.35
	3.25	0.75	23.08
4	2.64	0.68	25.76
	4.87	0.94	19.30
5	4.89	1.36	27.81
	3.33	0.38	11.41
	5.12	1.65	32.23
	5.51	1.85	33.58
	3.85	0.73	18.96
6	2.88	1.12	38.89
	5.86	1.63	27.82
	5.28	1.13	21.40
	6.12	1.42	23.20
	4.71	1.06	22.51
7	4.02	1.33	33.08
	3.01	0.56	18.60
	4.39	0.84	19.13
	2.44	0.31	12.70
8	5.70	1.16	20.35
	5.28	1.15	21.78
	5.36	1.79	33.40
	5.31	1.14	21.47
9	4.62	1.03	22.29
	3.50	0.99	28.29
	5.59	1.52	27.19
	5.20	0.73	14.04
	4.97	0.95	19.11

Table 38. Continued.

Pot No.	Leaf Area*	Infected Area**	Percent Infection
10	3.93	0.69	17.56
	2.84	0.64	22.54
	2.10	0.51	24.29
	2.31	0.81	35.06
	3.65	0.54	14.79
11	5.88	1.57	26.70
	6.82	1.45	21.26
	6.40	1.78	27.81
	4.11	0.77	18.73
	2.59	1.01	39.00
12	3.07	0.42	13.68
	3.37	1.06	31.45
	3.72	1.06	28.50
	4.28	0.95	22.20
	5.24	1.15	21.95
13	3.44	0.57	16.57
	3.34	0.98	29.34
	2.15	0.53	24.65
14	5.13	1.18	23.00
	5.91	1.12	18.95
	3.75	0.74	19.73
	3.69	0.77	20.87
	3.87	0.77	19.90

Treatment total for 62 tetraploids = 1483.04
Mean percent infection = 23.92

* = Total measured area of the leaf (square inches).

** = Total measured area of lesions on the leaf (square inches).

Table 39. Analysis of variance, Satterthwaite's "F", and multiple range analysis of mean percent infections for experiment 8.

Source of Variation	d.f.	M.S.	F
Dosage	3	674.69	15.14**
Among Pots	40		
Among Pots/Dosage	37	44.57	
Among Plants/Pots	117		
Total	157		

** = Significant at 0.01 level.

Satterthwaite's "F" = 15.14**

Multiple Range Analysis

Genotype =	ht	ht ht	ht ht ht ht	ht ht ht
Mean =	38.82	24.99	23.92	22.41

(Means not underscored by the same line are significantly different).

The diploids, triploids, and tetraploids all showed about the same level of susceptibility. The monoploids were significantly more susceptible than either the diploids, triploids, or tetraploids.

Figure 12 shows seedling leaves of W153R from the four dosage levels after inoculation with H. turcicum. The monoploid leaves are much more wilted than those of the diploids, triploids, or tetraploids.

Figure 12. Infected seedling leaves of W153R following inoculation with H. turcicum. Paired leaves from left to right: monoploids, diploids, triploids, and tetraploids.

Interpretation of the data.

It should be clarified at this point that resistant seedlings did not necessarily show less percent infection than susceptible seedlings. Since the two disease phenotypes are quite different from one another, each was expected to show a range of percent infection totally independent from the other.

It was originally hypothesized that a linear increase in resistance would occur with increasing doses of the Ht allele. This hypothesis was based upon the incomplete dominance of the allele. However, there was no difference in resistance between monoploids and homozygous diploids containing one and two doses, respectively, of Ht. There was also no difference in resistance between triploids and tetraploids containing three and four doses, respectively, of Ht. It was always apparent that three and four doses conferred a higher level of resistance than did one or two doses. Heterozygous diploids were always the least resistant of any of the Ht dosage levels.

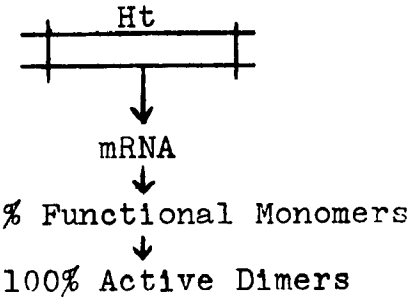
Diploids, triploids, and tetraploids containing two, three, and four doses, respectively, of the ht allele showed no difference in susceptibility. Monoploid (ht) seedlings were always significantly more susceptible than the other ht dosage levels. Many of the monoploid seedlings died after infection.

The data appear to conform to a model of protein interaction, which is based upon differences in gene product

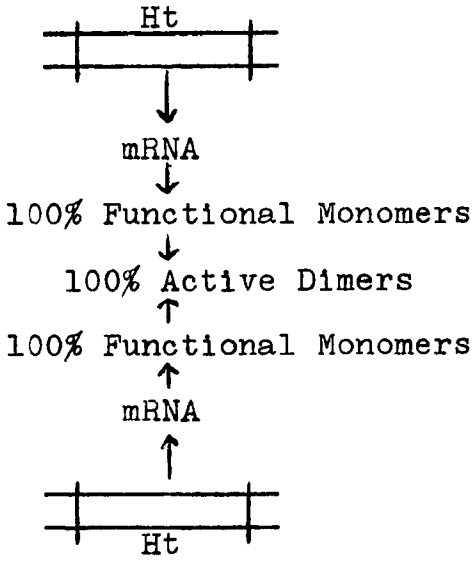
of the two alleles. It is well established that the product of a gene is a protein which is often enzymatic. The differences among the various Ht dosage levels can be reconciled if it is assumed that the product of the Ht locus is a polymeric protein, i.e., a protein composed of more than one polypeptide chain. The data fit the model best if it is assumed that this protein is actually a dimer. The product of the Ht Ht genotype would then be identical monomers which polymerize to form active dimers. The product of the Ht (monoploid) genotype would be the same as that of the diploid, except that quantitatively less product would be formed. It is assumed that the amount of gene product at the monoploid chromosome level would be sufficient to confer the diploid level of resistance.

The surprising difference between the heterozygous diploids and the monoploids can be explained by assuming that the recessive allele produces a defective protein monomer. The defective monomer can polymerize with a normal monomer, but the resulting hybrid dimer will be inactive. Figure 13 shows how the heterozygous diploid plants could be less resistant than the monoploids.

MONOPLOID



HOMOZYGOUS DIPLOID



HETEROZYGOUS DIPLOID

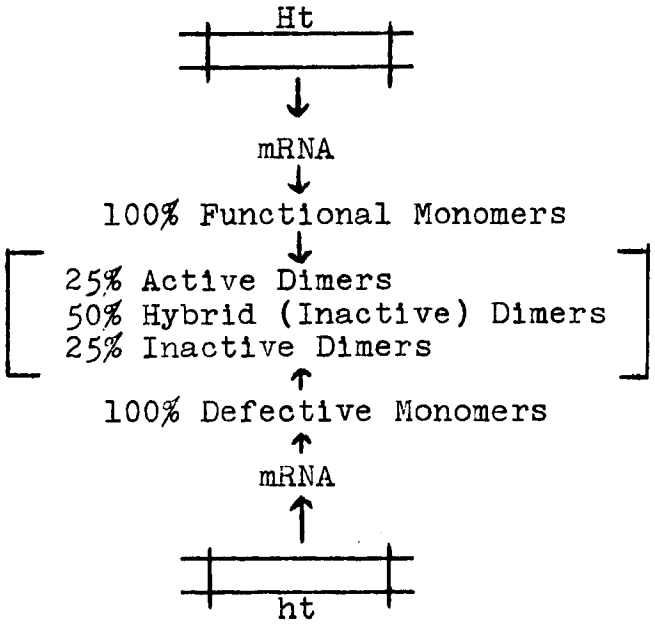


Figure 13. Production of protein dimers by monoploid, homozygous diploid, and heterozygous diploid resistant plants.

The monoploids and homozygous diploids produce all active dimers. The heterozygous diploids produce only 25 percent active dimers because of random association between defective and normal monomers. Thus the heterozygote can produce some active gene product, but not as much as the monoploids and homozygous diploids. Translating this into an expression of the phenotype, the heterozygotes will be less resistant than either the monoploids or homozygous diploids. It is pertinent to note that incomplete dominance of the Ht allele can be explained by using the same model.

Triploids and tetraploids produce more gene product at the Ht locus and at all other loci. It is assumed that the increase in "resistance product" is sufficient to confer a higher level of resistance on the two polyploids than on the monoploids or homozygous diploids. The increase in gene product at certain other loci, such as those which control increased vigor, thicker stalks and leaves, larger plant parts, and increase in protein content, might favorably augment the added "resistance product." It is assumed that the amount of gene product formed by a tetraploid would not be sufficient to make it more resistant than a triploid. The data suggest that an increase of two doses of the Ht allele might be required to give a substantial increase in disease resistance.

With regard to the recessive allele, monoploids were much more susceptible than diploids. At first it was thought that this difference might be due to hypomorphic gene action. However, the triploids and tetraploids were equally as suscep-

tible to the disease as were the diploids. Hypomorphism was thus ruled out as an explanation because additional doses of the recessive allele did not decrease susceptibility. The difference was then thought to be due to an interaction between deleterious recessive genes at other loci and susceptibility to the pathogen. Deleterious recessive genes are often masked in a diploid plant but are expressed in a monoploid. Susceptibility in a monoploid may then be augmented by deleterious recessives, resulting in increased susceptibility. Theoretically, all ht dosage levels produce qualitatively the same gene product, i.e., all defective monomers. On this basis there should be no difference in susceptibility between any of the ht dosage levels. However, the augmentation of susceptibility by the deleterious recessive genes appears to explain the one clear difference that was found.

Suggestions for the use of tetraploid (Ht Ht Ht Ht) plants.

Additional doses of certain endosperm genes, such as waxy (wx) or amylose extender (ae), offer possibilities for better kernel quality (15, 19, 25, 74). If corn were being grown in an area where incidence of northern corn leaf blight was high, tetraploid resistant plants would offer the double advantage of improved kernel quality as well as a high level of disease resistance. Such plants could also tolerate areas having high wind intensity because of the added thickness of the stalks.

A selection program would have to be undertaken to

compensate for the lower level of fertility in tetraploids. Considering the advantages of tetraploid (Ht Ht Ht Ht) corn, such a breeding program might be well worthwhile, especially in areas where H. turcicum is a problem.

Suggestions for future study.

It would be feasible to obtain trisomics for chromosome two in which the extra chromosome was carrying the Ht or ht allele. A test could be set up to compare zero, one, two, and three doses of the Ht allele in the following manner (the trisomic dose of the allele is indicated by parentheses):

ht ht (ht)

ht ht (Ht)

ht Ht (Ht)

Ht Ht (Ht)

Other combinations are possible which would elicit more information on the relationship between gene dosage and the incomplete dominance of the Ht allele. Some of the differences probably resulting from physiological or modifying factors at other loci might also be reduced.

The true effect of gene dosage can be studied only when a duplication is produced at the Ht locus itself by irradiation or other means. Such a duplication would require elegant cytological techniques both in induction and detection.

SECTION V

CONCLUSIONS

Dosage effects of the Ht allele.

Monoploid and homozygous diploid seedlings containing one and two doses, respectively, of Ht did not differ in their degree of resistance to northern corn leaf blight. There was also no difference in resistance between triploids and tetraploids containing three and four doses, respectively, of Ht. However, three and four doses of the Ht allele conferred a higher level of resistance on seedlings than did one or two doses. Heterozygous diploids (Ht ht) were always the least resistant to the disease.

Dosage effects of the ht allele.

Diploids, triploids, and tetraploids containing two, three, and four doses, respectively, of ht did not show any difference in susceptibility to the disease. Monoploid (ht) seedlings were much more susceptible to infection than were seedlings from the other three ht dosage levels.

SECTION VI

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Appendix table 1. Frequency of monoploidy in the resistant and susceptible lines used in this study.

Variety and Exp't. No.	No. Seeds	Purple Endosperm White Embryo	No. Without Purple Root	No. Diploid	No. Monoploid	Frequency per 1000
R223 (No. 2)	4816	34	16	0	16	3.3
R223 (No. 3)	4627	55	21	4	17	3.7
R223 (No. 4)	3149	45	14	2	12	3.8
W153R (No. 6)	4327	41	26	10	16	3.6
W153R (No. 7)	5270	161	16	5	11	2.1
W153R (No. 8)	40091	74	18	7	11	0.3
65-225-1 (No. 5)	82625	181	138	32	106	1.3

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