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BIOASSAY TESTS FROM LEAF DIFFUSATES OF HELMINTHOSPORIUM TURCICUM ON ZEA MAYS

ALFONSO G. CALUB

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BIOASSAY TESTS FROM LEAF DIFFUSATES OF *HELMINTHOSPORIUM TURCICUM* ON *ZEA MAYS*

by

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B.S., University of the Philippines, 1960
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A THESIS

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This thesis has been examined and approved.

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ABSTRACT

BIOASSAY TESTS FROM LEAF DIFFUSATES OF HELMINTHOSPORIUM TURCICUM ON ZEA MAYS

by

ALFONSO G. CALUB

The Ht gene induced the production of phytoalexin upon interaction of the host with the pathogenic organism Helminthosporium turcicum Pass.

The objectives of these studies were: 1) to determine the effect of some environmental and genetic factors on production of phytoalexin, 2) to determine the effect of different genetic background on production of phytoalexin by Ht lines, and 3) to discover whether phytoalexin or other inhibiting substances were produced in multigenic resistant plants and if so, to compare them with monogenic types.

Corn leaves for the bioassay tests were inoculated with spore drop suspension of H. turcicum in a completely saturated chamber at 21°C and light intensity of 300 foot-candles. The drop suspensions were then withdrawn and centrifuged. The supernatant, now free of spores and germ tubes, was termed the diffusate. Bioassay tests were conducted by mixing a drop of fresh spore suspension with the diffusate. Phytoalexin production was estimated by its effect on spore germination and germ tube length; diffusates which inhibited spore germination and delayed germ tube growth were assumed

to contain phytoalexin.

Homozygous resistant normal (HtHtBxBx) genotypes produced highly significant amounts of phytoalexin compared to resistant deficient (HtHtbxbx) and heterozygous resistant normal (HthtBxBx) which in turn produced significantly greater amounts than susceptible normal (hthtBxBx) ans suceptible deficient (hthtbxbx) genotypes. In the absence of the Ht gene, the Bx gene plays a significant role in the delay of spore germination.

Various treatments such as attached leaf, high inoculum concentration, pre-conditioning temperature at 21°C and inoculation at young leaf stage favor phytoalexin synthesis.

The incorporation of the Ht gene into any line induces that particular line to produce a significant amount of phytoalexin. The genetic background in which Ht is placed may influence the time, rate and amount of phytoalexin synthesis. Multigenic resistant lines produced significantly more inhibitory substances than monogenic lines. The incorporation of the Ht gene in a multigenic resistant line diluted the ability of that line to produce phytoalexin.

No correlation was observed between the bioassay tests and percent leaf infection, or between quantitative evaluation and visual rating system for amount of leaf infection.

SECTION I

INTRODUCTION

Northern corn leaf blight is undoubtedly one of the most important leaf diseases of corn, particularly in the corn belt areas. The severity of infection depends upon existing environmental conditions but is favored by cool temperature and high relative humidity.

The causal organism Helminthosporium turcicum , may infect the corn plants as early as the three-leaf seedling stage. Symptoms of the disease are long, elliptical, grayish green or tan spots on the leaves, which soon coalesce into necrotic wilt-type lesions (76,82). When temperature and humidity favor infection, yield is considerably reduced and, in severe cases, it may lead to the death of the plant. Early infection may also predispose the corn to stalk rot (83).

Two types of resistance to the disease are known. The first type, conditioned by many dominant genes (41,42), is characterized by a significant reduction in the number and size of the lesions. However, the lesions are necrotic and wilted like the susceptible type. They support abundant sporulation of the pathogen.

The second type of resistance, conditioned by a single dominant gene, is characterized by chlorotic lesions which are surrounded by a yellow to light brown margin. Wilting is absent and fungus sporulation is inhibited or greatly

reduced. The gene governing resistance has been designated Ht (34).

The discovery, that phytolaexin was produced in the resistant host following infection broadened the spectrum of the study (52). Phytoalexin was found to inhibit spore germination and because of this, they were useful in the evaluation of resistant plants. Factors influencing the production of phytoalexin have been extensively studied in legumes and other dicots but little has been done in corn to correlate the results of bioassay and inoculation studies, or to determine whether phytoalexin is produced in multigenic resistant lines.

The objectives of these studies were:

1. to determine the effect of some environmental and genetic factors on production of phytoalexin.

2. to determine the effects of different genetic background on production of phytoalexin by Ht lines.

3. to discover whether phytoalexin is produced in multigenic resistant plants and if so, to compare them with monogenic types.

4. to determine if there is any correlation between the visual rating system and the quantitative method of disease evaluation following standard inoculation.

SECTION II

LITERATURE REVIEW

NORTHERN CORN LEAF BLIGHT

Northern corn leaf blight was first noticed in 1922 but did not become economically important until the early 1940's. In 1942, heavy losses were inflicted by the disease in corn belt areas. If the disease becomes established 2 to 3 weeks before silking, corn grain yield may be reduced more than 50 percent (85).

Various environmental factors influence the degree of disease infection. Elliott and Jenkins (22) found that disease spreads rapidly during cool rainy days, but slowly during hot and dry conditions. The effects of cool temperature and high relative humidity have been found by other workers (2, 21, 29), to favor disease development.

Cultural variability and longevity.

Hilu (29) reported increased virulence and conidial production of the pathogen after passing it through a susceptible host. Rodriguez and Ullstrup (73) demonstrated that monoascosporic progenies of Trichometaspheria turcica varied in their reaction to susceptible plants. Some progenies attacked corn but not sudangrass, others attacked corn but not sorghum, etc. . Working on the same stage of the fungus, Lutrell (53) found that the number of ascospores normally varied within a given ascus, hence the difficulty of genetic

study of the organism.

The longevity of the organism Helminthosporium turcicum was studied by several investigators. Under natural environment, the organism overwinters in the field as conidia which retain their viability until the next planting season (3, 9, 39, 40, 70).

High temperature, high moisture and increasing age decreases the longevity of the fungus. Nelson and Scheifele (59) found that the ability of T. turcica to overwinter saprophytically on maize is influenced in part by the genotype of the host on which the pathogen overwinters. Races of the pathogen have differing abilities to survive in the overwintering stage and differing abilities to survive on different host genotypes. Infected leaves stored under ordinary conditions retain their potency after one year (70); while infected leaf inoculum stored in the refrigerator at 10°C remained pathogenic after 25 months (40). Robert (69) gave evidence that conidia of H. turcicum remain viable for 12 years when stored at 0°C and 49% relative humidity. On the other hand, Braverman and Crosier (10) indicated that under mineral oil an agar slant of this organism can be stored for 15 years and still remain pathogenic.

DISEASE INHERITANCE

Different races of the pathogen, Helminthosporium turcicum showed differences in host specificity (72). For example, the fungus was more virulent on sweet corn than on

sudangrass or johnson grass. Ullstrup (83) reported that isolates from a single corn host showed no differential pathogenecity within the same line.of the host.

Multigenic inheritance.

Resistance to northern corn leaf blight was found by Elliott and Jenkins (22) to be transmitted from generation to generation. Jenkins and Robert (42) demonstrated the polygenic nature of resistance. Resistance was partially dominant and controlled by many genes, some of which produced major effects. The importance of additive gene action has been indicated by the success of phenotypic recurrent selection for resistance (46) and the relative unimportance of line x tester interactions in studies evaluating the potential breeding lines (43, 44, 45). Hughes and Hooker (41) studied the nature of gene action conditioning the quantitative type of resistance in maize to northern corn leaf blight. They found that additive, dominance and epistatic action are operative,but nonadditive effects are generally small. They concluded that blight resistance is conditioned by a relatively low number of genes, primarily additive in effects.

Similarly, Bogyo (8) studied the inheritance of resistance to T. turcica in maize. He indicated that additive effects are more important than epistatic or dominant effects in determining resistance to T. turcica.

Jenkins and co-workers (44, 47) located some of the factors for resistance by means of chromosomal translocations.

Findley and Leffel (24) utilized gene marker stocks to identify factors conditioning resistance and concluded that at least 12 chromosomal arms are involved. Maximum lesion size and total frequency of the susceptible type lesions were associated by Nelson et al (57) with the number of known chromosomal arms carrying genes for resistance.

Robert and Sprague (71) investigated the adaptation of the fungus to resistant and susceptible host. They reported a tendency of isolates from a particular line to be more virulent on plants of that line than of other lines. The same multigenically resistant stocks were tested by Fleming and Kozelnicky (25) in six different geographical areas and they found significant differences from area to area. These variations were heritable and they attributed these to mutation and/ or residual segregation.

Sources of multigenic resistance have been reported in several lines and inbreds, such as Mo21A, NC34, C123, and L97 (24, 61). Resistance to the same disease has also been reported in other countries (66, 67, 74, 80). Their findings showed that most, if not all, of their sources of resistance came from inbreds or lines indigenous or native to their respective countries. Imported lines or varieties were usually susceptible.

Monogenic resistance.

Monogenic resistance was first reported by Hooker (33) in the inbred G3440 and in ladyfinger popcorn. Further studies by Hooker, associates and others (32, 35, 36, 38, 77) showed that resistance was controlled by a simple dominant

gene which was later given the symbol Ht.

Patterson and co-workers (60, 61) tried to locate the Ht in the arm of chromosome 2. In 1968 (62), they were able to clarify the gene order and distance as follows:

V_4 27.6 W_3 10.3 Ht 34.0 Ch.

Subsequent studies on chlorotic lesion infection produced several sources of monogenic resistance. Hilu and Hooker (30), Hooker (33, 35, 37), Saxena and Hooker (75) and Ullstrup (83) reported such materials. Examples of resistance are: Hastings Prolific dent corn, the sweet corn inbreds EES 647 and EES 650, dent corn inbred W37A, line PI 2174407 and others.

Comparing **Ht** gene on one starchy and four sweet corn backgrounds Wilson and Rhodes (86) found no difference in the chlorotic lesions. Indications were that the mode of inheritance from the sweet corn lines was identical, allelic or closely linked to the Ht gene from the starchy corn source.

Ullstrup (84) compared polygenic and monogenic resistant hybrids in a three year trial. He found that single crosses possessing polygenic resistance average 57.1, 27.3, and 58.0 bushels per acre more than the average yields of their susceptible counterpart. On the other hand, hybrids with monogenic resistance average 38.0, 20.1 and 31.6 bushels per acre more than their corresponding susceptible lines. He pointed out that monogenic resistant genotypes infected with H. turcicum had extensive chlorosis, resulting in a debilitating effect on grain yield. Severity of the blight was positively associated with reduction in yield.

Host-Pathogen interaction.

Host-pathogen interactions were studied by Hilu and Hooker (31). They found that the initial stage of infection of Helminthosporium turcicum on corn was similar for the susceptible, multigenic resistant and monogenic resistant lines. The main difference was in the subsequent spread of the organism inside the host. In a susceptible host, the hyphae readily entered the mesophyll and clogged the xylem and tracheids. In a multigenic resistant host, the hyphae also became established in the xylem and tracheids but growth was very much curtailed and clogging of the xylem was not severe. In a monogenic resistant host, hyphae was sparsely established in the xylem, growth through the mesophyll was slow, and rapid killing of the cells was not apparent.

Gene Dosage.

The only study on gene dosage in relation to resistance was that by Dunn and Namm (20). They worked with monogenic chlorotic lesion resistance to northern corn leaf blight. They found that one and two doses of the Ht gene did not result in difference in level of resistance. Also, no differences were observed between three and four doses. However, triploid and tetraploid levels of Ht conferred a higher degree of resistance than did haploid or diploid. On the other hand, 2, 3, and 4 doses of ht did not show any differences in susceptibility, while haploid ht plants were extremely susceptible.

PHYTOALEXIN STUDIES

Recent studies showed that resistance to many diseases may be chemical in nature, following host-pathogen interaction. Muller et al, as cited by Klarman and Gardeman (49), found that resistance to Phytophthora infestans was due to the ability of resistant plants to produce a toxin in response to infection and he called this toxin which killed the invading fungus a phytoalexin. Similarly, it was found that potatoes produced phytoalexin when inoculated with three non-pathogenic fungi (50).

Muller (54) placeddrops of a fungal suspension on the uninjured inner epidermal layer of immature bean pods; he demonstrated that as infection occurred, phytoalexin diffused into the drops of water. He later (55) pointed out that cells of Phaseolus vulgaris infected with Sclerotinia fructicola or Phytophthora infestans produced phytoalexin. He suggested that a diffusable substance produced by the fungus was the agent that initiated the reaction (56).

Resistant corn plants inoculated with Helminthosporium carbonum were reported by Kúc et al (50) to produce phytoalexin. Condon and Kúc (12) found that carrots inoculated with Ceratocystis fimbriata also produced a phytoalexin. Other plants in which phytoalexin was apparently produced in response to resistant host-pathogen interactions were: soybean (64), rice (81), peas (16), beans (65), alfalfa (27, 28), safflower (1), cotton (4), and peperonia (78).

Phytoalexin in corn.

Lim et al (52) first reported production of phytoalexin in corn infected with H. turcicum in 1968. In their exploratory investigation, they found that phytoalexin inhibiting spore germination was present in diffusates and extracts from inoculated resistant leaves. No inhibition was found in non-infected or susceptible leaves.

Later, the same workers (51) isolated the chemicals and called them phytoalexin A1 and phytoalexin A2, with paper chromatography Rf values of 0.87 and 0.97, maximum uv absorption spectra of 280 nm. and 270 nm., respectively. Both are blue fluorescent compounds and are produced when spore suspensions of Helminthosporium turcicum from corn were incubated on both intact and detached leaves. Different fungal isolates were found to have varied response to phytoalexin. Spores of a highly pathogenic isolate germinated better than those of a weakly pathogenic isolate at the same phytoalexin concentration. Also, the former induced the production of a higher concentration of phytoalexin than did the latter.

Couture and co-workers (14, 15) reported that gene BxBx mediated the production of cyclic hydroxamates and related compounds which are implicated in the resistance mechanism of maize. Resistant deficient (HtHtbxbx) and susceptible deficient (hthtbxbx) genotypes were compared with resistant normal (HtHtBxBx) and susceptible normal (hthtBxBx) genotypes. The percentage of leaf infection was significantly higher in the

bxbx genotype due to an increase in the number and size of lesions. In hthtbxbx plants, the lesions enlarged and became flaccid. Non-infected tissue distal to the lesions became dessicated , suggesting plugging of xylem vessels. In HtHt-bxbx plants, the chlorotic lesions became large and transparent before becoming flaccid, while non-infected tissue distal to the lesions remained turgid.

In a preliminary report, Calub et al (11) showed that HtHt genotypes produce phytoalexin earlier in the intact than in the detached leaf. Leaf diffusates decreased rate and percent spore germination within 2-3 days in the former, and 4-5 days in the latter. Diffusates from HtHtBxBx leaves were more inhibitory on spore germination than diffusates from HtHtbxbx. The diffusates from hthtBxBx were slightly more inhibitory than diffusates from hthtbxbx, and the former delayed the growth of germinated spores. Diffusates from homozygous resistant leaves (HtHtBxBx) inhibited spore germination much more than diffusates from the heterozygous genotypes (HthtBxBx).

Factors affecting phytoalexin production.

The influence of temperature on phytoalexin production has been studied in some plants. Pre-inoculation treatment of detached pods of Phaseolus vulgaris at various temperatures affected the production of phytoalexin and the disease reaction of this host to Sclerotinia fructicola (48). Bell (5) reported that varieties of Gossypium sp. resistant to a defoliating strain of Verticillum albo-atrum were all susceptible

at 22°C; at 32°C, all were resistant; and at 25-29°C susceptible and resistant varieties gave expected reactions. On the other hand, rate of phytoalexin synthesis was maximum at 27.5 to 35°C. Below 27°C the rate decreased rapidly, and became negligible at 15°C.

Paxton and Chamberlain (63) reported that sorghum stem tissue becomes more resistant to Phytophthora megasperma var. sojae with age. Consequently, the ability to produce phytoalexin decreased. Contradictory results were found by Bell (4) who reported that very young root or stem tissue of cotton inoculated with Verticillium albo-atrum produced little or no phytoalexin.

Bell (4) investigated the effect of different spore concentrations and found that less than 10^4 conidia/ml induced only traces of phytoalexin, while 10^5 to 10^8 conidia/ml caused about a two fold increase in phytoalexin content of inoculated stem sections with Verticillium albo-atrum of cotton.

Bell and Presley (7) heat-killed and heat-inhibited conidia of Verticillium albo-atrum by incubating them in water at 50°C and 30-40°C, respectively, for ten minutes. They found that both heat-killed and heat-inhibited conidia induced phytoalexin synthesis in inoculated plants which markedly or completely inhibited spore germination.

Perrin and Cruickshank (68) reported that heavy metal ions, especially silver, mercury and copper stimulated pisatin (phytoalexin) formation in Pisum sativum.

The same workers further found that (17) heat treat-

ment (45°C) or anaerobic storage was dependent on the duration of exposure. Pisatin formation was stimulated by several saprophytic fungi. They also reported (19) that oxygen tension lower than 10 percent significantly reduced the rate of pisatin and phaseolin biosynthesis. Maximum fungal growth occured at one percent oxygen where phytoalexin synthesis was greatly inhibited.

Chamberlain and Paxton (13) demonstrated that phytoalexin was transported from the resistant to the susceptible plants by a string wick in sufficient quantity to protect the susceptible plants.

Bell (6) investigated the possible relationship between phytoalexin synthesis and peroxides in cotton, and found that a peroxidase enzyme is involved in the induction and synthesis of gossypol.

Frank and Paxton (26) studied soybeans to determine the time and sequence of production of phytoalexin. They found that phytoalexin production and fungal development occured in both resistant (Harosoy 63) and susceptible (Harosoy) varieties within 4 hours after inoculation. Differences in the host parasite interaction of the two varieties became apparent between 4 and 8 hours after inoculation. Phytoalexin was no longer detectable in Harosoy after 8 hours and the disease developed, while in the resistant line, production of phytoalexin continues. The pathogen was killed after 72 hours, production of phytoalexin ceased and the existing phytoalexin began to disappear.

SECTION III

MATERIALS AND METHODS

Description of materials.

All materials except the bx gene in this study were obtained from Dr. Albert L. Hooker, Professor of Plant Pathology at the University of Illinois. The bx gene came from Mr. Roger Couture's thesis materials from the University of New Hampshire, originally from Dr. R.H. Hamilton, of Pennsylvania State University.

Monogenic lines. Lines 65-225-1 is chlorotic lesion resistant to H. turcicum. The pedigree line is W153R crossed to GE440, backcrossed six generations to W153R, and self fertilized until homozygous for the Ht gene. Lines, 65-225-1 (HtHt) and W153R (htht) are isogenic and identical in maturity and morphology.

Line 59C32-1 was segregating 3:1 for the normal (Bx) versus the deficient mutant (bx). This line differed considerably from W153R and 65-225-1. The bx gene was incorporated in the material with the Ht gene to provide a similar genetic background in the following manner[1/]. The resistant deficient (HtHtbxbx) genotype was produced by crossing resistant normal (HtHtBxBx) and susceptible deficient (hthtbxbx) genotypes. The progenies were self fertilized until the F_2 and the deficient genotypes (bxbx) were detected by the negative root tip reac-

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1/ Couture, R. M. 1970. Role of Hydroxamic Acids in Monogenic Resistance of Maize to Helminthosporium turcicum. M. S. thesis, University of New Hampshire.

tion with ferric chloride. F_2 segregating lines HtHtbxbx and Hthtbxbx were distinguished and established by inoculation in the F_3 generation. The hthtbxbx genotype used in the study was extracted from the cross, hthtBxBx x hthtbxbx, the latter line being the one obtained from R.H. Hamilton.

RHy-2Ht is a resistant line with Ht incorporated into Hy, a susceptible inbred. It is an established line at Illinois developed from Illinois high yield selection. Incorporation of the Ht gene was done in the following manner[2/]. Hy inbred was crossed with a stock having the gene Ht. The hybrid was then backcrossed with Hy and the resistant Ht backcrossed plants identified. These plants were again crossed with Hy for a total of 5 or more backcrosses. At the end of the fifth backcrossed generation, the heterozygous Htht plants were selfed for 3 generations where homozygous HtHt selections were identified and increased. During backcrossing and selfing, selections were made for the Hy plant and ear types. The genetic background of Hy and RHy-2Ht are therefore very similar except for HtHt versus htht gene (and closely linked genes) differences.

The same procedure was followed for the rest of the succeeding lines used in the study. This includes the multigenic and the combination of mutligenic and monogenic lines.

RWF9-Ht is a resistant line Ht incorporated into WF9 susceptible inbred. This was developed at Indiana from open

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2/ Letter of Dr. A.L. Hooker, Professor of Plant Pathology, University of Illinois to Dr. G.M. Dunn, Professor of Plant Science, University of New Hampshire, dated March 14, 1972.

pollinated variety at Wilson Farm.

RW64A-Ht is W64A, a susceptible inbred, to which Ht has been added. ROh43-Ht is Oh 43, a susceptible inbred to which Ht had been added.

Multigenic Lines. H55 is a resistant line developed in Indiana. It originally came from the cross (Hy x Mol1a) x Hy. H49, is a resistant line also developed in Indiana but from the cross (WF9 x L97) x WF9.

Combined multigenic and monogenic lines. These lines were originally multigenic resistant with the Ht gene incorporated.

RH55Ht is H55 with Ht added. RH49Ht was developed similarly from H49.

Planting and growth of materials.

Planting materials were divided into two groups as follows: those planted for inoculation in the inoculation chamber, designated greenhouse evaluation; and those planted for bioassay test, designated bioassay evaluation.

For greenhouse evaluation, two 4-inch plastic pots were planted with 6-7 seeds per pot per genotype. After germination, the seedlings were thinned to five seedlings per pot. For bioassay evaluation, three seeds of each genotype were planted in 3-inch peat moss pots arranged within a plastic flat and later thinned to two seedlings per pot. Both seedlings for greenhouse and bioassay evaluation were grown under the same greenhouse conditions, 27°C (80°F) during the day and 18°C

(65°F) during the night. Seedlings subjected to pre-disposed temperatures were grown inside the growth chamber continuosly at the prescribed day and night temperature. In all instances, supplementary light was used to extend the daylength to 16 hours during short days.

Culture of the Pathogen.

The medium used for cultivating 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	1000.0	ml.

This medium was utilized as suggested by Hooker. The mixture was heated until fairly homogenous, then autoclaved for 20 minutes at 15 pounds pressure at 120°C. While the media was still hot, it was poured on petri dishes under aseptic condition. About 20 ml of media was put on each plate. The media was allowed to solidify and cool, then stored in the refrigerator until used.

Leaf lesion isolates of H. turcicum were obtained from Dr. Hooker. The lesions were cut into small squares and the surfaces sterilized in a ten percent solution of commercial chlorox. The sections were agitated in the solution until the edges became transparent. Sections were then dried on absorbent blotting papers and transfered aseptically to petri dishes containing nutrient agar medium. The dishes were incubated

at 23°C (74°F) for four weeks. Cultures 21-25 days old were used for inoculation, although cultures up to five weeks old usually showed satisfactory virulence. Spores from six weeks old cultures were dried out and non-virulent. For subsequent inoculations, the lesions came from susceptible infected plants.

Preparation of the inoculum.

Inoculum for seedlings. Cultures were cut into small blocks and placed in a flask containing distilled water. The flask was shaken vigorously to dislodge the spores and filtered through a fine wire mesh into another flask. A blank nutrient agar medium without the organism was thoroughly ground in a Waring blendor and the filtered spores added while the blendor was still running. The ratio of blank agar plate to culture plate was 1:3. For every culture plate, 100 ml. of distilled water was mixed for inoculation of 50 seedlings. Two drops of Tween 20 were added to every 100 ml. of solution. The inoculum suspension was then transferred to a sprayer for inoculation.

Inoculum for bioassay. Distilled water was added to culture plates of the organism. The surface of the culture was lightly scraped with a spatula. This suspension was then filtered through glass wool into a flask with the aid of a vacuum suction.

Spore concentration for all inoculum was determined as follows: Inoculum was stirred until thoroughly mixed; then, a 10-15 ml. sample was withdrawn. Sampling was done before

Tween 20 was added. The sample was shaken vigorously and a drop of spore suspension was placed on the Howard Mold Counting Chamber Slide, having a volume of .15 mm^3. A cover slip was put on the top of the drop to obtain a uniform thickness of suspension. The slide was then observed under the low power objective of the microscope. Spore count of the entire field was facilitated with the aid of cross sectioned lines attached to the eyepiece of the microscope. After counting the spores of that particular field, a marker was noted on the far end of the field and the slide move slowly with the marker traversing the field diametrically until it disappeared. A second count was started until five field counts were made. A second drop of the sample suspension, thoroughly mixed, was observed in a similar fashion. The ten observations were added, and the average number of spores per observation was computed. This was multiplied by the factor 6666 to give the approximate number of spores per ml.

Inoculation.

The inoculated seedlings were three to four weeks old. At this age the seedlings were at the third leaf stage with the fourth leaf about an inch long. For the bioassay test, this stage was not feasible because of its small size. Instead, the 6th, 9th and 12th leaf stages were used to determine the effect of age of plant.

The inoculation chamber was built inside a growth chamber control room 4 feet by 8 feet long and sealed with heavy plastic sheets. A thermostatically controlled heater was placed

on the floor of the room to maintain a constant temperature of 20°C (68°F).

A humidifier was placed at the corner of the chamber and attached to a clock which allowed it to operate up to 25 minutes every hour. The seedlings were left in the chamber for 18 hours. A humidistat control the humidifier when the humidity reached 100 percent, thus avoiding excessive spore wash-off. A ten gallon plastic can was used to supply the humidifier. An inline deionizer was attached between the water container and the humidifier to remove any traces of copper.

Greenhouse evaluation. One hour before inoculation the chamber was sealed and the humidifier turned on until the humidity of the chamber reached 100 percent. Plants for greenhouse evaluation were then placed inside the chamber and the humidifier turned off. A suspension of inoculum was sprayed on the plants from above until all leaves were covered with fine droplets. The chamber was then resealed and the clock reset on the sixteen hour cycle of mist spray. The plants were placed back in the greenhouse for 7 to 9 days before readings were made. Seedlings predisposed at different temperatures were all placed under 21°C after inoculation.

Bioassay evaluation. Two assay methods were used, the attached and detached leaf.

For the attached leaf method, plants were placed inside the growth chamber provided with benches. Room temperature was adjusted to 20-21°C (68-70°F). Labelled plants in peat pots

were arranged in a single row at random in a plastic flat. The flat was then lowered beside the bench and tied in such a position that the base of the top leaves of the plants were on the same level as the bench.

Clear plastic boxes 3 x 11 x 15 inches with covers were used as inoculating chambers. On one side of the plastic box, 9 spaced square holes about an inch size were made. Cellulo-cotton layers were placed on the inside bottom of the box and saturated with distilled water. The purpose of the water was to increase the humidity inside the chamber and thereby prevent drying of the drop suspension. On top of the wet cellulo-cotton, a rubber screen was placed so that the leaves would not come in direct contact with the water. One or two of the youngest fully expanded leaves were selected for inoculation. The selected sample was then inserted in each of the square holes to the base of the leaf and flattened carefully on the rubber screen. To hold the leaf in place, small stoppered vials with water were used as weights strategically placed on top of the leaves. The vials were also used to attached labels of the plant genotype. Masking tape one inch wide was used to cover the holes where the leaves were inserted and to anchor the leaf in the box (Figure 1).

Inoculation was done by placing several drops of spore suspension on each of the entire leaves, then replacing the cover of the box. A drop of fresh spore suspension was added to the suspension on the leaf every day to prevent drying of the inoculum. This condition was maintained for 5-7 days or until

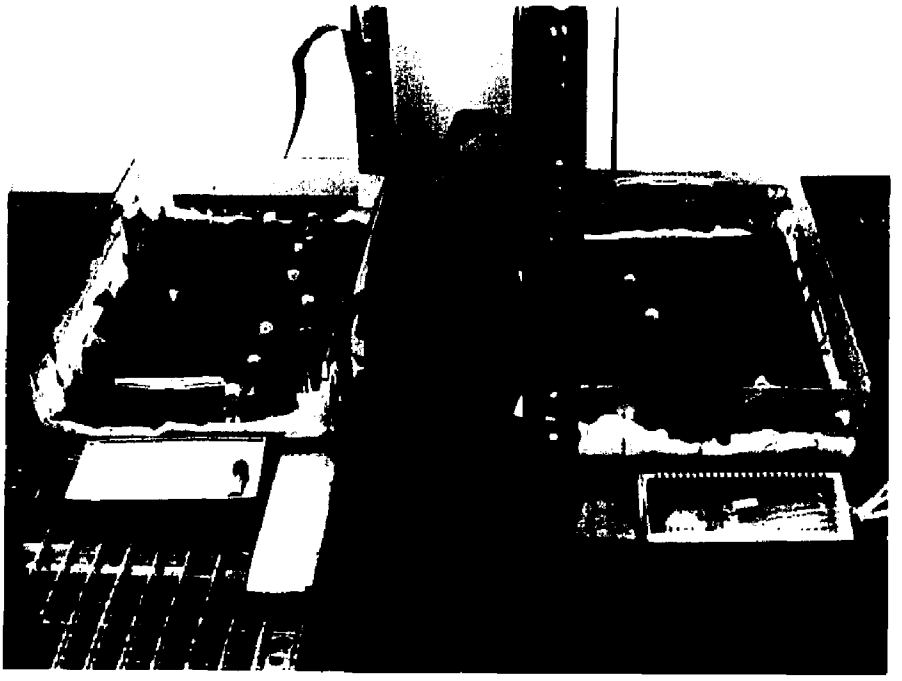

Figure 1. Arrangement of attached leaf technique for bioassay test.

more than 50 percent of the leaves became yellow. For the control, the HtHtBxBx genotype was used and the suspension was distilled water unless otherwise specified.

For the detached leaf, the plastic boxes without holes were used and a solution containing 20 ppm of N^6-Benzyladenine + 5% sucrose was used to saturate the cellulo-cotton instead of water (52). Rubber screen was not provided. The purpose of the solution was to delay yellowing or senescence of the leaves. The youngest fully expanded leaves were then cut at the base and laid out with the lower surface of the leaves directly in

contact with the solution and labelled as above. Figure 2 illustrates the set-up of the experiment.

Figure 2. Close-up view of the detached leaf technique. This was at the start of the experiment. Note the green color of the leaves.

Disease evaluation.

Two types of evaluation were made. The first was a visual rating. The same leaf was re-evaluated quantitatively using a modified Namms'[3/] method.

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3/

Namm, T. 1968. Gene Dosage Effects on Monogenic Chlorotic Lesion Resistance to Northern Corn Leaf Blight. Ph. D. Dissertation, University of New Hampshire.

Visual rating. A single leaf from each seedling was evaluated for disease reaction. Observations were taken from the emerging unopened leaf at the time of inoculation. Each leaf was carefully examined for lesions and the percentage infection based on the entire leaf area was roughly estimated visually. The lesions were then classified into either chlorotic or necrotic. Scores were assigned as follows:

1 = 0 infection
2 = 1-10% infection with chlorotic lesion
3 = 1-10% infection with necrotic lesion
4 =11-25% infection with chlorotic lesion
5 =11-25% infection with necrotic lesion
6 =26-50% infection with cholrotic lesion
7 =26-50% infection with necrotic lesion
8 =51% or more lesion, either necrotic or chlorotic

Quantitative evaluation. The same leaf used in the visual rating was used in this method. The area of the leaf was measured with transparent grid. Modifications of Namms' method were that instead of only 25 dots per square inch, 100 evenly space dots/in^2 (15.50 dots/cm^2) were used. Also, instead of positioning the leaf randomly under the grid, one edge of the leaf was aligned under a column of dots, directly. This method gives a rapid estimate of the area and is ten or more times faster than that of Namms'. The area of the lesion was determined in the same manner. Percent infection was then expressed as follows:

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

Bioassay test.

Twenty four hours after the spore suspensions were placed on the leaves, samples for the bioassay test were taken eve-

ry day for 5-7 days. Five to ten drops of the supension were withdrawn at random by a pipette and collected in a properly labelled centrifuge tube. The same procedure was followed for the control. These were then centrifuged at 25,000 x G for 10 minutes to remove the spores and germ tubes. The centrifuge suspension, now free of germ tube and spores, was termed the diffusate. For each genotype 0.4 cc of the diffusates was withdrawn from the tube with a syringe and 0.2 cc each was placed in separate glass well slides. The two slides represented two replications. A drop of fresh spore suspension was added to each slide. All slide experiments were incubated in a clear plastic chamber lined with wet cellulo-cotton at the bottom, covered and placed at room temperature overnight. After incubation, the spores were killed and stained with IKI and the percentage of germination and the germ tube length were determined.

Each slide was examined under the low power objective and the number of germinated and ungerminated spores was counted. The percentage of spore germination was then computed.

For germ tube length, germinating spores were measured at random with the use of occular micrometer which had been previously calibrated. Thirty or more germinating spores were measured for each replication and the average was taken.

Analysis of variance.

A randomized complete block design involving a factorial treatment arrangement was used in the experiment.

The data for disease infection and spore germination in percentages were normally distributed. Hence, conversion to arcsine angles was not made and the percentages were used directly in the analysis of variance. For the greenhouse evaluation, the experimental units was the individual pot, while for the bioassay test it was the drop suspension.

Since the factors involved vary from experiment to experiment, it is not feasible to show the analysis of variance for each. However, a typical analysis was as follows:

Source of Variation	d.f.
Day	(d-1)
Stage of plant growth	(s-1)
Stage x Day	(s-1) (d-1)
Genotype	(g-1)
Genotype x Day	(g-1) (d-1)
Genotype x Stage	(g-1) (s-1)
Genotype x Stage x Day	(g-1) (s-1) (d-1)
Replication	(r-1)
Error	(r-1) (dsg-1)
TOTAL	(rdsg-1)

Duncan's multiple range test, as outlined by Steel and Torrie (79), was used to test for differences among the different means.

SECTION IV

RESULTS

Length of spore viability

Spore suspensions used for germination count and stored in the refrigerator at 4 - 6°C were tested for length of viability. Table 1 shows the daily percentage count on spore germination.

Table 1. Effect of storage on spore viability (Based on 2 trials).

Number of Days Stored	Number of spores		%
	Counted	Not germinated	Germinated
1	368	1	99.8
2	350	1	99.7
3	217	7	96.7
4	409	10	97.6
5	295	1	99.7
6	438	4	99.1
7	314	8	97.4
8	261	7	97.3
9	222	10	95.5
10	299	23	92.3
11	337	10	97.0
12	406	32	92.1

The data indicate that a spore suspension could be

stored up to twelve days without significantly reducing spore viability. Based on this indication, a spore suspension was prepared at the beginning of each experiment, stored in the refrigerator and used daily till the end of that experiment. Each experiment usually lasted for 5-7 days.

Factors influencing the production of phytoalexin.

Studies on the factors influencing the production of phytoalexin were centered on the following genotypes: HtHtBxBx = homozygous resistant normal; HthtBxBx = heterozygous resistant normal; HtHtbxbx = homozygous resistant deficient; hthtBxBx = susceptible normal; and hthtbxbx - susceptible deficient.

All of these genotypes were tested as controls by inoculating them with distilled water. Their leaf diffusates showed no differences in spore germination in several trials. Also, two types of control treatments were tried. The first was the used of distilled water and the second with killed spores. A spore suspension of H. turcicum was killed by boiling the inoculum for 5 minutes. Replicated trials showed almost no inhibition of spore germination by either treatment. The term control in the text therefore referred to the genotype HtHtBxBx inoculated with water.

All bioassays, unless otherwise specified, were on the: 5th-6th leaf stage; inoculated at 20-21^{o}C; with 9.9 x 10^{4} spores per ml.; and under a light intensity of 300 foot candles.

The data presented in the succeeding tables were the means of two or more separate trials or experiments. The production of phytoalexin was then evaluated in terms of per-

cent spore germination compared to the control using a water suspension only. An increased amount of phytoalexin resulted in a lower percent germination and presumably decreased the rate of growth of the germ tube. Length of the latter was measured in microns.

Effect of attached and detached leaf. Table 2 shows the percent spore germination by the different genotypes under the various treatments. Analysis of the data is shown in table 3 as well as the comparison of the means of the different factors and their levels. The analysis of variance indicated that F values were highly significant for all factors involved including their corresponding interactions.

Table 2. Percent spore germination in bioassay test of diffusates from attached and detached leaves at 2 inoculation temperatures. (Average of 2 replications)

Genotypes	Attached Leaf						Detached Leaf					
	18°C			24°C			18°C			24°C		
	Days			Days			Days			Days		
	1	2	3	1	2	3	1	2	3	1	2	3
HtHtBxBx	13.5	6.2	15.6	20.8	4.2	5.8	17.9	57.5	52.3	85.2	80.8	61.5
HtHtbxbx	24.4	8.8	47.6	10.8	8.0	9.4	88.8	80.8	78.2	84.0	93.2	74.6
HthtBxBx	85.3	37.0	33.9	77.7	12.6	23.8	89.4	85.6	80.1	90.8	80.0	79.2
HthtBxbx	81.4	77.6	63.6	73.2	33.1	71.2	88.5	17.2	85.5	83.6	87.4	78.5
hthtBxBx	90.3	89.3	84.2	84.3	91.0	85.8	89.5	89.1	88.5	89.3	96.4	80.8
hthtBxbx	89.3	95.2	80.0	91.2	91.1	84.8	87.8	87.8	93.6	89.1	87.2	91.9
hthtbxbx	90.6	92.6	92.4	90.2	91.2	91.0	86.2	78.7	91.5	95.4	91.1	83.3
Control	95.2	94.8	94.9	88.4	71.0	95.3	87.2	93.8	94.5	92.0	95.2	82.0

Table 3. Analysis of variance and Duncan's multiple range test of the means of table 2.

Source of Variation	d.f.	M.S.	F
Days	2	1442.34	134.65**
Temperature	1	75.12	7.01**
Temp. x Day	2	162.11	15.13**
Leaf Condition	1	20777.09	1939.69**
Leaf x Day	2	218.34	20.38**
Leaf x Temp.	1	1809.94	168.97**
Leaf x Day x Temp.	2	734.30	68.55**
Genotype	7	9607.66	896.94**
Gen. x Day	14	488.97	45.64**
Gen. x Temp.	7	197.75	18.46**
Gen. x Temp. x Leaf	14	107.33	10.02**
Gen. x Leaf	7	4693.55	438.17**
Gen. x Leaf x Day	14	388.92	31.64**
Gen. x Leaf x Temp.	7	207.38	19.36**
Gen. x Leaf x Temp. x Day	14	305.80	28.54**
Replication	1	5.36	0.50^{NS}
Error	95	10.71	
Total	191		

** Significant at 0.01 level ; NS = not significant

MEANS

HtHtBxBx	HtHtbxbx	HthtBxBx	HthtBxbx
35.1^{a}	50.7^{b}	64.6^{c}	70.7^{d}
hthtBxBx	hthtBxbx	hthtbxbx	Control
88.2^{e}	89.0^{e}	89.5^{e}	90.4^{e}
Attached	Detached	18°C	24°C
62.3^{a}	83.1^{b}	73.4^{a}	72.1^{b}
Day 2	Day 3	Day 1	
68.9^{a}	71.1^{b}	78.0^{c}	

Means not followed by the same letter differ significantly at 1% level.

Comparison of the attached and detached leaf showed that the former produced significantly more phytoalexin than the latter as shown by its lower percent spore germination. A closer look at the data revealed that the resistant genotypes with attached leaves started to produce phytolaexin twenty four hours after inoculation with the suspension. This increased progressively up to the third day. The same genotype with the detached leaves apparently did not produce the substance until the second day and at a much lesser amount.

The resistant normal genotype (HtHtBxBx) inhibited spore germination by about 55% based on the control. This inhibition was significantly higher than any other genotype at the one percent level. This was followed by HtHtbxbx, HthtBxBx, and HthtBxbx in that order with highly significant differences among these three genotypes. On the other hand, hthtBxBx, htht-Bxbx, hthtbxbx did not differ significantly from the control.

Inoculation temperatures of 18° and 24°C differ significantly. However the small difference requires that further study is needed before a definite conclusion can be made. Appearance of the attached and detached leaves several days after inoculation is shown in figures 3 and 4, respectively. These are explained under discussion.

Further studies on the effects of attached and detached leaf are shown in tables 4-7. The percent spore germination and germ tube length are shown in tables 4 and 6, while their corresponding analysis are presented in tables 5 and 7, respectively.

Figure 3. Close-up of attached leaves 2 days after inoculation with spore suspension. Note the water soaked lesions underneath the drop suspension. The first 2 leaves on the left were the control and the 2nd and 3rd leaves from the right were the susceptible genotypes.

Figure 4. Appearance of the detached leaves 5 days after the suspension was placed on the leaves. The genotypes from left to right were: Control, hthtbxbx, hthtBxBx, HtHtbxbx, HthtBxBx, and HtHtBxBx.

Table 4. Percent spore germination on attached and detached leaf on 65 day old seedlings. (Average of 2 experiments with two replications each)

Leaf Condition	Genotypes	Days after inoculation						
		1	2	3	4	5	6	7
Attached	HtHtBxBx	12.4	4.3	6.2	16.1	21.4	14.4	69.8
	HthtBxBx	23.0	17.2	18.2	28.9	40.5	90.6	80.8
	HtHtbxbx	42.1	8.4	21.5	28.0	17.8	70.5	70.7
	hthtBxBx	67.1	41.4	77.7	89.9	73.4	84.8	90.6
	hthtbxbx	53.2	84.4	87.4	90.6	88.4	89.2	91.8
	Control	98.2	97.2	96.9	96.7	95.6	95.6	99.0
Detached	HtHtBxBx	86.7	65.1	78.2	83.2	88.0	89.9	96.9
	HthtBxBx	80.6	69.1	82.8	85.8	84.0	92.5	91.4
	HtHtbxbx	93.0	81.6	79.2	87.6	82.9	88.2	83.5
	hthtBxBx	87.8	97.9	97.7	98.0	90.8	88.7	90.9
	hthtbxbx	90.8	91.8	93.5	98.1	93.0	91.8	92.9
	Control	99.2	97.1	99.1	97.9	97.2	97.4	97.8

Table 5. Analysis of variance and Duncan's multiple range test of the means of table 4.

Source of Variation	d.f.	M.S.	F
Days	6	1540.79	51.77**
Leaf Condition	1	9367.64	314.76**
Day x Leaf	6	41.56	1.39^{NS}
Genotype	5	17329.54	582.28**
Gen. x Day	30	556.48	18.69**
Gen. x Leaf	5	1269.58	42.65**
Gen. x Leaf x Day	30	258.64	8.55**
Replication	1	48.75	1.63^{NS}
Error	83		
Total	167		

** Significant at 0.01 level; NS = not significant

MEANS

HtHtBxBx	HtHtbxbx	HthtBxBx	hthtBxBx	hthtbxbx	Control
52.3^{a}	61.1^{b}	63.3^{b}	84.1^{c}	86.6^{c}	97.5^{d}

Day 2	Day 1	Day 3	Day 5	Day 4	Day 6	Day 7
63.0^{a}	69.5^{b}	69.9^{b}	72.7^{b}	75.1^{b}	82.4^{c}	86.4^{d}

Attached	Detached
59.9^{a}	88.9^{b}

Means not followed by the same letter differ significantly at the 1% level.

Table 6. Germ tube length of H. turcicum on attached and detached leaf on 65 days old seedlings. (Average of 2 experiments, with 2 replications each in microns).

Leaf Condition	Genotypes	Days of inoculation						
		1	2	3	4	5	6	7
Attached	HtHtBxBx	26.2	10.5	31.5	10.5	47.2	26.2	47.2
	HthtBxBx	42.0	26.2	21.0	21.0	73.5	341.2	115.5
	HtHtbxbx	36.8	15.8	26.2	15.8	21.0	26.2	26.2
	hthtBxBx	31.5	26.2	26.2	26.2	26.5	31.5	36.8
	hthtbxbx	36.8	36.8	36.8	36.8	47.2	42.0	136.5
	Control	456.8	383.2	393.8	388.5	393.8	304.5	299.2
Detached	HtHtBxBx	231.0	26.2	63.0	141.8	115.5	233.2	173.2
	HthtBxBx	94.5	57.8	57.8	173.2	178.5	183.8	57.8
	HtHtbxbx	147.0	63.0	173.2	120.8	115.5	94.5	26.2
	hthtBxBx	131.2	73.5	168.0	294.0	152.2	120.8	147.0
	hthtbxbx	315.0	110.2	120.8	141.7	168.0	63.0	115.5
	Control	551.2	393.8	462.0	399.0	294.0	288.8	309.8

Table 7. Analysis of variance and Duncan's multiple range test of the means of table 6.

Source of Variation	d.f.	M.S.	F
Days	6	12235.05	39.84**
Leaf Condition	1	493946.25	1608.58**
Day x Leaf	6	3324.24	10.82**
Genotype	5	229222.56	746.48**
Gen. x Day	30	10912.07	35.53**
Gen. x Leaf	5	134562.00	438.21**
Gen. x Leaf x Day	30	6430.23	20.94**
Replication	1	15882.86	51.72**
Error	83	317.06	
Total	167		

** Significant at .01 level

MEANS

HtHtbxbx	HtHtBxBx	hthtBxBx	HtHtBxBx	hthtbxbx	Control
64.9^{a}	84.5^{b}	92.3^{b}	103.1^{c}	103.1^{c}	379.9^{d}

Day 2	Day 7	Day 3	Day 5	Day 6	Day 4	Day 1
101.9^{a}	124.2^{b}	131.7^{b}	136.1^{b}	146.3^{c}	147.4^{c}	175.0^{d}

Attached	Detached
100.1^{a}	174.9^{b}

Means not followed by the same letter differ significantly at the 1% level.

Similar results were observed for the effect of leaf attachment in tables 4 and 6. In addition, the attached leaf produced a shorter germ tube length than the detached leaf. The attached leaf producedphytoalexin at a much faster rate and amount than the detached leaf. This was clearly demonstrated in figure 5.

The genotypes were in the same order of arrangement in table 2 as in table 4. Unlike the first experiment, however, the susceptible genotypes (hthtBxBx and hthtbxbx) decreased germination very significantly compared to the control, suggesting the production of some phytoalexin by these genotypes. The amount , however, was probably not sufficient for good inhibition of spore germination and reduction of the germ tube. Interestingly, the germ tube of the control was four times longer than the resistant genotypes and three and one half times longer than the susceptible genotypes. Production of phytoalexin (table 4) started on the first day and increased to its peak on the second day. It then invariably decreased and almost entirely disappeared by the 6th or 7th day, particularly on the detached leaves. Figures 6, 7, 8 and 9 show the effect of the genotypes on the germination and germ tube length of H. turcicum.

The effect of inoculum concentration. The effects of inoculum concentration upon the production of phytoalexin substances are presented in tables 8 and 10.

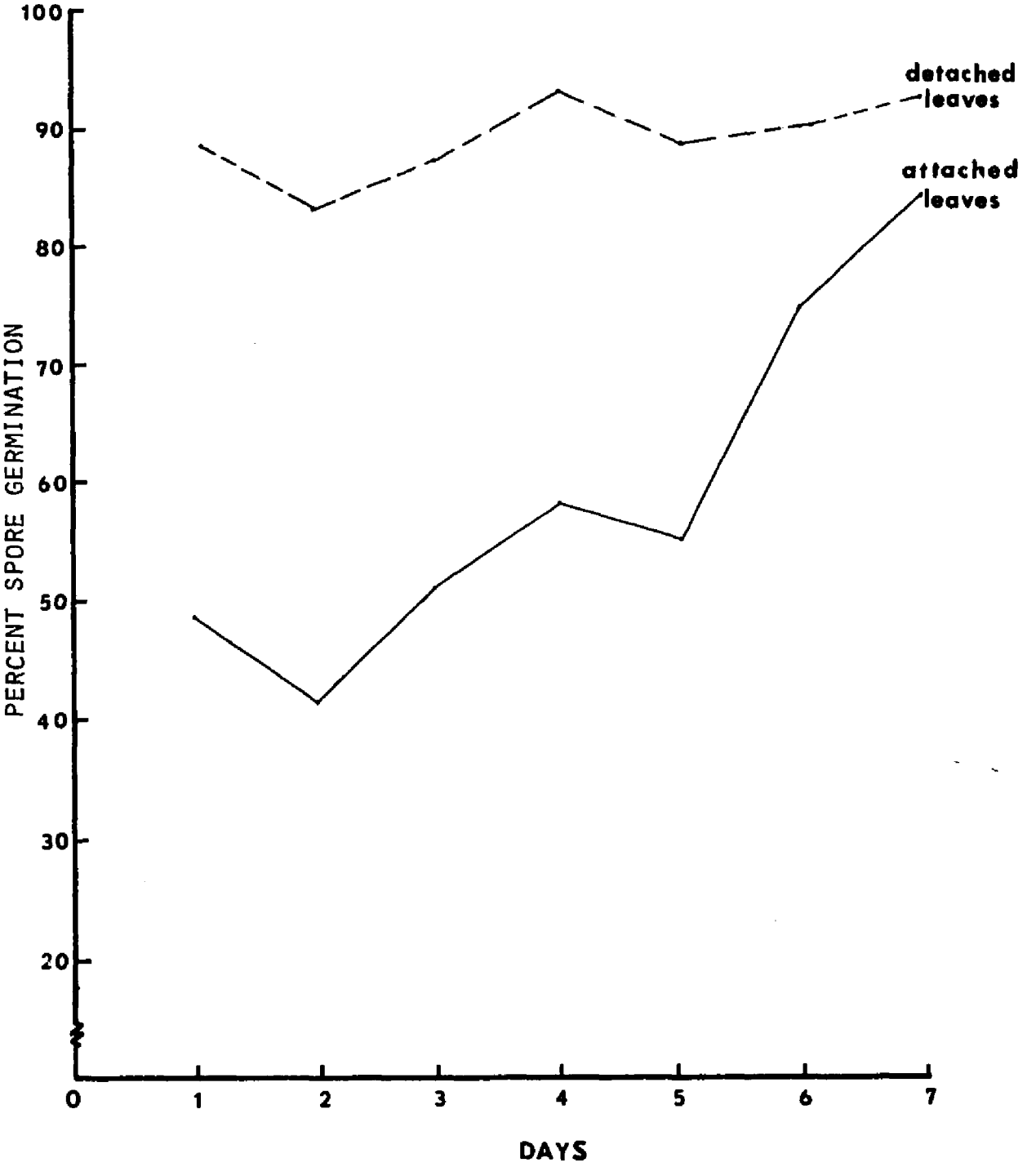


Figure 5. Effect of attached and detached leaves on the time of phytoalexin production and percentage spore germination.

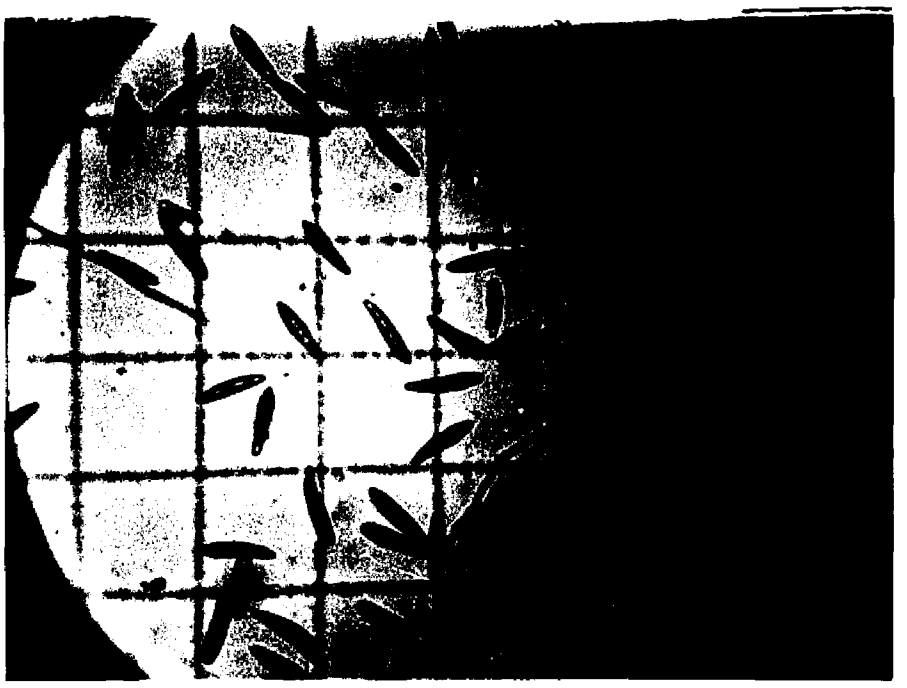


Figure 6. Effect of the diffusate from the genotype HtHtBxBx on the spore germination of H. turcicum. The square grid is inserted into the eyepiece of the microscope to facilitate counting.

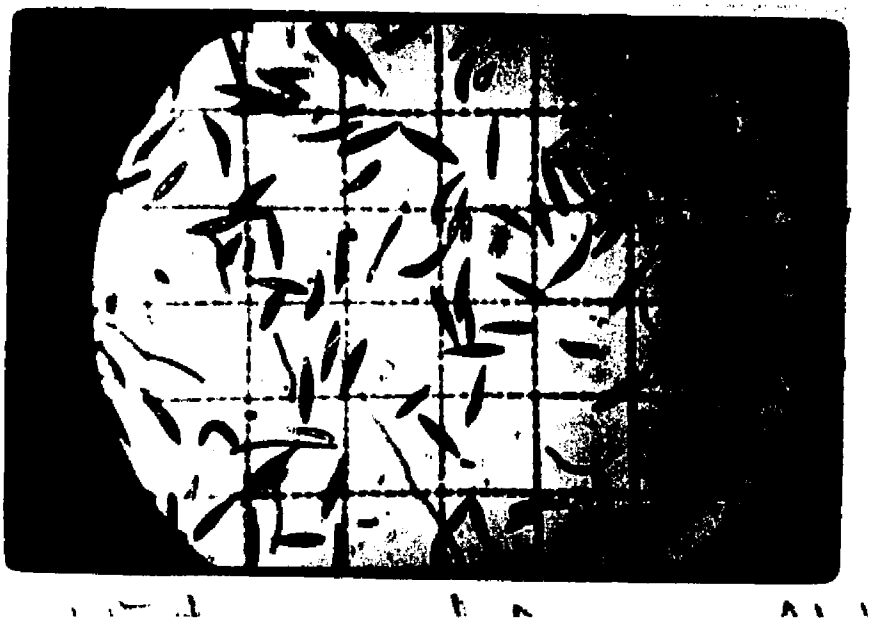


Figure 7. Effect of the diffusate from the genotype HthtBxBx or hthtBxBx on the spore germination of H. turcicum. Germination and germ tube length are usually reduced.

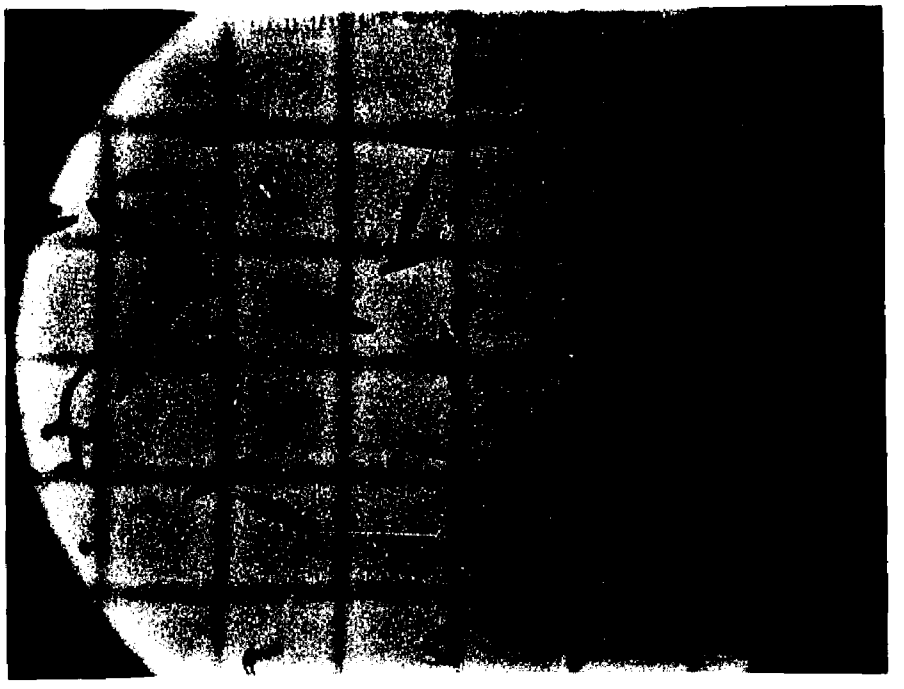


Figure 8. The effect of the diffusate from the genotype hthtbxbx or hthtBxBx on the spore germination of H. turcicum. Germ tube length and spore germination is very slightly reducedas compared to the resistant genotypes.

Figure 9. Effect from the distilled water in the control on the spore germination of H. turcicum. There was no inhibition of germination and growth of the germ tube.

Table 8. Percent spore germination at two inoculum concentrations on the attached leaf of 65 days old seedlings. (Average of 2 experiments with 2 replications each)

Concentration	Genotypes	Days of inoculation						
		1	2	3	4	5	6	7
Low	HtHtBxBx	92.8	20.2	5.8	9.1	20.1	24.3	82.0
	HthtBxBx	78.4	72.6	15.4	23.8	27.0	36.1	80.4
	HtHtbxbx	70.1	53.2	16.6	16.9	21.6	24.4	68.5
	hthtBxBx	97.1	87.0	27.6	78.8	94.0	96.8	89.3
	hthtbxbx	93.9	90.7	90.6	90.7	94.1	88.4	94.2
	Control	99.4	97.0	97.4	97.8	98.7	95.6	96.8
High	HtHtBxBx	12.4	4.3	6.25	16.1	21.4	14.4	69.8
	HthtBxBx	23.0	17.2	18.2	28.9	40.5	90.6	80.8
	HtHtbxbx	42.1	8.4	21.5	28.0	17.8	70.5	70.7
	hthtBxBx	67.1	41.4	77.7	89.9	73.4	84.8	90.6
	hthtbxbx	53.2	84.4	87.4	90.6	88.4	89.2	91.8
	Control	98.2	97.2	96.9	96.7	95.6	95.6	99.0

Table 9. Analysis of variance and Duncan's multiple range test of the means of table 8.

Source of Variation	d.f.	M.S.	F
Days	6	3637.35	224.24**
Concentration	1	20756.98	1279.67**
Day x conc.	6	354.42	21.85**
Genotype	5	18048.93	1112.71**
Gen. x Day	30	1152.88	71.07**
Gen. x Conc.	5	1591.29	98.10**
Gen. x Conc. x Day	30	378.25	23.31**
Replication	1	30.00	1.84 NS
Error	83	16.22	
Total			

** Significant at 0.01 level; NS not significant

MEANS

HtHtBxBx	HtHtbxbx	HthtBxBx	hthtBxBx	hthtbxbx	Control
28.5[a]	37.9[b]	45.2[c]	78.2[d]	87.7[e]	97.3[f]

Day 3	Day 4	Day 2	Day 5	Day 6	Day 1	Day 7
46.8[a]	55.6[b]	56.2[b]	57.7[b]	67.5[c]	69.0[c]	84.5[d]

High	Low
59.3[a]	65.6[b]

Means not followed by the same letter differ significantly at the 1% level.

Table 10. Germ tube length of H. turcicum at 2 inoculum concentrations on attached leaf on 65 days old seedlings. (Average of 2 experiments, with 2 replications each, in microns).

Concentration	Genotypes	Days after inoculation						
		1	2	3	4	5	6	7
Low	HtHtBxBx	73.5	15.8	10.5	15.8	21.0	26.2	36.8
	HthtBxBx	57.8	21.0	15.8	15.8	15.8	52.5	26.2
	HtHtbxbx	26.2	15.8	15.8	15.8	15.8	26.2	21.0
	hthtBxBx	52.5	26.2	26.2	26.2	26.2	68.2	36.8
	hthtbxbx	115.5	63.0	84.0	73.5	147.0	57.8	210.0
	Control	488.2	393.8	283.5	262.5	225.8	288.8	304.5
High	HtHtBxBx	26.2	10.5	31.5	10.5	47.2	26.2	47.2
	HthtBxBx	42.0	26.3	21.0	21.0	73.5	341.2	115.5
	HtHtbxbx	36.8	15.8	26.2	15.8	21.0	26.2	26.2
	hthtBxBx	31.5	26.3	26.2	26.2	26.2	31.5	36.8
	hthtbxbx	36.8	36.8	37.8	37.8	47.2	42.0	136.5
	Control	456.8	383.2	393.8	388.5	393.8	304.5	299.6

Table 11. Analysis of variance and Duncan's multiple range test of the means of table 10.

Source of Variation	d.f.	M.S.	F
Days	6	6649.33	31.58**
Concentration	1	451001.87	2142.23**
Conc. x Day	6	6231.96	29.60**
Genotype	5	240725.00	1143.42**
Gen. x Day	30	4309.40	20.46**
Gen. x Conc.	5	124071.12	589.33**
Gen. x Conc. x Day	30	5222.37	24.80**
Replication	1	10584.65	50.27**
Error	83	210.52	
Total	167		

** Significant at 0.01 level

MEANS

HtHtbxbx	HtHtBxBx	hthtBxBx	HthtBxBx	hthtbxbx	Control
21.8[a]	28.5[a]	33.4[a]	60.4[b]	80.2[c]	356.4[d]

Day 4	Day 3	Day 2	Day 5	Day 6	Day 1	Day 7
72.7[a]	76.6[a]	81.4[a]	93.6[b]	108.9[b]	120.3[c]	128.6[d]

Low	High
93.4[a]	100.1[b]

Means not followed by the same letter differ significantly at the 1% level.

The spore counts for high and low inoculum concentration were 13.3×10^4 and 3.3×10^4 spores per ml, respectively. The experiment showed that the high concentration inhibited spore germination by 40% compared to the low concentration of 34% (table 9). These are highly significant differences. Similar results were obtained with the growth of the germ tube (table 10). A shorter tube growth was observed at high than at low concentration. The analysis of variance for tables 8 and 10 are shown in tables 9 and 11, respectively.

A graph of concentration and genotype (figure 10) showed that all the genotypes under high concentration produced more phytoalexin than the same genoytpes under low concentration. This was most pronounced with the homozygous resistant normal (HtHtBxBx) genotype.

The genotypes and their ability to produce phytoalexin were arranged in decreasing order as follows: HtHtBxBx, HtHtbxbx, HthtBxBx, hthtBxBx, hthtbxbx, and control. Their means for spore germination were all highly significant, one from another. Figure 11 clearly illustrates the relationship between the different genotypes and the rate of appearance of phytoalexin. The graph shows that all the resistant genotypes produced significant amounts of phytoalexin twenty-four hours after inoculation and the phytoalexin increased substantially until a maximum peak on the third day. Thereafter, phytoalexin decreased until it almost disappeared on the seventh day. On the other hand, the susceptible genotypes, particularly hthtBxBx, showed a very slight production of phytoalexin until the

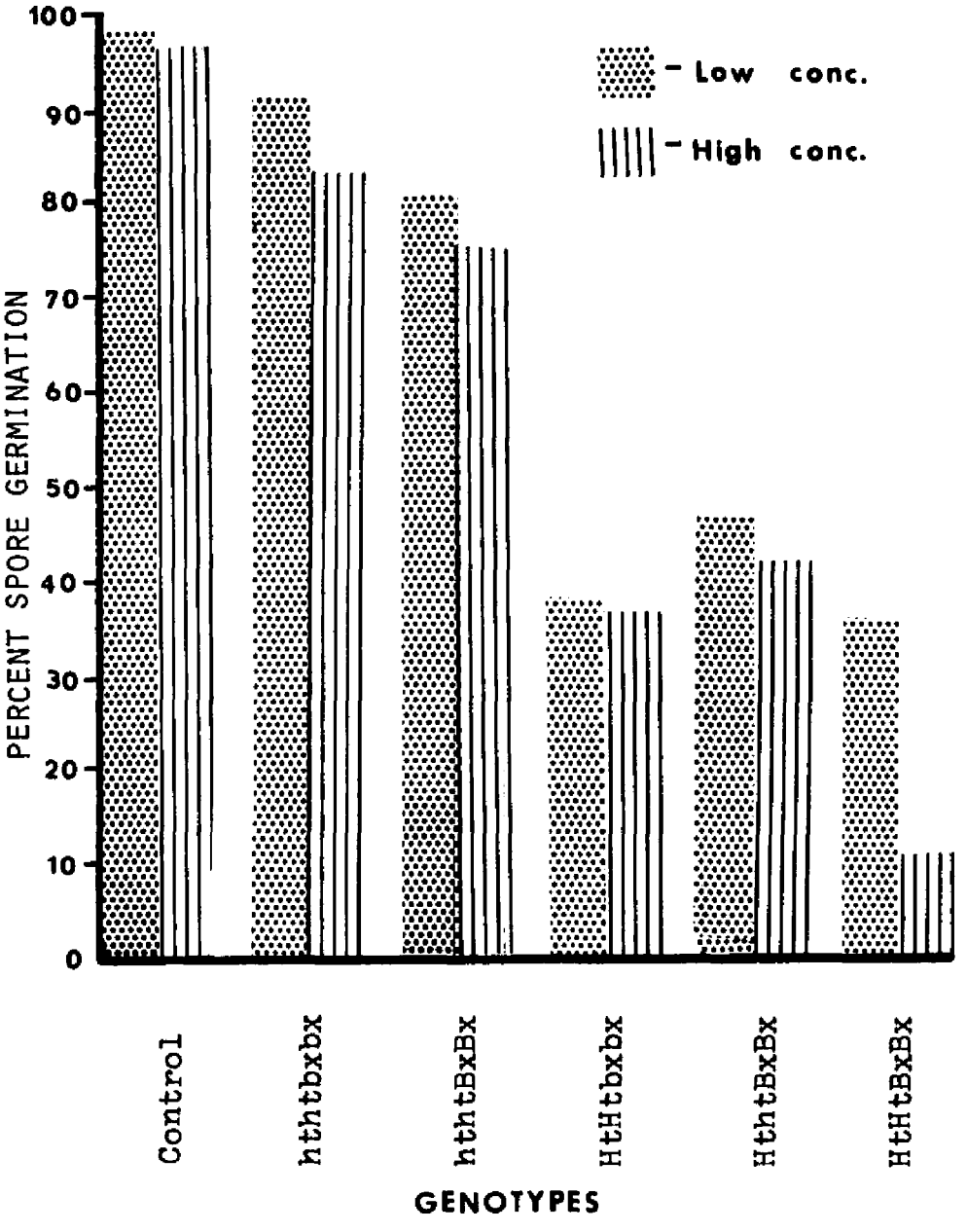


Figure10. Effect of genotype and inoculum concentration on percent spore germination.

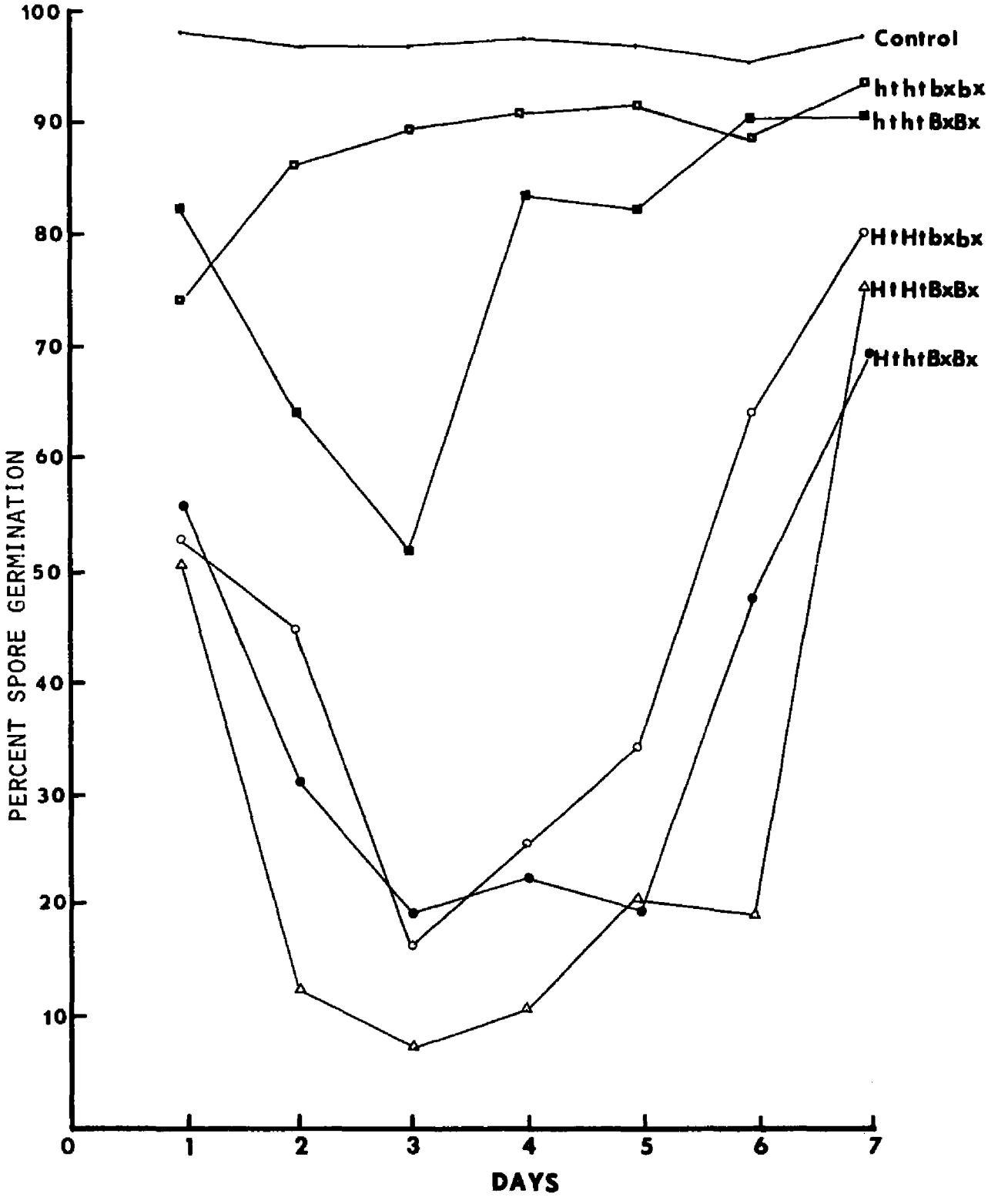


Figure 11. Effect of genotypes and time of phytoalexin production on percent spore germination.

third day, then disappeared. In most instances, the susceptible lines behaved like the control.

Measurement of germ tube growth showed a less pronounced effect (table 10). The resistant genotypes did not differ significantly from each other. However, they gave significantly shorter germ tube than the susceptibles and control.

Effect of inoculation temperature. The effect of temperature during the inoculation period on the production of phytoalexin is shown in tables 12 and 14. Tables 13 and 15 present the analysis of variance for the percent spore germination and germ tube length, respectively.

Table 12. Percent spore germination at 2 inoculation temperatures on attached leaf. (Average of 2 experiments, with 2 replications each)

Temperatures	Genotypes	Days of inoculation				
		1	2	3	4	5
16°C	HtHtBxBx	75.0	57.7	22.8	50.4	54.3
	HthtBxBx	79.4	69.0	49.6	57.6	63.5
	hthtBxBx	81.7	74.8	71.9	68.9	79.3
	hthtbxbx	87.4	81.7	81.6	81.2	82.2
	Control	84.3	85.5	86.3	85.0	93.6
27-34°C	HtHtBxBx	74.2	62.7	20.6	53.0	54.2
	HthtBxBx	76.2	67.1	46.2	72.2	67.0
	hthtBxBx	73.9	71.4	70.8	77.6	75.4
	hthtbxbx	85.0	79.3	80.6	80.0	80.8
	Control	88.2	88.6	88.0	87.8	90.2

Table 13. Analysis of variance and Duncan's multiple range test of the means of table 12.

Source of Variation	d.f.	M.S.	F
Days	4	916.97	54.55**
Temperature	1	39.31	2.33^{NS}
Temp. x Day	4	145.95	8.68**
Genotype	4	3935.49	234.15**
Gen. x Day	16	282.13	16.78**
Gen. x Temp.	4	34.58	2.05^{NS}
Gen. x Temp. x Day	16	105.75	6.29**
Replication	1	1.66	0.09^{NS}
Error	49	16.80	
Total	99		

** Significant at 0.01 level; NS - not significant

MEANS

HtHtBxBx	HthtBxBx	hthtBxBx	hthtbxbx	Control
52.5^{a}	64.6^{b}	74.6^{c}	82.0^{d}	87.8^{e}

Day 3	Day 2	Day 5	Day 4	Day 1
61.8^{a}	71.4^{b}	73.8^{b}	73.8^{b}	80.0^{c}

27-32^{o}C	16^{o}C
71.6 ^{a}	72.9^{a}

Means not followed by the same letter differ significantly at 1% level.

Table 14. Germ tube length of H. turcicum at 2 inoculation temperatures on attached leaf. (Average of 2 experiments, with 2 replications each, in microns).

Temperatures	Genotypes	Days of inoculation				
		1	2	3	4	5
16°C	HtHtBxBx	160.2	144.4	41.8	47.2	128.6
	HthtBxBx	220.5	193.2	105.0	144.4	157.5
	hthtBxBx	154.8	144.4	91.8	160.1	283.5
	hthtbxbx	199.5	217.8	183.7	196.8	328.1
	Control	422.6	196.8	385.8	320.2	396.4
21-32°C	HtHtBxBx	183.7	136.4	49.8	55.1	126.0
	HthtBxBx	244.1	165.4	81.4	157.4	131.1
	hthtBxBx	165.4	147.0	107.6	133.8	272.8
	hthtbxbx	244.1	196.8	173.0	210.0	307.0
	Control	417.4	386.4	343.8	328.7	399.1

Table 15. Analysis of variance and Duncan's multiple range test of the means of table 14.

Source of Variation	d.f.	M.S.	F
Days	4	34598.53	82.79**
Temperature	1	9.85	0.02^{NS}
Temp. x Day	4	5924.76	14.17**
Genotype	4	225329.00	539.19**
Gen. x Day	16	5908.12	14.13**
Gen. x Temp.	4	17875.39	42.77**
Gen. x Temp. x Day	16	7909.00	18.92**
Replication	1	345.94	0.82^{NS}
Error	49	417.90	
Total	99		

** Significant at 0.01 level; NS - not significant

MEANS

HtHtBxBx	HthtBxBx	hthtBxBx	hthtbxbx	Control
107.3^{a}	160.0^{b}	166.1^{b}	225.7^{c}	382.8^{d}

Day 3	Day 4	Day 2	Day 1	Day 5
156.4^{a}	175.4^{b}	215.9^{c}	241.2^{d}	253.0^{d}

16°C	27-32°C
208.1^{a}	208.7^{a}

Means not followed by the same letter differ significantly at 1% level.

The two inoculation temperatures did not significantly affect the production of phytoalexin. Similar results were observed from the percent spore germination (table 12) and germ tube length (table 14). The behavior of genotypes behaved similarly to that shown in previous experiments. The resistant genotypes inhibited spore germination and produced much shorter germ tubes than did the susceptible lines and the control. Production of phytoalexin increased up to the third day then decreased considerably.

While no significant difference was found between the two inoculation temperatures, several problems were encountered with the higher temperature. At 27-32^{o}C, it was more difficult to maintain the drop suspension on the leaves. Even with complete saturation of the inoculating chamber, drying of the drop suspension occured. Inoculation at this temperature is therefore not recommended.

Effect of preconditioned temperature. The effect of preconditioning temperature upon the induction of phytoalexin is shown in tables 16 and 18. Results for percent spore germination are listed in table 16. The germ tube growth is shown in table 18. Their corresponding analyses of variance are presented in tables 17 and 19, respectively.

Table 16. Percent spore germination at two pre-conditioning temperatures on attached leaf at the 8-9th leaf stage (Average of 3 experiments, with 2 replications each).

Temperatures	Genotypes	Days after inoculation						
		1	2	3	4	5	6	7
27-32°C	HtHtBxBx	72.6	47.4	17.6	48.4	15.3	16.0	75.4
	HthtBxBx	79.9	46.8	52.9	54.9	50.6	28.6	88.4
	HtHtbxbx	81.1	47.4	18.0	45.8	46.0	32.8	84.2
	hthtBxBx	80.7	60.8	83.8	76.2	78.0	77.0	81.2
	hthtbxbx	88.2	82.6	85.6	77.7	84.9	86.0	87.2
	Control	93.2	93.2	94.1	93.9	93.2	95.2	95.9
21°C	HtHtBxBx	69.3	28.0	14.6	17.2	18.5	13.2	20.0
	HthtBxBx	78.2	36.4	19.8	50.3	28.1	24.6	17.2
	HtHtbxbx	75.5	46.0	24.0	44.8	28.2	22.6	21.7
	hthtBxBx	87.0	77.6	28.5	74.5	53.2	49.1	79.6
	hthtbxbx	88.6	87.0	79.4	85.6	85.0	84.2	86.3
	Control	86.0	92.4	89.2	92.0	90.0	91.9	93.0

Table 17. Analysis of variance and Duncan's multiple range test of the means of table 16.

Source of Variation	d.f.	M.S.	F
Days	6	2680.47	290.62**
Pre-Temp.	1	10998.16	1192.45**
Pre-Temp. x Day	6	270.46	29.32**
Genotype	5	14682.44	1591.92**
Gen. x Day	30	533.31	57.82**
Gen. x Pre-Temp.	5	667.88	72.41**
Gen. x Pre-Temp. x Day	30	176.53	19.14**
Replication	1	0.35	0.03^{NS}
Error	83	9.22	
Total	167		

** Significant at 0.01 level; NS - not significant.

MEANS

HtHtBxBx	HtHtbxbx	HthtBxBx	hthtBxBx	hthtbxbx	Control
33.8^{a}	44.2^{b}	46.9^{c}	72.1^{d}	84.9^{e}	92.4^{f}

Day 6	Day 3	Day 5	Day 2	Day 4	Day 7	Day 1
51.8^{a}	52.5^{a}	55.9^{b}	62.1^{c}	63.4^{c}	69.2^{d}	81.7^{e}

21°C	27-32°C
57.2^{a}	67.6^{b}

Means not followed by the same letter differ significantly at 1% level.

Table 18 Germ tube length of H. turcicum at two pre-conditioning temperatures on attached leaf at the 8-9th leaf (Average of 3 experiments, with 2 replications each, in microns).

Temperatures	Genotypes	Days after inoculation						
		1	2	3	4	5	6	7
27-32°C	HtHtBxBx	127.8	126.8	66.5	54.2	35.0	47.2	68.2
	HthtBxBx	195.0	143.5	143.5	141.8	91.0	76.1	314.9
	HtHtbxbx	204.8	245.0	112.0	157.5	119.0	86.6	199.5
	hthtBxBx	292.2	113.8	59.5	106.8	98.0	89.2	121.0
	hthtbxbx	234.5	190.8	169.8	110.2	110.2	73.5	99.8
	Control	292.4	308.0	332.5	344.8	306.2	299.2	420.0
21°C	HtHtBxBx	63.0	113.8	54.2	70.0	40.2	42.0	35.8
	HthtBxBx	143.5	96.2	94.5	82.2	77.0	52.5	47.2
	HtHtbxbx	147.0	75.2	71.8	61.2	82.2	47.1	40.5
	hthtBxBx	190.8	68.2	119.0	115.5	61.2	49.8	57.8
	hthtbxbx	304.5	89.2	157.2	92.8	99.8	81.4	199.5
	Control	434.0	339.0	339.5	577.6	360.5	304.5	477.8

Table 19. Analysis of variance and Duncan's multiple range test of the means of table 18.

Source of Variation	d.f.	M.S.	F
Days	6	30642.65	13.52**
Pre-Temp.	1	336027.12	148.46**
Pre-Temp. x Day	6	6577.77	2.90**
Genotype	5	202807.25	89.60**
Gen. x Day	30	4290.36	1.89^{NS}
Gen. x Pre-Temp.	5	89421.12	39.50**
Gen. x Pre-Temp. x Day	30	5157.16	2.27**
Replication	1	5289.76	2.33^{NS}
Error	83	2263.40	
Total	167		

** Significant at 0.01 level; NS - not significant.

MEANS

HtHtBxBx	hthtBxBx	HtHtbxbx	HthtBxBx	hthtbxbx	Control
67.5^{a}	110.2^{b}	117.8^{b}	121.4^{b}	149.1^{c}	371.1^{d}

Day 6	Day 5	Day 3	Day 4	Day 2	Day 7	Day 1
110.6^{a}	123.4^{a}	142.9^{b}	159.6^{b}	164.1^{c}	173.5^{c}	219.1^{d}

21°C	27-32°C
145.5^{a}	166.8^{b}

Means not followed by the same letter differ significantly at 1% level.

Bioassay on corn leaves pre-conditioned at two temperature levels before inoculation influenced the rate and amount of phytoalexin produced. Highly significant differences were obtained for both percent germination and germ tube length.

Means of the genotypes were significantly different from each other with HtHtBxBx inhibiting about 66% germination based on the control. The effects of pre-conditioning temperatures and genotypes upon the rate and production of phytoalexin are shown in figure 12. The bars clearly indicated that the resistant genotypes at 21°C synthesized phytoalexin faster and in a larger amount than at 27-32°C. No significant differences were observed with the susceptible genotypes at these two different temperatures. Similarly, the control did not differ at these temperatures.

Somewhat erratic results were obtained in determining the time of production of phytoalexin. Percent spore germination and germ tube growth indicated that phytoalexin reached its peak on the sixth day. This is contrary to the previous findings. The result of this particular experiment showed that phytoalexin was synthesized increasingly up to the third and sixth day, but disappeared on the seventh day. The reason for this is not known, but could be attributed to sampling of the drop suspension.

Effect of the stage of plant growth. The effects of two stages of plant growth on the production of phytoalexin are shown in tables 20, 21, 22 and 23. The percent spore germination and its analysis are presented in tables 20 and 21.

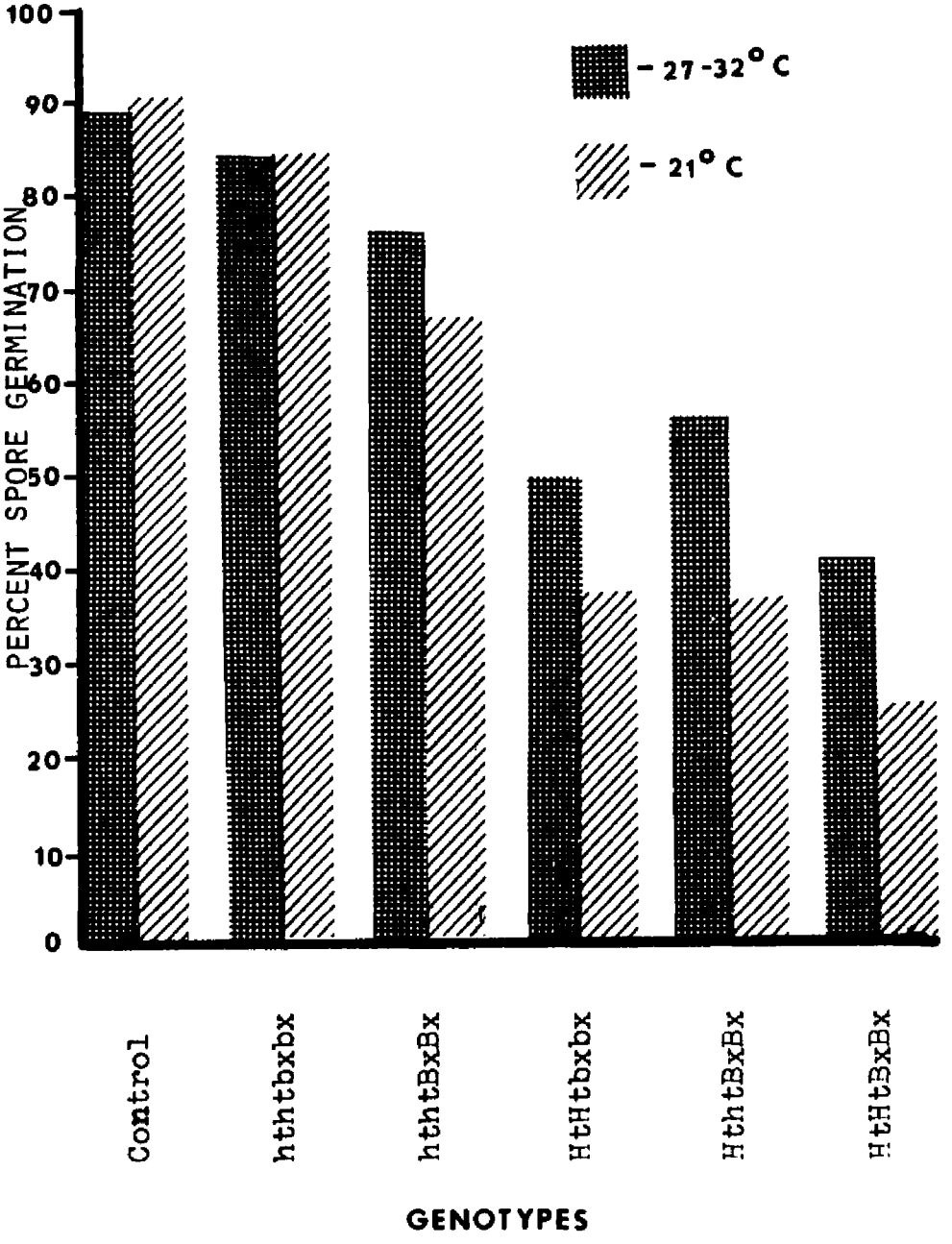


Figure 12. Genotype x pre-conditioned temperature interaction on percent spore germination.

Table 20. Percent spore germination for 2 stages of plant growth on the attached leaf (Average of 2 experiments, with 2 replications each).

Genotypes	6-7th leaf stage					9-10th leaf stage				
	Days after inoculation					Days after inoculation				
	1	2	3	4	5	1	2	3	4	5
HtHtBxBx	74.6	51.4	40.8	36.0	19.8	41.8	36.8	29.4	46.2	73.7
HthtBxBx	83.4	48.0	48.8	50.8	49.2	77.6	62.0	80.8	54.6	79.9
HtHtbxbx	76.4	53.1	34.8	24.2	29.0	79.2	78.4	44.8	36.4	68.3
hthtBxBx	83.5	64.0	80.9	78.4	77.0	88.2	87.8	61.8	75.8	83.8
hthtbxbx	76.6	84.2	86.3	81.7	89.1	90.0	86.9	87.8	87.9	88.8
Control	93.4	92.7	94.4	94.6	95.2	92.6	90.3	96.0	95.2	94.7

Table 21. Analysis of variance and Duncan's multiple range test of the means of table 20.

Source of Variation	d.f.	M.S.	F
Days	4	952.77	149.26**
Stages of growth	1	1411.10	221.06**
Stage x Day	4	512.12	88.22**
Genotypes	5	7458.81	1168.50**
Gen. x Day	20	290.87	45.56**
Gen. x Stage	5	293.48	45.97**
Gen. x Stage x Day	20	269.83	42.27**
Replication	1	87.55	13.71**
Error	59	6.38	
Total	119		

** Significant at 0.01 level.

MEANS

HtHtBxBx	HtHtbxbx	HthtBxBx	hthtBxBx	hthtbxbx	Control
45.0[a]	52.4[b]	63.5[c]	78.1[d]	85.9[e]	93.9[f]

Day 4	Day 3	Day 2	Day 5	Day 1
63.9[a]	65.6[b]	69.6[c]	70.7[c]	94.4[d]

6-7th leaf	9-10th leaf
66.4[a]	73.3[b]

Means not followed by the same letter differ significantly at 1% level.

Table 22. Germ tube length of H. turcicum for 2 stages of plant growth on attached leaf (Average of 2 experiments, with 2 replications in microns).

Genotypes	6-7th leaf stage					9-10th leaf stage				
	Days after inoculation					Days after inoculation				
	1	2	3	4	5	1	2	3	4	5
HtHtBxBx	60.4	62.8	42.0	26.0	26.2	53.8	94.5	81.4	76.1	68.2
HthtBxBx	131.2	81.4	49.8	49.8	52.4	157.4	110.2	86.6	70.8	105.0
HtHtbxbx	49.8	49.8	44.6	37.2	47.2	118.1	110.2	157.4	44.6	41.9
hthtBxBx	105.0	63.0	49.8	42.0	78.8	118.1	70.8	55.1	109.4	112.8
hthtbxbx	120.7	102.4	105.0	91.8	91.8	128.6	105.0	81.4	86.6	120.8
Control	299.2	357.0	354.4	375.4	246.8	325.5	320.2	254.6	296.6	291.4

Table 23. Analysis of variance and Duncan's multiple range test of the means of table 22.

Source of Variation	d.f.	M.S.	F
Days	4	4480.16	15.52**
Stage of Growth	1	10419.74	36.10**
Stage x Day	4	510.59	1.76^{NS}
Genotypes	5	183438.50	635.69**
Gen. x Day	20	1894.70	6.56**
Gen. x Stage	5	3850.48	13.34**
Gen. x Stage x Day	20	1497.61	5.18**
Replication	1	4008.98	13.89**
Error	59	288.56	
Total	119		

** Significant at 0.01 level; NS - not significant.

MEANS

HtHtBxBx	HtHtbxbx	HthtBxBx	hthtBxBx	hthtbxbx	Control
59.1^{a}	64.5^{a}	80.8^{b}	89.5^{b}	103.4^{c}	312.1^{d}

Day 5	Day 3	Day 4	Day 2	Day 1
107.0^{a}	108.9^{a}	109.1^{a}	127.3^{b}	139.0^{c}

6-7th leaf	9-10th leaf
109.8^{a}	126.7^{b}

Means not followed by the same leter differ significantly at 1% level.

The germ tube length measurements are given in tables 22 and 23. The seedlings in the 6th-7th leaf stage were 23 days old, while in the 8-9th leaf stage they were 37 days old. Both groups of plants were grown in the greenhouse prior to inoculation.

Plant age affected the production of phytoalexin. The 6-7th leaf stage inhibited germination 34% as compared to 26% at the 9-10th leaf stage. The difference is highly significant. The same pattern was observed with the germ tube length (table 22 and 23). The relative effect of genotype on percent spore germination was similar to previous experiments. Production of phytoalexin decreased in the following order: HtHtBxBx, HtHtbxbx, HthtBxBx, hthtBxBx, hthtbxbx, and control. Differences among genotypes were all highly significant. Germ tube growth gave a similar pattern except that the first and second genotypes and the third and fourth genotypes were not different form each other. Maximum production of phytoalexin was observed on the third and fourth day after inoculation.

Further effects of stages of growth are shown in the following tables. The percent germination is given in table 24 and its analysis in table 25. Germ tube length is given in table 26 and its analysis in table 27. The young stage (5-6th leaf stage) was 15 days old, the mature stage (8-9th leaf) was 34 days old, and the old satge (11-12th leaf) was 49 days old. The 11-12th leaf plants were tasseling at this stage.

Table 24. Percent spore germination at 3 stages of plant growth on the attached leaf (Average of 2 experiments, with 2 replications each).

Leaf Stages	Genotypes	Days after inoculation						
		1	2	3	4	5	6	7
5-6th	HtHtBxBx	52.0	51.8	17.2	38.6	27.7	14.0	22.0
	HthtBxBx	87.8	72.4	56.2	46.6	50.3	20.0	32.8
	HtHtbxbx	60.4	67.4	19.8	53.4	41.8	73.4	72.5
	hthtBxBx	81.9	83.0	84.2	82.3	85.1	80.0	82.4
	hthtbxbx	85.8	87.6	88.8	85.7	83.9	87.0	90.0
	Control	96.0	94.2	92.6	92.2	93.0	92.4	95.0
8-9th	HtHtBxBx	76.8	67.6	25.7	53.6	21.2	10.8	75.4
	HthtBxBx	84.6	80.8	80.1	79.0	77.6	29.4	88.4
	HtHtbxbx	89.2	77.1	52.3	79.9	78.1	33.2	84.2
	hthtBxBx	85.0	81.2	89.8	82.6	85.0	75.2	81.2
	hthtbxbx	91.3	88.8	88.3	87.0	89.4	81.2	87.2
	Control	95.4	97.8	96.5	95.4	93.6	94.0	95.9
11-12th	HtHtBxBx	60.0	49.2	44.8	22.4	54.2	23.4	25.4
	HthtBxBx	79.3	79.8	61.8	50.5	71.4	30.1	75.5
	HtHtbxbx	69.8	65.8	78.8	82.6	80.0	37.2	75.8
	hthtBxBx	87.9	87.4	90.2	87.6	80.4	86.2	84.4
	hthtbxbx	92.2	89.6	91.7	92.6	89.8	90.9	90.8
	Control	90.2	94.8	95.2	94.4	93.1	96.6	96.0

Table 25. Analysis of variance and Duncan's multiple range test of the means of table 24.

Source of Variation	d.f.	M.S.	F
Days	6	1956.51	212.40**
Stages of Growth	2	7636.60	829.06**
Stage x Day	12	207.59	22.53**
Genotype	5	14894.51	1617.02**
Gen. x Day	30	592.22	64.29**
Gen. x Stage	10	491.68	53.37**
Gen. x Stage x Day	60	188.57	20.47**
Replication	1	63.79	6.92**
Error	125	9.21	
Total	251		

** Significant at 0.01 level.

MEANS

HtHtBxBx	HthtBxBx	HtHtbxbx	hthtBxBx	hthtbxbx	Control
39.7[a]	57.1[b]	65.4[c]	83.9[d]	88.5[e]	94.5[f]

Day 6	Day 3	Day 5	Day 4	Day 7	Day 2	Day 1
58.6[a]	69.6[b]	71.8[c]	72.5[c]	75.3[d]	78.7[d]	81.4[e]

5-6th leaf	8-9th leaf	11-12th leaf
67.2[a]	76.3[b]	74.3[c]

Means not followed by the same letter differ significantly at the 1% level.

Table 26. Germ tube length of H. turcicum at 3 stages of plant growth on attached leaf. (Average of two experiments, with two replications each, in microns).

Leaf Stage	Genotypes	Days after inoculation						
		1	2	3	4	5	6	7
5-6th	HtHtBxBx	76.2	42.0	42.0	52.8	28.6	15.8	26.2
	HthtBxBx	160.1	65.6	42.0	42.0	23.6	**26.2**	32.0
	HtHtbxbx	78.7	55.1	47.1	88.4	101.4	273.0	325.5
	hthtBxBx	183.6	68.2	44.6	42.0	78.7	97.2	92.0
	hthtbxbx	110.0	112.8	76.1	91.8	**91.8**	110.2	136.5
	Control	372.8	372.8	273.0	427.8	372.8	425.2	420.0
8-9th	HtHtBxBx	149.6	112.8	76.1	68.2	36.8	69.2	118.2
	HthtBxBx	231.0	238.8	183.8	178.5	100.0	97.2	315.0
	HtHtbxbx	275.6	338.6	136.5	210.0	141.8	94.5	199.5
	hthtBxBx	391.0	144.4	57.7	139.2	89.2	84.0	121.0
	hthtbxbx	294.0	236.2	196.8	118.1	112.8	105.0	997.5
	Control	338.6	383.2	404.2	307.0	349.1	378.0	420.0
11-12th	HtHtBxBx	84.0	107.6	160.1	65.6	105.0	57.8	92.2
	HthtBxBx	133.8	254.6	165.4	99.7	136.6	99.5	139.2
	HtHtbxbx	199.5	275.6	199.5	217.8	212.6	147.0	120.8
	hthtBxBx	181.1	186.4	228.4	194.2	168.0	294.0	247.2
	hthtbxbx	215.4	291.4	283.4	246.8	168.0	157.5	230.8
	Control	278.1	357.0	320.2	361.4	135.4	350.2	320.2

Table 27. Analysis of variance and Duncan's multiple range test of the means of table 26.

Source of Variation	d.f.	M.S.	F
Days	6	19994.65	25.17**
Stages of Growth	2	565674.00	712.11**
Stage x Day	12	8463.12	10.65**
Genotypes	5	168518.43	212.14**
Gen. x Day	30	8894.27	11.19**
Gen. x Stage	10	42144.47	53.05**
Gen. x Stage x Day	60	5401.01	6.79**
Replication	1	1361.75	1.71 NS
Error	125	794.35	

** Significant at 0.01 level; NS - not significant.

MEANS

HtHtBxBx	HthtBxBx	hthtBxBx	hthtbxbx	HtHtbxbx	Control
74.8 a	108.6 b	146.5 c	165.7 d	178.0 e	362.9 f

Day 5	Day 6	Day 3	Day 4	Day 7	Day 2	Day 1
148.4 a	159.9 a	163.2 a	165.4 a	192.0 b	202.4 b	202.5 b

5-6th leaf	8-9th leaf	11-12th leaf leaf
133.0 a	194.9 b	202.2 c

Means not followed by the same letter differ significantly at the 1% level.

Inoculation of plants at the young stage induced production of more phytoalexin than inoculation in the older plants. Results of the bioassay showed that tasseling plants (11-12th leaf stage) produced less phytoalexin as evidenced by the higher percent germination and longer germ tube length.

Among the genotypes this was the first instance that the heterozygous **resistant normal** (HthtBxBx) outranked the homozygous **resistant deficient** (HtHtbxbx). This was true for both the percent spore germination and germ tube length. The other genotypes behaved as before in ranked means. The effect of stages in plant growth and genotype on the percent spore germination is illustrated in Figure 13. The graph distinctly indicates that the resistant genotypes at the 5-6th leaf stage are consistently lower germinating than the 8-9th or the 11-12th leaf stage. The susceptible genotypes showed a similar trend, but their differences were not significant.

The genotypic means from the entire experiments involving the HtBx genes are shown in figure 14. Briefly it could be described as a right triangle with the longest side representing the control and the shortest side representing the homozygous resistant normal genotype. Figure 15 represents the average of sixteen experiments involving the HtBx genes on the time of phytoalexin production as measured by percent spore germination and germ tube length. The shape of the bar graph could be described as a U or V shaped.

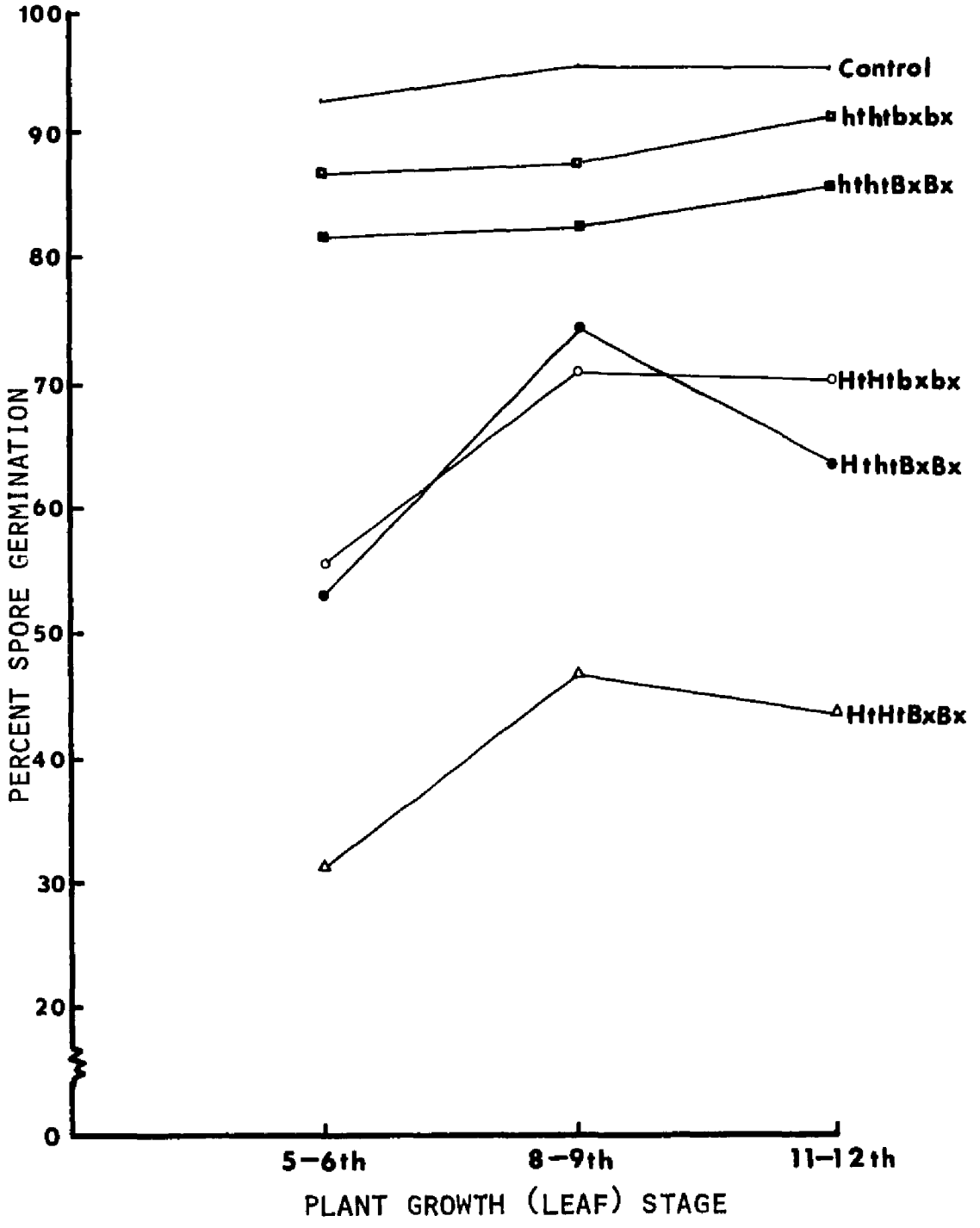


Figure 13. Relationship of percent spore germination and different stages of plant growth on the plant genotypes.

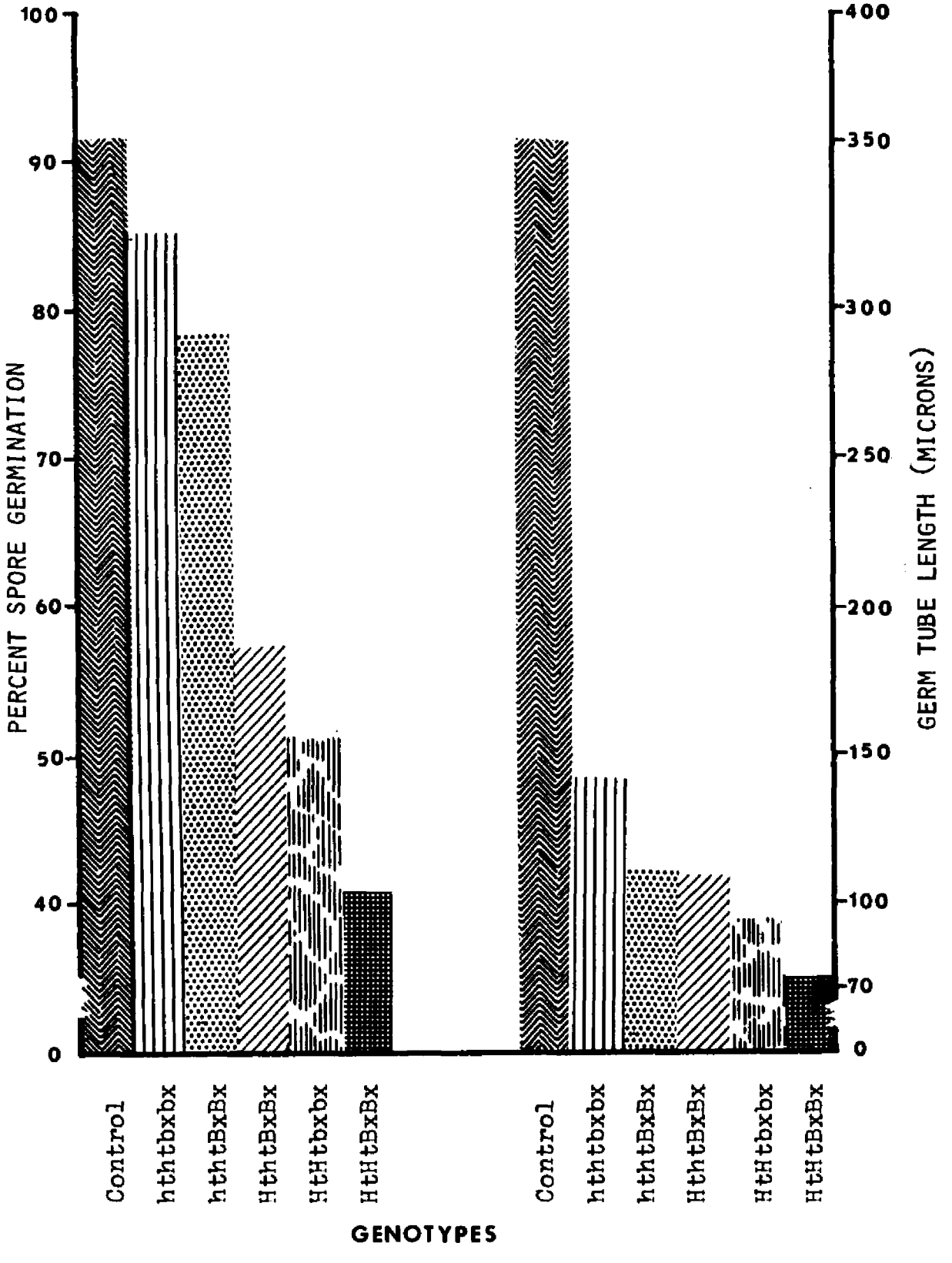


Figure 14. Average percent germination and germ tube length of 16 experiments on the different genotypes.

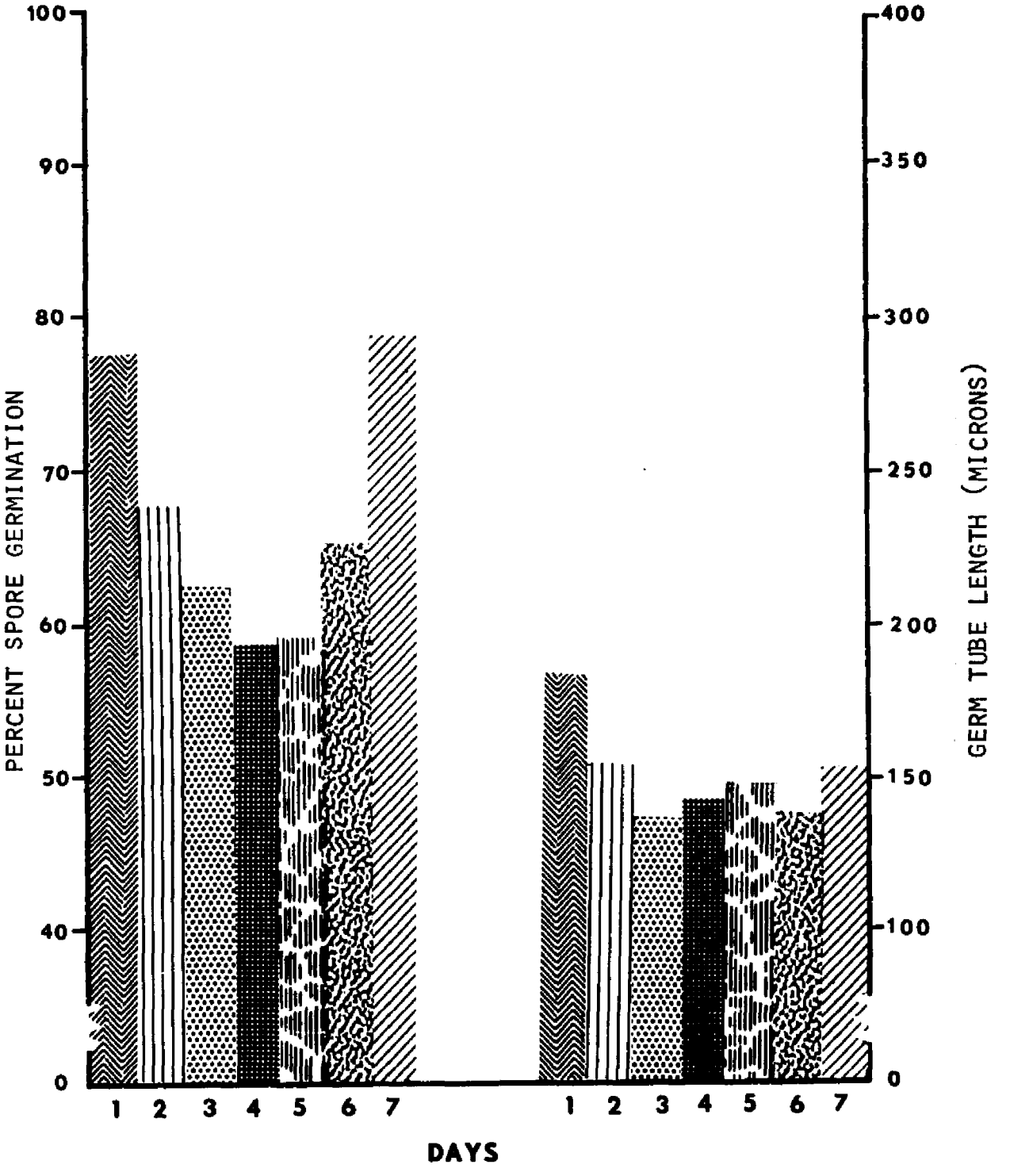


Figure 15. Average percent spore germination and germ tube length of 16 experiments on time of phytoalexin production.

Influence of Ht background on phytoalexin production.

Tables 28 and 30 show the percent spore germination and germ tube length, respectively, of the various resistant and susceptible monogenic lines. The corresponding analyses of variance and comparison of the means are shown in tables 29 and 31. Comparisons of the different monogenic lines were made on three levels: dominant homozygous (HtHt), dominant heterozygous (Htht), and recessive (htht).

Means of the three levels of resistance show phytoalexin production to be greatest in the HtHt lines, followed by Htht and htht in that order. These differences are highly significant (table 29). Similar results were obtained for germ tube length (table 31). Further observation revealed that while the HtHt lines produced more phytoalexin than Htht lines, the rate and trend of production were the same (figure 16). The control shown in figure 16 was based on a simultaneous experiment with the various lines but it was not included in the data.

The interaction between the Ht background or line and level of resistance was interesting. In the HtHt level line 65-225-1 produced significantly more phytoalexin (table 29) than any other line. Lines RHy-2Ht and RW64Ht produced less phytoalexin. This relationship, however, did not hold true for the germ tube length (table 31), since line 65-225-1 gave the longest tube length. It should be pointed out that germ tube length is not as reliable as percent spore germination in evaluating phytoalexin production

Table 28. Percent spore germination in bioassay test from diffusates of attached leaves of various resistant and susceptible monogenic lines following incorporation of the Ht gene on 65 days old seedlings. (Average of 4 experiments with 2 replications each).

Type of resistance	Lines	Days after inoculation						means
		1	2	3	4	5	6	
Dominant homozygous (HtHt)	65-225-1	33.6	27.8	14.7	19.4	21.0	21.2	22.9
	RW64Ht	42.9	36.7	17.4	44.4	46.9	54.6	40.4
	RWF9Ht	51.4	34.0	12.8	15.4	18.2	67.6	32.3
	RHy-2Ht	41.8	33.6	14.8	27.7	34.5	82.0	39.1
	ROh43Ht	45.0	32.8	13.4	26.0	21.7	61.6	33.4
	means	42.9	33.0	14.6	26.6	28.5	57.4	
Dominant heterozygous (Htht)	65-225-1	60.0	54.0	44.1	45.4	37.0	68.4	51.6
	RW64Ht	58.7	45.2	31.8	51.4	66.6	73.5	54.5
	RWF9Ht	62.4	48.2	24.8	22.8	27.8	72.1	43.0
	RHy-2Ht	67.8	54.8	33.9	45.0	53.4	84.4	56.6
	ROh43Ht	53.2	44.2	20.1	28.5	33.0	70.0	41.5
	means	60.4	49.4	31.0	38.6	42.8	73.7	
Recessive (htht)	65-225-1*	79.1	78.2	74.3	80.0	82.0	78.2	78.6
	W64	79.7	82.6	83.3	87.6	88.8	88.8	85.1
	WF9	81.2	88.2	89.5	89.4	89.8	91.6	88.3
	Hy	86.4	84.0	81.0	88.0	87.2	89.8	86.1
	Oh43	85.6	86.4	80.0	83.3	80.8	83.0	83.2
	means	82.4	83.9	81.6	85.6	85.7	86.3	

* = This is actually W153R which is isogenic to 65-225-1(HtHt)

Table 29. Analysis of variance and Duncan's multiple range test of the means of table 28.

Source of variation	d.f.	M.S.	F
Day	5	3216.51	115.88**
Resistance	2	2417.07	87.08**
Resist. x Day	10	41.48	1.49*
Lines	4	16610.80	598.42**
Line x Day	20	299.78	10.80**
Line x Resist.	8	1727.63	62.24**
Line x Resist. x Day	40	194.70	7.01**
Replication	1	0.02	0.01 NS
Error	89	27.76	
Total	179		

** Significant at 0.01 level; * Significant at 0.05 level; NS - not significant.

MEANS

Day 3	Day 4	DAy 5	Day 2	Day 1	Day 6
42.4[a]	50.3[b]	52.3[a]	55.4[c]	61.9[d]	72.5[e]

dominant homozygous	dominant heterozygous	recessive
33.6[a]	49.5[b]	84.3[c]

line x resistance

HtHt	Htht	htht
65-225-1 - 22.9[a]	ROh43Ht - 41.5[a]	65-225-1 - 78.6[a]
RWF9Ht - 32.3[b]	RWF9Ht - 43.0[a]	Oh43 - 83.2[b]
ROh43Ht - 33.4[b]	65-225-1- 51.6[b]	W64 - 85.1[b]
RHy-2Ht - 39.1[c]	RW64Ht - 54.5[b]	Hy - 86.1[b]
RW64Ht - 40.4[c]	RHy-2Ht - 56.6	WF9 - 88.3[b]

Means not followed by the same letter differ significantly at the 1% level.

Table 30. Germ tube length of H. turcicum in bioassay test from diffusates of attached leaves of various resistant and susceptible monogenic lines following incorporation of the Ht gene on 65 days old seedlings. (Average of 4 experiments with 2 replications each).

Type of resistance	Lines	Days after inoculation						means
		1	2	3	4	5	6	
Dominant homozygous (HtHt)	65-225-1	85.4	57.8	43.3	77.4	171.9	115.5	98.4
	RW64Ht	90.4	53.8	51.2	42.2	86.6	99.8	70.7
	RWF9Ht	143.3	52.4	22.3	18.4	38.0	43.6	53.0
	RHy-2Ht	126.8	94.5	40.7	40.7	53.7	57.2	68.9
	ROh43Ht	87.9	52.5	35.4	27.6	60.4	22.2	46.6
	means	106.7	62.2	38.6	41.3	82.1	67.7	
Dominant heterozygous (Htht)	65-225-1	124.6	124.7	49.8	44.6	168.3	142.0	109.0
	RW64Ht	136.5	120.8	89.8	45.4	152.8	169.7	119.2
	RWF9Ht	291.4	173.2	81.4	52.5	126.0	165.0	148.2
	RHy-2Ht	176.6	129.0	60.2	43.2	70.1	120.6	100.0
	ROh43Ht	81.0	123.4	118.2	91.1	107.3	119.8	106.8
	means	162.0	134.2	79.9	55.4	124.9	143.4	
Recessive (htht)	65-225-1*	164.0	169.3	120.7	144.4	152.3	136.5	147.8
	W64	243.1	173.2	118.2	200.8	241.6	241.8	203.1
	WF9	267.8	317.6	228.4	238.9	249.4	260.4	260.4
	Hy	266.4	172.0	116.8	112.9	136.5	299.3	184.0
	Oh43	199.5	169.3	109.0	95.2	158.8	144.4	146.0
	means	228.2	200.2	138.6	158.4	187.7	216.5	

* = This is actually W153R which is isogenic to 65-225-1(HtHt)

Table 31. Analysis of variance and Duncan's multiple range test of the means of table 30.

Source of variation	d.f.	M.S.	F
Day	5	31136.57	79.73**
Resistance	2	1535.10	3.93*
Resist. x Day	10	2391.58	6.12**
Lines	4	101007.12	258.63**
Line x Day	20	2552.03	6.53**
Line x Resist.	8	22624.76	57.93**
Line x Resist. x Day	40	2482.80	6.36**
Replication	1	9.47	0.02^{NS}
Error	89	390.54	
Total	179		

** Significant at 0.01 level; * significant at 0.05 level; NS - not significant.

MEANS

Day 4	Day 3	Day 5	Day 2	Day6	Day 1
85.0^{a}	85.7^{a}	131.6^{b}	132.0^{b}	142.5^{c}	165.6^{d}

dominant homozygous	dominant heterozygous	recessive
67.7^{a}	116.6^{b}	188.3^{c}

line x resistance

HtHt		Htht		htht	
ROh43Ht	- 47.6^{a}	RHy-2Htht	- 100.0^{a}	Oh43	- 146.0^{a}
RWF9Ht	- 53.0^{a}	ROh43Htht	- 106.0^{a}	65-225-1	- 147.8^{a}
RHy-2Ht	- 68.9^{b}	65-225-1	- 109.0^{a}	Hy	- 184.0^{b}
RW64Ht	- 70.7^{b}	RW64Htht	- 119.2^{a}	W64	- 203.1^{c}
65-225-1	- 98.4	RWF9Htht	- 148.2^{b}	WF9	- 260.4^{d}

Means not followed by the same letter differ significantly at the 1% level.

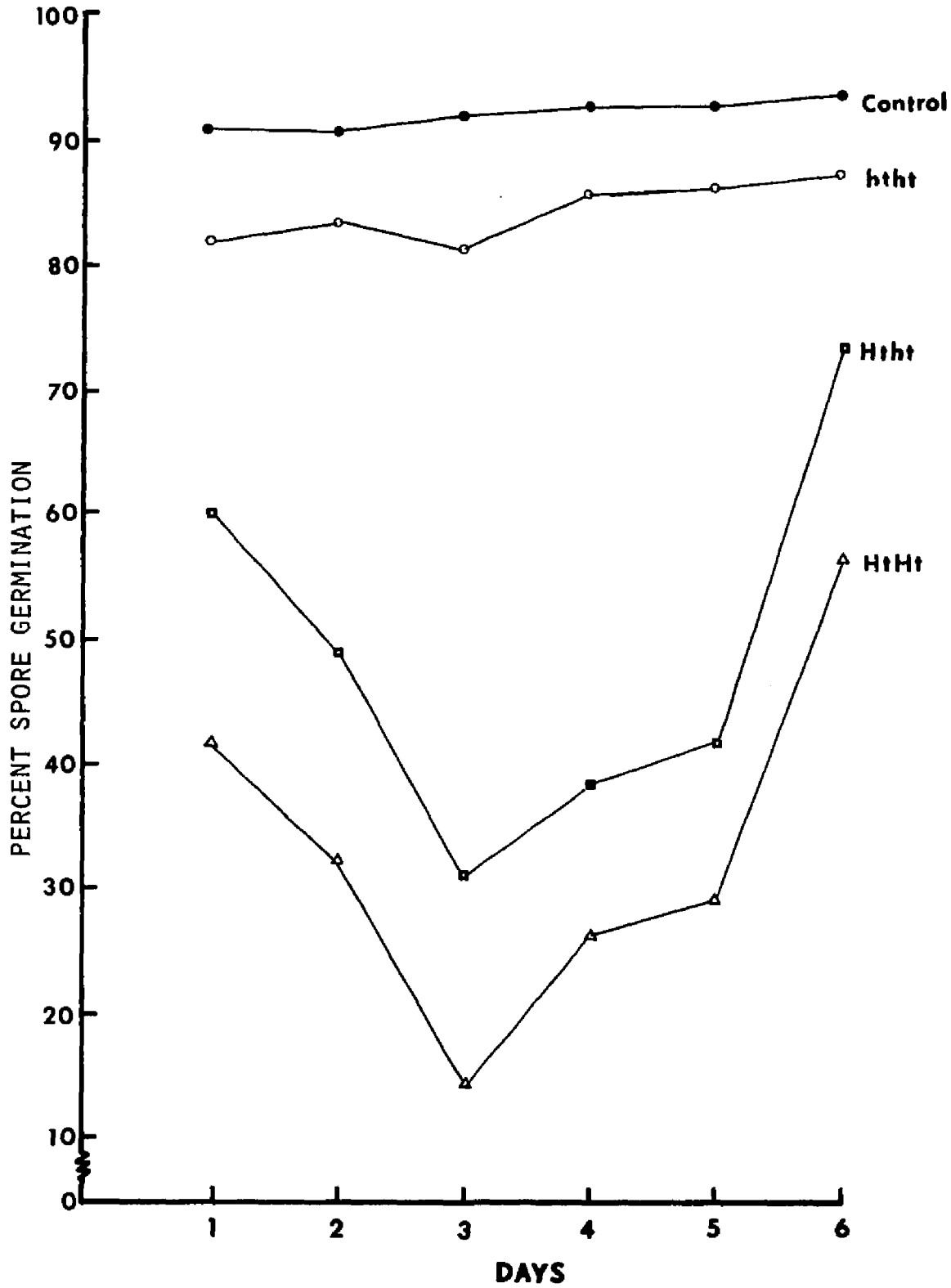


Figure 16. Effects of the different types of resistance and time of phytoalexin production on percent spore germination.

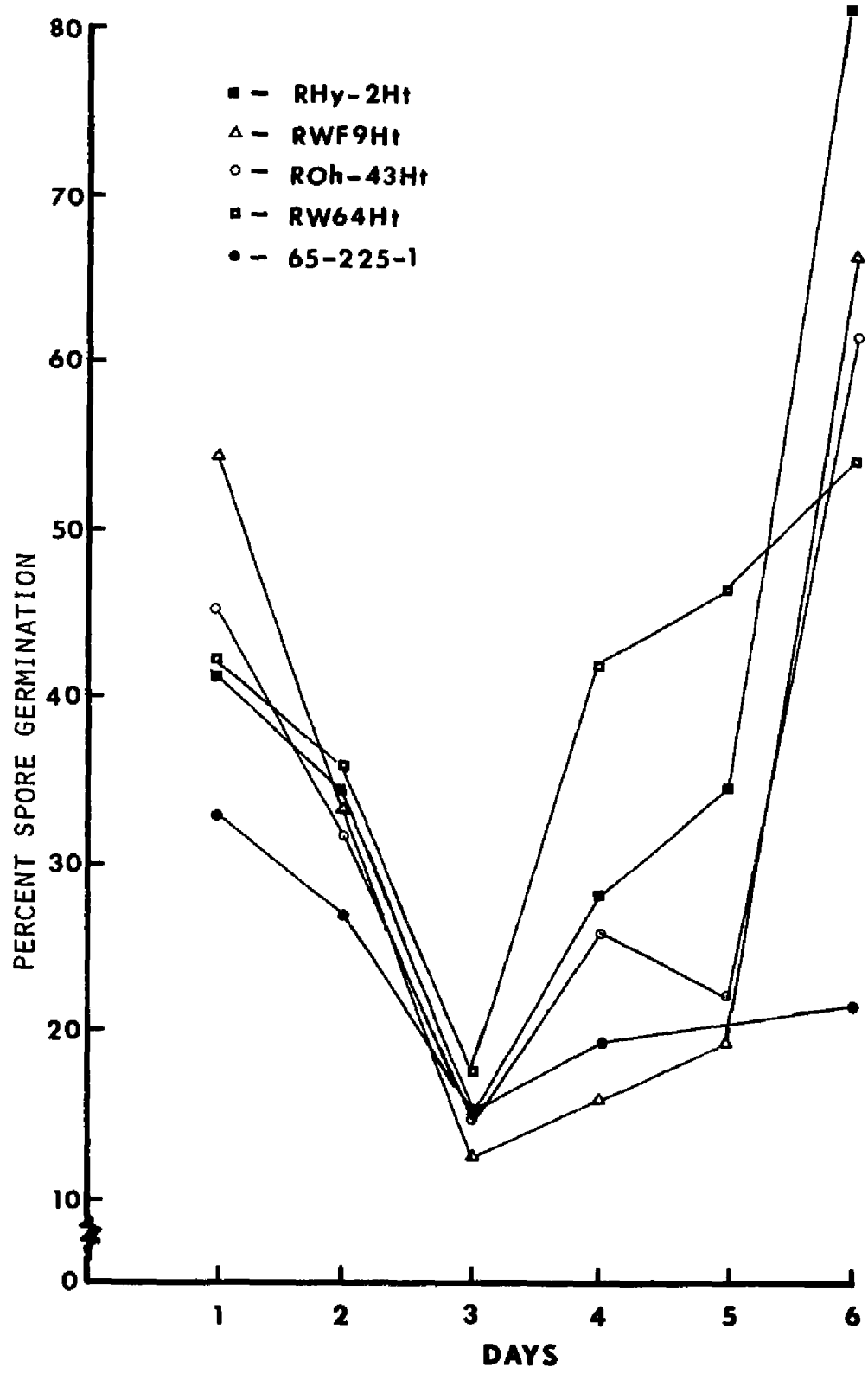


Figure 17. Comparison of the different types of monogenic resistant lines (Ht background) on the time of phytoalexin production and percent spore germination.

(see discussion). While differences were found among the different lines, particularly in the HtHt level, the pattern of phytoalexin development among these lines was similar. Figure 17 indicates that all the homozygous dominant lines reached their maximum phytoalexin production on the third day which gradually diminished until the sixth day. The exception was line 65-225-1 which apparently produced considerable phytoalexin until the sixth day.

Results in the heterozygous (Htht) level show that the same lines were less variable in the production of phytoalexin. This was indicated by reduced mean differences between lines. These differences were very small at the recessive (htht) level, suggesting little if any effect of the ht gene. The effect of the 3 levels of resistance on the mycelial growth of H. turcicum is illustrated in figure 18.

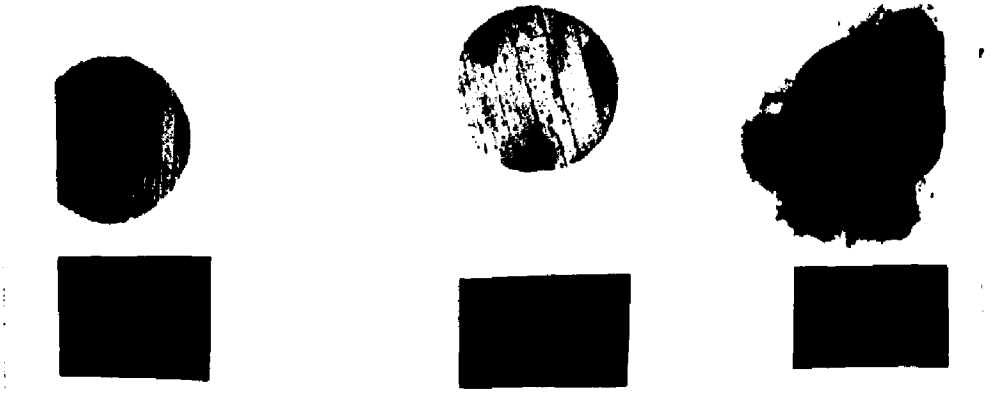


Figure 18. Mycelial growth of H. turcicum 36 hours after incubation on a nutrient agar medium. Lesions were taken from plants 9 days after inoculation.

Effect of type of disease inheritance on phytoalexin production.

The effects of monogenic, multigenic and mono-multigenic types of disease inheritance to H. turcicum on the production of inhibitory substances are shown in tables 32 and 34. Tables 33 and 35 present the analyses of variance for percent spore germination and germ tube length, respectively.

Table 32. Percent spore germination in bioassay tests from diffusates of attached leaves of monogenic, multigenic and mono-multigenic resistant lines on 65 day old seedlings. (Average of 4 experiments with 2 replications each).

Type of resistance	Lines	Days after inoculation						means
		1	2	3	4	5	6	
Monogenic	65-225-1	33.6	27.8	14.8	19.4	21.0	2.2	33.4
	RW64Ht	42.9	36.7	17.4	44.4	46.9	54.6	40.5
	means	38.2	32.3	16.1	32.0	33.9	37.9	
Multigenic	H55	14.8	13.8	15.9	11.0	18.9	28.5	17.2
	H49	13.6	13.3	15.8	18.4	18.3	35.0	19.1
	means	14.2	13.5	15.8	14.6	18.6	31.8	
Mono-multigenic	RH55Ht	47.4	40.5	30.6	16.4	28.7	34.0	32.9
	RH49Ht	46.0	41.1	24.4	20.0	19.0	28.4	29.8
	means	46.7	40.8	27.6	18.2	23.9	31.2	

Table 33. Analysis of variance and Duncan's multiple range test of the means of table 32.

Source of variation	d.f.	M.S.	F
Days	5	400.15	16.51**
Inheritance	2	1710.78	70.58**
Inherit. x Day	10	45.12	1.86^{NS}
Lines	1	3.12	0.13^{NS}
Line x Day	5	127.58	5.26**
Line x inherit.	2	700.97	28.92**
Line x Inherit. x Day	10	240.48	9.92**
Replication	1	133.93	5.52*
Error	35	24.24	
Total	71		

** Significant at 0.01 level; * significant at 0.05 level; NS - not significant.

MEANS

Day 6	Day 2	Day 5	Day 1	Day 4	Day 3
57.9^{c}	39.6^{b}	36.8^{b}	33.0^{b}	21.6^{a}	19.8^{a}

multigenic	mono-multigenic	monogenic
18.2^{a}	31.4^{b}	37.0^{c}

Means not followed by the same letter significantly differ at the 1% level.

Table 34. Germ tube length of H. turcicum in bioassay test from diffusates of attached leaves of monogenic, multigenic and mono-multigenic resistant lines on 65 day old seedlings. (Average of 4 experiments with 2 replications each).

Type of inheritance	Lines	Days after inoculation						means
		1	2	3	4	5	6	
Monogenic	65-225-1	85.4	57.8	43.3	77.4	171.9	115.5	91.9
	RW64Ht	90.4	53.8	51.2	42.2	86.6	99.8	54.0
	means	87.9	55.8	47.2	59.8	129.3	107.6	
Multi-genic	H55	128.5	120.8	49.2	61.5	81.4	119.4	93.5
	H49	97.1	44.6	49.9	44.6	55.2	93.9	64.2
	means	112.8	82.7	49.6	53.0	68.3	106.6	
Mono-multigenic	RH55Ht	148.3	86.6	53.8	53.8	67.1	120.8	88.4
	RH49Ht	107.6	56.4	47.2	31.4	32.8	44.6	53.3
	means	128.0	71.6	50.5	42.6	49.9	82.7	

Table 35. Analysis of variance and Duncan's multiple range test of the means of table 34.

Source of variation	d.f.	M.S.	F
Days	5	7286.42	44.30**
Inheritance	2	245.85	1.49^{NS}
Inherit. x Day	10	1327.88	8.07**
Lines	1	5006.68	30.44**
Line x Day	5	2128.11	12.94**
Line x Inherit.	2	5560.42	33.81**
Line x Inherit. x Day	10	759.28	4.62**
Replication	1	1005.03	6.11**
Error	35	164.47	
Total	71		

** Significant at 0.01 level; * Significant at 0.05 level; NS - not Significant.

MEANS

Day 3	Day 4	Day 2	Day 5	Day 6	Day 1
49.1^{a}	51.8^{a}	70.0^{b}	82.5^{b}	99.0^{c}	109.6^{c}

mono-multigenic	monogenic	multigenic
70.8^{a}	73.8^{a}	78.8^{b}

Means not followed by the same letter differ significantly at the 1% level.

The type of inheritance was found to affect production of inhibitory substances. Multigenic lines produced significantly more inhibitory substances than both the monogenic and mono-multigenic lines. This was observed in percent spore germination (table 33). The monogenic lines produced the least amount of inhibitory substances. In germ tube length however, the multigenic lines gave the longest measurement (table 35). The effect of the 3 types of disease resistance on the mycelial growth of the pathogen is illustrated in figure 19.

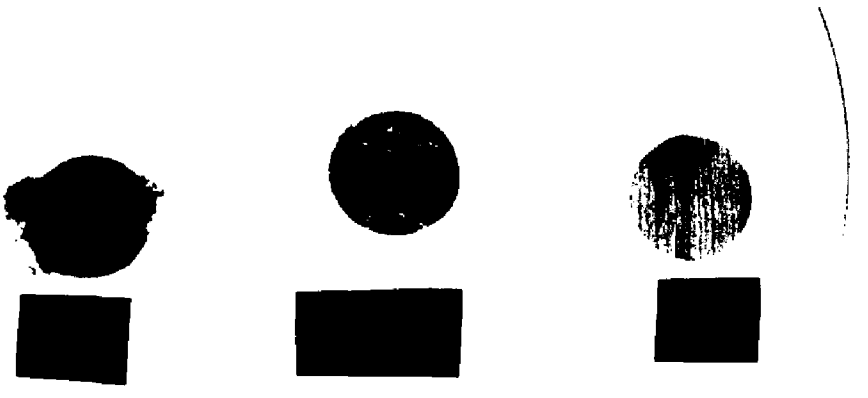


Figure 19. Mycelial growth of H. turcicum 36 hours after incubation in a nutrient agar medium. Lesions were taken from plants, 9 days after inoculation. Mu= multigenic, M-M= combined mono-multigenic, Mo= monogenic. M-M produced a very small amount of nycelial growth at this stage which is not visible in the photograph.

The relationship between the type of disease resistance and time of production of inhibitory substances is illustrated in figure 20. While there are differences in the amount produced,

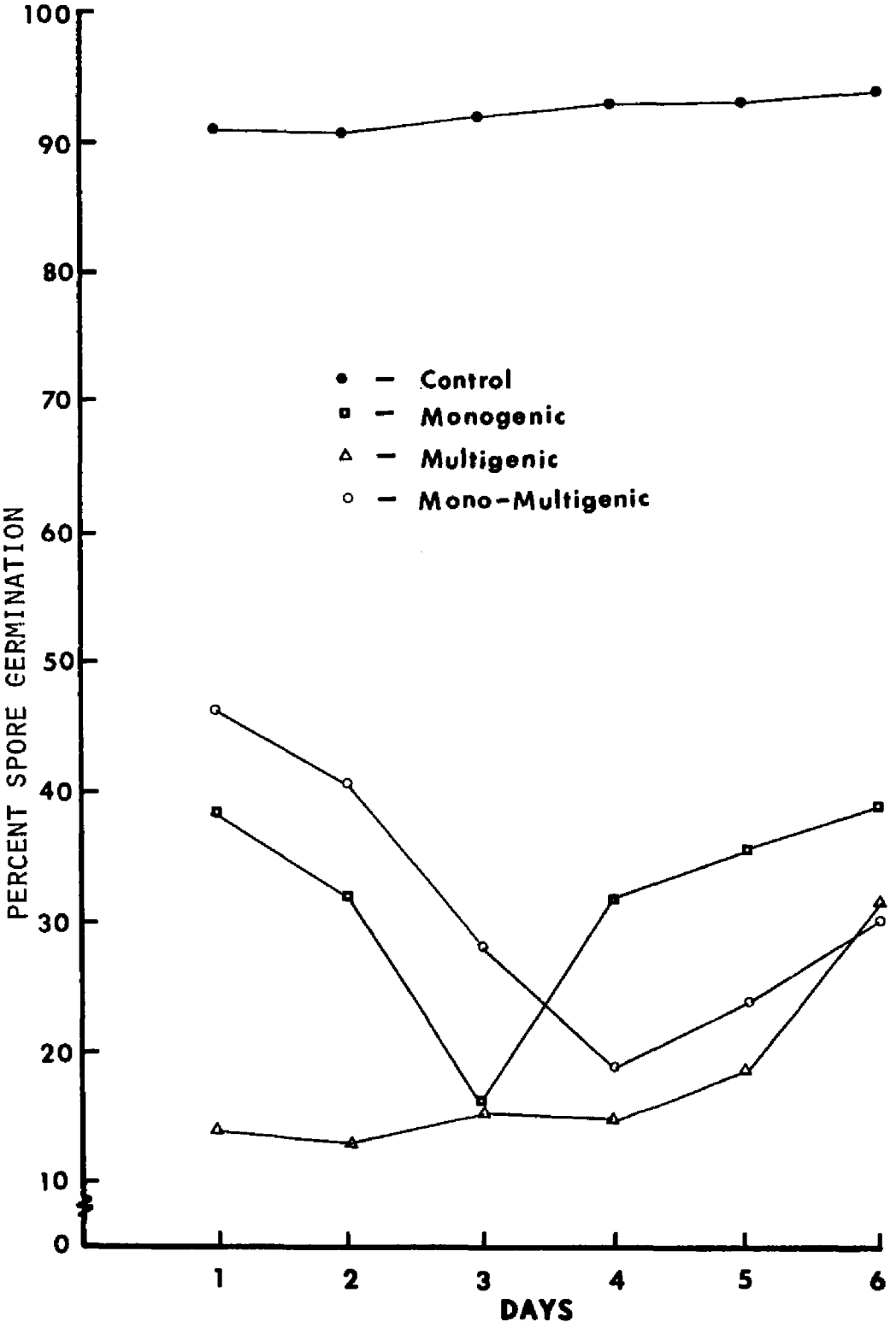


Figure 20. Effect of the type of disease inheritance and time of phytoalexin production on percent spore germination.

their patterns in production are similar.

Comparisons among lines within a given type of inheritance revealed that only the monogenic lines gave significant differences.in the production of inhibitory substances.These were lines 65-225-1 and RW64Ht. This difference was expected as shown in the previous experiment (table 29). In all types of inheritance, the maximum peak production of the inhibitory substances was reached on the third day as previously reported.

Disease evaluation following standard inoculation on monogenic lines.

The percent leaf infection of the various resistant and susceptible monogenic lines pre-conditioned at 3 temperature levels and evaluated 9 days after inoculation is shown in tables 36-40. Tables 36 and 37 present the quantitative method of evaluation and its corresponding analysis, while tables 38 and 39 present the visual evaluation and analysis, respectively.

Table 36. Percent leaf infection on various resistant and susceptible Ht monogenic lines with different genetic backgrounds, pre-conditioned at 3 temperatures levels, 9 days after inoculation. (Average of 3 experiments with 2 replications each).

Type of resistance	Lines	Temperature (°C)			means
		16	21	27-32	
Dominant homozygous (HtHt)	65-225-1	10.7	6.8	6.4	8.0
	RW64Ht	11.2	9.7	9.4	15.6
	RWF9Ht	8.4	13.0	7.0	9.4
	RHy-2Ht	11.6	9.2	9.8	10.2
	ROh43Ht	7.3	8.0	5.4	8.1
	means	9.8	9.3	7.6	
Dominant heterozygous (Htht)	65-225-1	9.3	6.3	7.6	7.8
	RW64Ht	14.2	9.5	11.3	11.6
	RWF9Ht	11.2	10.0	11.0	10.7
	RHy-2Ht	15.4	11.1	16.9	14.4
	ROh43Ht	10.0	7.9	5.1	7.7
	means	12.0	10.0	10.4	
Recessive (htht)	65-225-1*	10.6	10.6	7.8	9.7
	W64	19.0	15.4	13.2	15.8
	WF9	13.4	13.1	10.3	12.2
	Hy	10.2	10.8	17.8	13.0
	Oh43	15.6	8.6	7.0	10.4
	means	13.8	11.7	11.2	

* = This actually W153R which is isogenic to 65-225-1(HtHt)

Table 37. Analysis of variance and Duncan's multiple range test of the means of table 36.

Source of variation	d.f.	M.S.	F
Temperature	2	40.88	13.19**
Resistance	2	11.19	3.61*
Resist. x Temp.	4	11.43	3.69*
Lines	4	34.97	11.28**
Lines x Temp.	8	7.18	2.32*
Line x Resist.	8	41.40	13.36**
Line x Resist. x Temp.	16	11.40	3.68**
Replication	1	1.37	0.44NS
Error	44	3.10	
Total	89		

** Significant at 0.01 level; * significant at 0.05 level; NS - not significant.

MEANS

27-32°C	21°C	16°C
9.7^{a}	10.0^{a}	11.9^{b}

recessive	dominant heterozygous	dominant homozygous
12.2^{a}	10.4^{b}	10.3^{b}

line x resistance

HtHt		Htht		htht	
65-225-1	8.0^{a}	ROh43Ht	7.7^{a}	65-225-1	9.7^{a}
ROh43Ht	8.1^{a}	65-225-1Ht	7.8^{a}	Oh43	10.4^{a}
RWF9Ht	9.4^{a}	RWF9Ht	10.7^{b}	WF9	12.2^{a}
RHy-2Ht	10.2^{a}	RW64Ht	11.6^{b}	Hy	13.0^{a}
RW64Ht	15.6^{b}	Rhy-2Ht	14.4^{c}	W64	15.8^{b}

Means not followed by the same letter differ significantly at the 1% level.

Table 38. Visual evaluation on leaf infection of various resistant and susceptible Ht monogenic lines with different genetic backgrounds, pre-conditioned at 3 temperature levels, 9 days after inoculation. (Average of 3 experiments with 2 replications each)

Type of resistance	Lines	Temperature (°C)			means
		16	21	27-32	
Dominant homozygous (HtHt)	65-225-1	2.1	4.0	2.1	2.7
	RW64Ht	2.6	4.5	2.7	3.3
	RWF9Ht	2.6	3.6	3.0	3.1
	RHy-2Ht	4.5	2.5	2.6	3.2
	ROh43Ht	2.1	2.8	3.0	2.6
	means	2.8	3.5	2.7	
Dominant heterozygous (Htht)	65-225-1	3.0	2.5	3.5	3.0
	RW64Ht	2.5	2.5	3.5	2.8
	RWF9Ht	3.1	4.8	3.4	3.8
	RHy-2Ht	4.0	2.0	4.0	3.3
	ROh43Ht	3.1	2.8	2.3	2.7
	means	3.1	2.9	3.3	
Recessive (htht)	65-225-1*	4.3	3.5	4.0	3.0
	W64	5.0	4.0	5.0	4.7
	WF9	3.8	4.1	5.0	4.3
	Hy	5.1	3.3	4.6	4.3
	Oh43	4.8	4.6	4.0	4.4
	means	4.6	3.9	4.5	

* = This actually W153R which is isogenic to 65-225-1(HtHt)

Table 39. Analysis of variance and Duncan's multiple range test of the means of table 38.

Source of variation	d.f.	M.S.	F
Temperature	2	0.24	0.58^{NS}
Resistance	2	3.27	7.98^{**}
Resist. x Temp.	4	0.60	1.47^{NS}
Lines	4	3.76	9.18^{**}
Line x Temp.	8	1.97	4.82^{**}
Line x Resist.	8	1.74	4.24^{**}
Line x Resist. x Temp.	16	1.01	2.45^{*}
Replication	1	0.20	0.50^{NS}
Error	44	0.41	
Total	89		

** Significant at 0.01 level; * significant at 0.05 level; NS - not significant.

MEANS

dominant homozygous	dominant heterozygous	recessive
3.0^{a}	3.1^{a}	4.3^{b}

line x resistance

HtHt		Htht		htht	
ROh43Ht	$- 2.6^{a}$	ROh43Ht	$- 2.7^{a}$	65-225-1	$- 3.9^{a}$
65-225-1	$- 2.7^{a}$	RW64Ht	$- 2.8^{a}$	Hy	$- 4.3^{a}$
RWF9Ht	$- 3.1^{a}$	65-225-1	$- 3.0^{a}$	Oh43	$- 4.4^{a}$
RHy-2Ht	$- 3.2^{a}$	RHy-2Ht	$- 3.3^{a}$	WF9	$- 4.3^{a}$
RW64Ht	$- 3.3^{a}$	RWF9Ht	$- 3.8^{b}$	W64	$- 4.7^{a}$

Means not followed by the same letter differ significantly at the 1% level.

Pre-conditioning the maize seedlings at temperatures of 27-32° or 21°C did not significantly affect total area of leaf infection. At 16°C, however, total area of leaf infection was significantly increased (table 37). This difference was not obtained with the visual rating system (table 39). The homozygous dominant (HtHt) lines gave significantly less area of leaf infection compared to the recessive (htht) level. Very slight differences among lines within a particular Ht level were detected with the quantitative method of evaluation but almost no differences were observed with the visual evaluation. It is interesting to point out, however, that significant differences were obtained between lines 65-225-1 and RW64Ht in the dominant homozygous (HtHt) condition. This result supported the findings of the bioassay tests (table 29).

Disease evaluation following standard inoculation on different types of disease inheritance.

Percent leaf infection on monogenic, multigenic and mono-multigenic resistant lines pre-conditioned at 3 temperature levels, and evaluated 9 days after inoculation is shown in tables 40-43. Evaluation of the quantitative method and its analysis is presented in tables 40 and 42. Tables 42 and 43 show the visual evaluation and its corresponding analysis.

Table 40. Percent leaf infection on monogenic, multigenic and mono-multigenic resistant lines at 3 preconditioned temperatures, 9 days after inoculation with H. turcicum. (Average of 3 experiments with 2 replications each).

Type of inheritance	Lines	Temperature (°C)			means
		16	21	27-32	
Monogenic	65-225-1	10.7	6.8	6.4	8.0
	RW64Ht	11.2	9.7	9.4	15.8
	means	10.9	8.2	7.9	
Multigenic	H55	5.8	7.2	4.0	5.6
	H49	5.8	10.0	5.0	7.4
	means	5.8	8.6	4.5	
Mono-multigenic	RH55Ht	7.2	7.8	6.4	7.1
	RH49Ht	9.2	6.8	5.2	7.1
	means	8.2	7.3	5.8	

Table 41. Analysis of variance and Duncan's multiple range test of the means of table 40.

Source of variation	d.f.	M.S.	F
Temperature	2	17.41	9.93**
Inheritance	2	15.42	8.94**
Inherit. x Temp.	4	1.28	0.73^{NS}
Line	1	6.08	3.47^{NS}
Line x Temp.	2	3.48	1.99^{NS}
Line x Inherit.	2	14.62	8.34**
Line x Inherit. x Temp.	4	8.96	5.11**
Replication	1	0.64	0.36^{NS}
Error	17	1.75	
Total	35		

** Significant at 0.01 level; NS - not significant.

MEANS

27-32°C	21°C	16°C
6.1^{a}	8.0^{b}	8.3^{b}

multigenic	mono-multigenic	monogenic
6.5^{a}	7.1^{a}	11.9^{b}

Means not followed by the same letter differ significantly at the 1% level.

Table 42. Visual evaluation on leaf infection on monogenic, multigenic and mono-multigenic resistant lines at 3 pre-conditioned temperatures, 9 days after inoculation of H. turcicum. (Average of 3 experiments with 2 replications each).

Type of inheritance	Lines	Temperature (°C)			means
		16	21	27-32	
Monogenic	65-225-1	4.0	2.1	2.1	2.7
	RW64Ht	4.5	2.6	2.7	3.3
	means	4.2	2.4	2.4	
Multigenic	H55	2.5	2.0	2.5	2.3
	H49	2.3	2.0	2.1	2.1
	means	2.4	2.0	2.3	
Mono-multigenic	RH55Ht	3.0	2.1	2.0	2.4
	RH49Ht	2.8	2.0	2.4	2.4
	means	2.9	2.1	2.2	

Table 43. Analysis of variance and Duncan's multiple range test of the means of table 42.

Source of variation	d.f.	M.S.	F
Temperature	2	3.59	23.67**
Inheritance	2	0.75	4.96**
Inherit. x Temp.	4	0.39	2.60^{NS}
Lines	1	2.15	14.18**
Line x Temp.	2	0.55	3.64*
Line x Inherit.	2	0.71	4.70*
Line x Inherit. x Temp.	4	0.32	2.13^{NS}
Replication	1	0.07	0.47^{NS}
Error	17	0.15	
Total	35		

** Significant at 0.01 level; * significant at 0.05 level; NS - not significant.

MEANS

21°C	27-32°C	16°C
2.1^{a}	2.3^{a}	3.1^{b}

multigenic	mono-multigenic	monogenic
2.2^{a}	2.4^{a}	3.0^{b}

Means not followed by the same letter differ significantly at the 1% level.

The influence of temperature on the different types of inheritance was similar to that obtained in the previous experiment (table 37). Low temperature induced the maize plants to become more susceptible. Highly significant differences were found between 16° and 21°C and between 16° versus 27-32°C with the quantitative evaluation. With the visual rating system the significant differences were between 16°C on one hand, and 21° or 27-32°C on the other hand. Comparison of the different types of disease inheritance shows that multigenic lines had significantly less total area of leaf infection than the monogenic lines. No significance was obtained between multigenic and mono-multigenic lines. This observation was also noted in both types of evaluation.

Comparison between lines within the type of inheritance shows that only the monogenic types gave significance, that is between lines 65-225-1 and RW64Ht. As in the previous finding, the RW64Ht line gave a significantly higher disease reading.

Correlations.

Correlations between the different studies are shown in table 44.

Table 44. Linear correlation between leaf infection and visual rating, leaf infection and bioassay test, and visual rating and bioassay test.

Factors [a]			r		
			HtHt	Htht	htht
Leaf infection (quantitative)	vs.	Visual	0.783	0.398	0.884*
		Bioassay(max)	0.841*	0.059	0.505
		Bioassay($\bar{x}$)	0.712	0.634	0.532
Visual	vs.	Bioassay(max)	0.469	-0.058	0.532
		Bioassay($\bar{x}$)	0.729	-0.137	0.635
Bioassay(max)	vs.	Bioassay($\bar{x}$)	0.454	0.715	0.933*

$P_{.05} = .811$

[a] Leaf infection = quantitative method of evaluation
Visual = visual method of evaluation of leaf infection
Bioassay(max) = values taken from the maximum phytoalexin production which is the third day
Bioassay($\bar{x}$) = values taken from the mean of the 6 days of phytoalexin production

* = Significant at the 0.05 level

Most of the paired comparisons presented in table 44 were not significant. The effect of one factor- for example, low percent spore germination (high phytoalexin)- did not necessarily give a low percent of infected leaf area. The only factors which seemed to have a good correlation were: quantitative evaluation versus bioassay(max) at the HtHt level; quantitative evaluation versus visual rating at the htht level; and bioassay(max) versus bioassay ($\bar{x}$) at the htht level.

SECTION V

DISCUSSION

Effect of plant genotype.

The lowest spore germination was observed with HtHtBxBx, followed by HtHtbxbx, HthtBxBx, hthtBxBx, hthtbxbx, and control in that order. The same ranking was found in the germ tube length except that the distribution was not gradual (figure 14). The control produced germ tubes approximately three times longer than the susceptible deficient genotype (hthtbxbx).

Two important comparisons should be stressed. First, note the difference between hthtBxBx and hthtbxbx. Second, note the difference between hthtBxBx and hthtbxbx relative to the control. The first point concerns the role of BxBx gene. The highly significant differences between the two genotypes, especially for germ tube length, suggest its role is reducing or delaying growth of the organism. This result confirms Couture el al.'s (14) work who reported that a hydoxamic acid produced by the Bx gene reduced percent spore germination and delayed germ tube growth of H. turcicum. Secondly, the susceptible genotypes usually decreased percent spore germination and germ tube length compared to the control. This effect indicates that the susceptible genotypes do produce some inhibitory compounds although apparently not an amount sufficient to inhibit or delay tube growth as much as the resistant genotypes. These inhibitory compounds are probably also produced in the HtBx genotype and are reflected in its sig-

nificant inhibition of spore germination.

As stated earlier, germ tube length was not a very reliable measure of phytoalexin production. Several reasons could account for this effect. First, the number of germ tubes measured were usually between 30 and 50 per experiment as compared to 300 to 400 spores for germination determination. Second, as soon as the germ tube grew beyond 10 microns, it became crooked and twisted (see figure 6 and 7), making accurate measurement difficult. Third, it was observed in a few instances that while only very few spores germinated, their tube length were much longer than expected (table 35). On this basis we therefore favor the use of percent germination as a better criterion for phytolaexin evaluation.

Time of phytoalexin development.

Production of phytoalexin started twenty-four hours after inoculation, and increased daily until it reached its peak on the fourth or fifth day, after which it gradually disappeared (figure 15). The germ tube seemed to exhibit the same tendency although it was more variable.

It should be pointed out, however, that these means include the values of the susceptible lines as well as the control in each experiment. If these lines were excluded, since they produce little phytoalexin, the peak of phytoalexin production would be observed on the second or third day. In addition, older plants also caused a delayed peak of phytoalexin production because synthesis of this chemical at this stage presumably was slow. Another significant change

in the bar graph, with the exclusion of the control and susceptible lines, would be the proportional reduction in the bar height or percent spore germination and tube length.

Effect of attached-detached leaf.

Comparison of the attached and detached leaf technique definitely established that the rate and amount of phytoalexin produced was significantly larger in the attached leaves. Our results confirmed the work of Lim et al (51). They reported that the chemical was not produced in the detached resistant corn leaves until three days following inoculation.

Close observation of the detached leaves showed that 2 to 3 days after inoculation with the drop suspension, the color of the leaves started to turn purple. This probably was due to host-pathogen interaction. No discoloration was observed with the control until after the fifth or sixth day.

Observation of the attached leaves at the time the drop suspension were withdrawn, and up to seven days after inoculation revealed a striking differences in symptom development between treatments. Forty-eight hours after inoculation with the suspension, the susceptible leaves developed lesions with a yellow halo. These lesions rapidly increased in diameter beyond the area originally occupied by the drop. On the seventh day the entire leaf was almost totally yellow. In the resistant leaves, water-soaked lesions were observed under the drops forty-eight hours after inoculation. These later developed into chlorotic lesions on the 6th or 7th day. In the control no such changes occurred, but

on the 6th or 7th day the whole leaf started to become yellow due to senescence. The yellowing of the whole leaf on the 6th or 7th day was observed in all leaves including the control. This was attributed to low light intensity as was also shown by the pale green color of the leaves of the plants not included in the treatment.

The low ability of the detached leaves to produce phytoalexin may be explained by the initiation of senescence upon cutting which in turn activate protein and chlorophyll breakdown. In a similar situation, Farkas et al (23) reported 20-30% protein breakdown in detached leaves which consequently led to faster chlorophyll breakdown than in the attached leaves. The change of the leaf color to purple was probably due to the very rapid breakdown of the chlorophyll. While N^6-benzyladenine was present, the leaves could probably not absorb it as rapidly as needed to maintain chlorophyll formation.

Effect of inoculum concentration.

The greater production of phytoalexin in the resistant plants under the high spore concentration was probably due to the increased number of spores interacting with the host. It was interesting to note, however, that even at high concentration, four times more concentrated than at low level, its effect on spore germination was only 6.3 percent. Increasing the inoculum dosage four times does not give a corresponding proportional increase of phytoalexin. This suggests that at this concentration(3.3×10^4 spores/ml) the number of spores re-

quired to produce an optimum amount of phytoalexin has been reached. It is probable that the low spore concentration used in the study is actually high when compared to natural conditions. This reasoning seems to agree with Bell's (4) finding with Verticillium albo-atrum of cotton. He found that spore concentration of 10^4 conidia/ml induced only traces of phytoalexin, while 10^5 to 10^8 conidia/ml gave a two fold increase in production of phytolaxein.

Effect of stage of plant growth.

Two important observations were possible from studies at different growth stages. First, resistant plants in the 5-6th leaf stage produced significantly more phytoalexin than those in the 8-9th or 11-12th leaf stage. This maybe due to the greater ability of the younger plants to synthesize the materials as compared with the older plants or to the greater diffusing out of the substance. Phytoalexin therefore seems to play an important role in the resistant mechanism of young plants.

The other observation is that phytoalexin production in the older plants of the same genotypes was both reduced and delayed. The reduction in phytoalexin production is probably a true reflection of the loss of the ability by the older plant tissue to produce this material. The delayed appearance, however, maybe due to resistancein older plant tissue. Paxton and Chamberlain (63), working with the fungus Phytophthora megasperma in soybeans, demonstrated that such a mechanism is actually operating in resistant varieties.

Effect of pre-conditioned temperature.

Percent leaf infection. Pre-conditioning the seedlings at low temperature (16°C) from date of emergence to inoculation date tended to decrease resistance as shown by the increase area of disease infection. This could be attributed to the weaker condition of the seedlings. Plants exposed to this temperature were observed to be much slower in growth, smaller, had pale green leaves, at times producing whitish yellow streaks on the leaves and the leaves were somewhat brittle. The increase in infection could not be associated with favorable growth of the organism as shown by Hilu and Hooker (30) who reported that H. turcicum optimally sporulates and develops mycelial growth at 20° and 25°C, respectively. It may also be added that pre-conditioning the seedlings at this temperature is probably not justified since corn plants are not normally grown at this temperature.

Bioassay test. The change in the resistant corn leaves with a change in pre-conditioned temperature is not fully understood. Bell (5) studied the effect of temperature upon resistance and phytoalexin production in cotton inoculated with verticllium wilt. He showed that resistant cotton varieties were: a) at 22°C, all susceptible; b) at 32°C, all resistant; c) but at 25-29°C, susceptible gave a susceptible reaction, and resistant varieties gave a resistant reaction. Based on this report we could speculate that resistant corn lines may behave similarly to (c) above.

It should be mentioned that temperature levels at 21° C and 27-32°C were found to give significant effects under the bioassay test, but were not significant in the percent leaf infection. This would suggest that the former is a more sensitive measurement than the latter. This assumption will be discussed later.

Effect of genetic background of the Ht gene.

Percent leaf infection. There is a difference in the type of lesion produce by the HtHt lines (chlorotic) and the htht (necrotic) lines. The results showed that the htht lines gave more percent infection. This is because the susceptible lines do not inhibit or reduce growth of the organism inside the host. This is supported by the extent of *in vitro* mycelial growth from both Ht and ht lesions (figure 18). In the htht lesion, mycelial growth of the organism developed profusely 36 hours after incubation. Very sparse growth was observed in both the HtHt and Htht lesions. The same results were obtained by Hilu and Hooker (30).

Bioassay test. The incorporation of the Ht gene in a line or inbred greatly increased the formation of phytoalexin in that inbred regardless of its genetic background. Also, the level in which the Ht gene is introduced, whether homozygous or heterozygous, determines the amount of phytoalexin which it can synthesize.

All lines possessing the HtHt gene tended to exhibit a similar pattern of phytoalexin production. The amount and rate, however, may differ from one line to another. These dif-

ference may be attributed to the genetic backgrounds which may play an important role in the synthesis of the material. Some of these lines may contain other dominant genes for resistance, while others may contain dominant genes indistinguishable from the Ht gene used in the study. In the susceptible lines, this characteristic is of no advantage since very little if any phytoalexin is produced.

Significant differences among the resistant monogenic lines suggest that phytoalexin is a more accurate method of distinguishing the effect of resistant lines with different genetic background. This was confirmed by the percent leaf infection of the same materials (tables 37 and 39) which resulted in no significant differences. Wilson and Rhodes (86) studied the chlorotic lesion type of resistance to H. turcicum from one starchy and four sweet corn sources. Results of their inoculations showed no significant differences among the materials.

Effect of type of disease inheritance.

Percent leaf infection. The lesser percent infection of the multigenic lines as compared to the monogenic lines is possibly due to the different type of lesions produced. In multigenic resistant lines, the lesions are necrotic, but the action of the genes is to reduce the area of infection (42). In monogenic lines, the action of the gene is to modify the lesion produced by making it chlorotic (33) but it does not

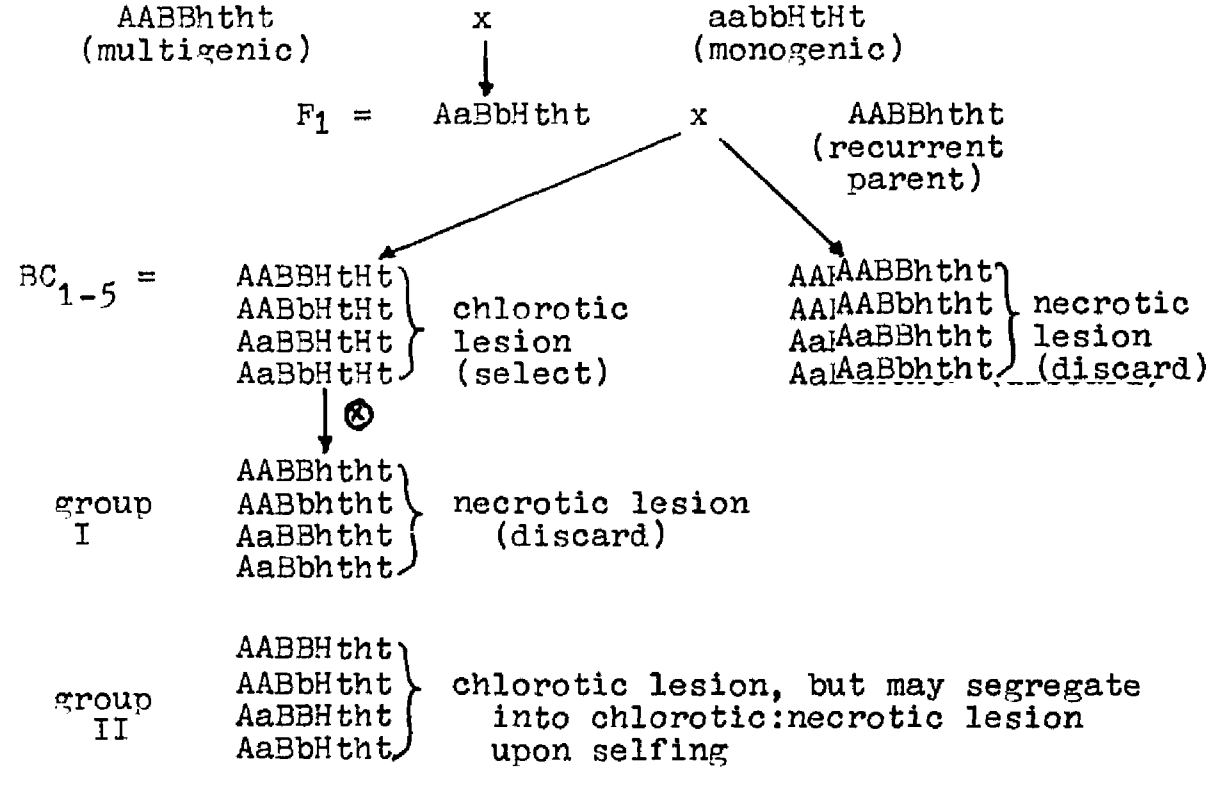
necessarily reduce the number of lesions. Hence, in computing the percent leaf infection, the monogenic lines may give a higher total lesion area than the multigenic lines as was observed in this trial. Similarly, Ullstrup (84) compared monogenic and polygenic resistant corn lines with reaction to H. turcicum and found the latter gave a higher yield than the former. He attributed this result to extensive chlorosis accompanying infection of the genotypes carrying monogenic resistance.

Preliminary study on the monogenic, multigenic, and combined mono-multigenic lesions showed that: in the multigenic, mycelial growth of the fungus started to develop 24 hours after incubation in an agar nutrient medium and rapidly increased after 36 hours; in the monogenic lesion, growth was not visible until after 48 hours; and in the combined mono-multigenic, very slight growth was observed after 36 hours. Similar results were observed by Hilu and Hooker (30) on the multigenic and monogenic resistant lines of corn. Sporulation of the organism under these types of disease inheritance needs further study.

Bioassay test. Results from this experiment showed three outstanding points. First it was demonstrated that inhibitory substances were produced in the multigenic lines. Second, not only were these substances produced but in much greater amount than in the monogenic level. Third, the addition of the Ht gene to the multigenic lines diluted the ability of these lines to produced phytoalexin.

Secondly, it is probable that in the multigenic lines there are more types of phytoalexin or inhibitory substances than are present in the monogenic resistant host. This assumption is based on the report that Ht produces two types of phytoalexin (51). It is therefore possible that with more genes, more phytoalexins or similar substances are involved.

Thirdly, a possibility exists that when a resistant gene (Ht) is added to other resistant genes (multigenic), a dilution of the effectiveness of the multigenic factors occurs. This concept could be approached in two ways, genetically and biochemically. From the genetic aspect, a model will be used to illustrate the concept. Assume for simplicity that the multigenic resistance is governed by 2 major genes, AABB. The incorporation of the HtHt genes would then be as follows:



group III

Line 1. AABBHtHt
Line 2. AABbHtHt
Line 3. AaBBHtHt
Line 4. AaBbHtHt

} does not segregate for chlorotic lesion, the AB gene may be segregating but cannot be detected.

⊗ Lines 1,2,3,and4 upon selfing will give:

Line 1. AABBHtHt (no segregation)
Line 2. AABBHtHt, AABbHtHt*, AAbbHtHt**
Line 3. AABBHtHt, AaBBHtHt*, aaBBHtHt**
Line 4. AABBHtHt, A_B_HtHt*, A_bbHtHt88, aaB_HtHt** , aabbHtHt**

* = heterozygous for A, B or both
** = recessive for A, B or both

From the model one could see that some if not the majority of the originally homozygous dominant multigenic factors were changed to the recessive condition. On this basis, it is therefore possible to obtain a reduction in resistance as well as in the production of the inhibitory substances.

From the biochemical point of view, it is possible that the phytoalexin induced by the monogenic resistant lines becomes antagonistic to those produced by the multigenic resistant lines. The consequence therefore may lead to the reduction in inhibitory effects such as were observed in the combined mono-multigenic lines. The dilution theory seems to be supported by the mycelial growth of the fungus as illustrated in figure 19. The photograph shows that the combined mono-multigenic lines behaves in a similar manner to the monogenic lines. This hypothesis will be proven or disproven by future biochemical studies. It is possible that this discovery may help shift the interest in future studies of phytoalexin to

multigenic resistant varieties.

Correlation studies.

No correlation was observed between the factors studied. This result could mean one of two things. First, that the factors under study were really independent of each other. Second, the method or system of taking the values may be faulty. For instance, a visual rating and quantitative evaluation for amount of leaf infection should logically be correlated. Since this was not observed, it could mean that either one or both methods for measuring disease infection were inaccurate.

SECTION VI

CONCLUSIONS

Homozygous resistant normal (HtHtBxBx) genotypes produced a significantly higher amount of phytoalexin, followed by resistant deficient (HtHtbxbx) and heterozygous resistant normal (HthtBxBx) genotypes in that order. This was reflected in low percent spore germination and shorter tube length of the H. turcicum organism. On the other hand, susceptible normal (hthtBxBx) and deficient (hthtbxbx) genotypes also produced inhibitory substances but at a very low level, with the former giving shorter germ tubes, suggesting a role in delaying spore germination.

Phytoalexin in resistant plants appeared 24 hours after inoculation, reached its maximum on the 2nd, 3rd or 4th day, then gradually disappeared.

The attached leaf technique is a more efficient method of studying phytoalexin than the detached leaf technique. High inoculum concentrations induced phytoalexin, at a much faster rate and quantity than low concentr
Similarly, younger plants synthesized the chemical much faster and in a greater amount than older plants. Inoculation test showed that pre-conditioning the host at a very low temperature (16^{o}C) tended to break down resistance which resulted in high leaf infection. Bioassay test indicated that pre-conditioning the plants at a very high (27-32^{o}C) temperature reduced its ability to produce phytoalexin.

Phytoalexin is a more useful method of determining the effects of the different genetic background of a gene than percent leaf infection. The rate and amount of phytoalexin varied depending upon the genetic background in which Ht gene was placed. However, the pattern of development in the different lines was the same. Multigenic lines produced inhibitory substances in larger amounts than the monogenic lines. Whether these are phytoalexins, pre-formed substances or combination of both remains to be studied. The addition of Ht gene to a multigenic resistant line diluted the ability of that line to produce these inhibitory substances.

SECTION VI

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