Factors Associated with the Resistance of Barley to Helminthosporium teres

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ABSTRACT

Helminthosporium teres caused smaller and fewer lesions on resistant, than on susceptible, barley. Sporulation of the fungus on excised leaves was less on resistant lines than on susceptible ones. No differences were found in spore germination, germ-tube growth, or number of penetrations by the fungus on leaves of resistant and susceptible barley. After penetration, however, the growth of the fungus was inhibited in resistant C.I. 4976 tissue, in which many infections did not progress beyond the penetrated cell.

At least one factor in the resistance of barley to net blotch appears to be the formation of an antifungal substance(s) produced as a result of the host-parasite interaction.

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The six-row spring barley, C.I. 4976, *Hordeum vulgare* L., highly resistant to net blotch caused by *Helminthosporium teres* Sacc., has been used extensively in crosses for the development of malting barleys resistant to this disease. In this line, lesions either fail to develop or remain restricted; whereas, extensive lesions with a netted appearance surrounded by chlorosis occur in susceptible cultivars such as 'Trophy' (C.I. 10647) (Fig. 1). Other six-row spring barley lines, C.I. 3902 and C.I. 11531 (ND B112), also used as sources of resistance to net blotch, usually develop abundant infections but the lesions are smaller than in susceptible barleys. Very little is known about the nature of resistance of barley to net blotch. These studies were made to identify factors associated with the resistance of barley to *H. teres,* and to elucidate the nature of this resistance.

MATERIALS AND METHODS.—The resistant line, C.I. 4976, two moderately resistant lines, C.I. 3902 and C.I. 11531 (ND B112), and a susceptible cultivar, 'Trophy' (C.I. 10647), were used. These lines and cultivar were originally obtained from the U.S. Department of Agriculture Collection of Spring Barleys, or from the

Dept. of Agronomy and Plant Genetics, University of Minnesota. Throughout the remainder of this paper, the term "line" will be used to designate both C.I. lines and cultivar Trophy. Ten to twelve-day-old seedlings grown in 10-cm diameter clay pots were used for all experiments.

The culture of *H. teres* was isolated from leaves of barley grown at Crookston, Minnesota. It sporulated abundantly on 2% rice cereal agar (Gerber's rice baby cereal) at 21 C when subjected to 12-hour periods of alternating light and dark. Plants were inoculated by spraying them with conidia at concentrations of 1,000-2,000 spores/ml of water, using a small sprayer attached to an air pump adjusted to deliver ~ 0.33 atmosphere (5 psi) air pressure. Tween 20 [polyoxyethylene sorbitan monolaurate] (20) was added to inoculum at rate of 1 drop/100 ml. In experiments that required uniform inoculation, the plants were placed on a turntable, revolved at 30 rpm, and sprayed for 30 seconds from a distance of 25-30 cm. Inoculated plants were incubated in a moist chamber maintained at 22 C ± 2.

RESULTS.—*Comparison of the host-parasite interaction as measured by lesion numbers, size of lesions, and sporulation.*—To obtain a quantitative comparison of the host-parasite interaction of *H. teres* on resistant and susceptible barley, lesions were counted and measured, and sporulation within lesions was measured. To obtain lesion counts, 12-day-old seedlings of C.I. 4976, C.I. 3902, C.I. 11531, and Trophy were inoculated uniformly with a conidial suspension and placed in a moist chamber. In 12-hour intervals up to 60 hours, pots of each line were removed from the moist chamber and placed in the greenhouse. Five days after inoculation, the number of lesions on the first foliage leaf of 12 plants of each line were counted for each moist period treatment. The number of lesions on C.I. 4976 consistently averaged fewer than half the number on Trophy (Fig. 2). Fewer lesions occurred on C.I. 3902 than on Trophy or C.I. 11531, but the numbers closely paralleled those of the latter two for each incubation period.

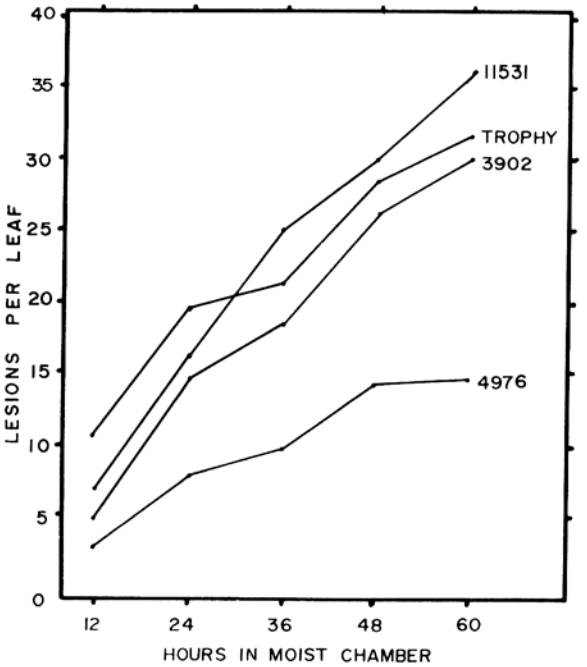

Fig. 2. Numbers of net blotch lesions on four barley lines after inoculation with *Helminthosporium teres* and incubation in a moist chamber for different lengths of time.

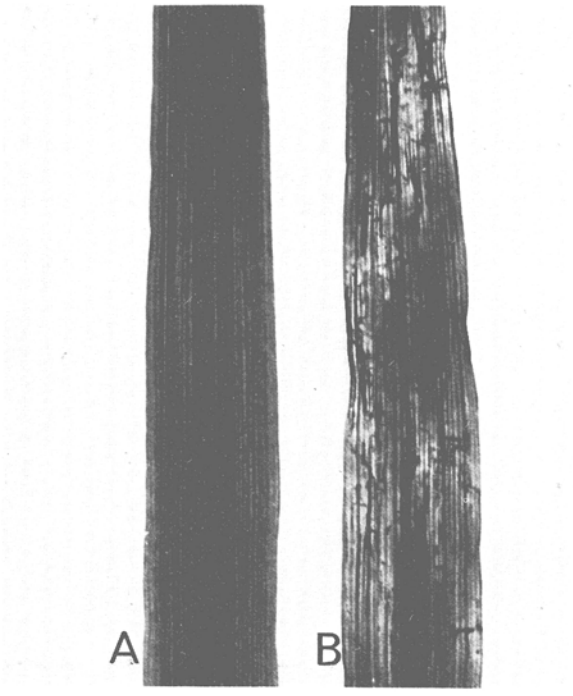

Fig. 1-(A, B). Disease reaction of **A)** resistant (C.I. 4976), and **B)** susceptible (Trophy) barley 10 days after inoculation with *Helminthosporium teres*.

Because of the irregular shape of net blotch lesions, the longest dimension was measured for comparison of lesion size in different barleys. Twelve-day-old seedlings of the four lines were inoculated uniformly, incubated for 36 hours in the moist chamber, and placed in the greenhouse. Eleven days after inoculation, the longest dimension of 20 lesions on the first foliage leaf of plants of each line was measured in each of four replicates. The mean lesion size on Trophy (7.3 mm) and C.I. 11531 (3.0 mm) were significantly ($P = 0.01$) larger than those of C.I. 3902 (1 mm) and C.I. 4976 (1 mm).

The inability of *H. teres* to sporulate in lesions could also be an important resistance mechanism. Mumford (4) found that, in general, *Helminthosporium sativum* sporulated poorly on resistant and abundantly on susceptible barleys. To test this aspect of development of *H. teres,* 12-day-old seedlings of the four lines were inoculated, placed in a moist chamber for 24 hours, and transferred to the greenhouse. One week after inoculation, leaf samples (6 cm long) of each line were cut and placed in petri dish moist chambers. The conidia produced in an area 3 mm in diameter on the leaf sections were counted using a stereomicroscope after 5 and 10 days of incubation. In Trophy at 10 days, few additional spores were produced over those formed in 5 days and most had fallen to the leaf surface and germinated or

TABLE 1. Number of spores produced by *Helminthosporium teres* on excised leaves of resistant and susceptible barley lines

Barley line[a]	Mean number of spores[b]	
	5 days	10 days
C.I. 4976	0.2 A	5.4 C
C.I. 3902	0.0 A	6.4 C
C.I. 11531	11.5 A	20.5 D
Trophy	67.6 B	

[a]"Line" as defined in this paper includes the susceptible cultivar Trophy.

[b]Each figure is the mean number of spores in an area 3 mm in diameter at two locations on eight leaves in four tests. Means followed by different letters are different ($P = 0.05$).

TABLE 2. Growth of *Helminthosporium teres* within leaf tissue of resistant and susceptible barley lines

Barley lines[a]	Mean hyphal growth (μm)[a]	
	24 hours after inoculation	48 hours after inoculation
C.I. 4976	102.8 A[b]	526.0 D
C.I. 3902	195.3 B	660.5 E
C.I. 11531	224.5 B	767.5 E
Trophy	322.8 C	1177.3 F

[a]"Line" as defined in this paper includes the susceptible barley cultivar Trophy.

[b]Each figure is the mean hyphal growth measured from the point of penetration to the greatest distance reached by the fungus for 60 penetrations at each time period in each of four tests. Means followed by different letters are significantly different $P = 0.05$.

TABLE 3. Length of germ tubes of *Helminthosporium teres* spores germinated in expressed sap from noninoculated and inoculated seedlings of the barley lines C.I. 4976 and Trophy

Barley lines[a]	Treatment	Mean length of germ tubes (μm)[b]	
		Sap filtrate	Nonfiltered
C.I. 4976	Inoculated	132 A[c]	121 C[c]
	Noninoculated	205 AB	181 D
Trophy	Inoculated	220 B	192 DE
	Noninoculated	269 B	211 E

[a]"Lines" as defined in this paper includes the susceptible barley cultivar Trophy.

[b]Each figure is the mean of 75 measurements in each of five tests.

[c]Each column of means was analyzed separately and means followed by different letters are significantly different ($P=0.05$).

shriveled. Counts were not made on this line at 10 days (Table 1). Significantly greater ($P = 0.05$) sporulation occurred on Trophy than on C.I. 11531, C.I. 4976 or C.I. 3902 in 5 days.

Germination, growth, penetration, and development of the infection of H. teres in resistant and susceptible barleys.—Since fewer lesions developed on C.I. 4976 than on other lines, tests were made to determine if the fungus was inhibited prior to or after penetration of the host had occurred. Seedlings of the four lines were inoculated and placed in the moist chamber. Leaves were removed every 12 hours up to 48 hours after inoculation; cleared of chlorophyll, stained in 0.1% acid fuchsin in lactophenol, and mounted on glass slides in 50% glycerine in water for observation. No significant differences occurred in spore germination, number and length of germ tubes, branching of germ tubes, and number of penetrations on the four barleys. The number of penetrations in each line, however, increased with increased incubation time up to 48 hours.

Development of the pathogen within the host tissue was studied using a modification of a technique described by Shipton and Brown (5). At 24 and 48 hours after inoculations 10 leaf pieces, 4-5 cm long, of each line were placed in 25 ml of alcoholic lactophenol cotton-blue (one part lactophenol cotton-blue to three parts 95% ethanol). The solution containing the leaf pieces was brought to a boil and simmered for 1 minute, then cooled until the leaf pieces sank and then immediately brought to a boil again for an additional 30 seconds. The leaf pieces were left in the stain solution at about 21 C for 48 hours, then washed with water and destained in a saturated aqueous solution of chloral hydrate for 30 to 40 minutes. The cleared leaf pieces were mounted on glass slides in 50% glycerine in water for observation. The extent of mycelial growth in leaf tissues was measured from the point of penetration to the greatest distance reached by the fungus during the allotted time (Table 2).

The growth of the fungus within C.I. 4976 in 24 hours was significantly less ($P = 0.05$) than in C.I. 3902 or C.I. 11531, and the fungus growth in the latter two barleys was significantly less than the growth in Trophy. The same general relationship of fungal growth was also observed after 48 hours. There were no apparent differences in the morphology of the mycelia in resistant and susceptible tissues. Within 24 hours only 43% of the penetrations in C.I. 4976 had progressed beyond the initially penetrated cell; whereas, 93% of those in Trophy had done so. At 48 hours after inoculation, 80 and 100% of the penetrations had progressed beyond the initially penetrated cells in C.I. 4976 and Trophy, respectively.

Production of an antifungal substance in barley as a result of infection with H. teres.—Millerd and Scott (1) found that barley infected with *Erysiphe graminis* produced a "phytopathogenic toxin" that they called a vivo toxin. They hypothesized that varietal differences in susceptibility to the pathogen may be explained in part by differences in hypersensitivity of the varieties to the toxin. To test the possibility that an antifungal substance may be produced in barley in response to infection with *H. teres,* nonfiltered sap and sap filtrates from infected and noninfected leaves of C.I. 4976 and Trophy were tested for their effect on the germination and growth of *H. teres*. Ten- to twelve-day-old seedlings of C.I. 4976 and Trophy were inoculated with a suspension of 8,000 spores/ml. This resulted in a large number of penetrations in each line and abundant sites for host-parasite interaction and production of inhibitor. The inoculated as well as comparable noninoculated plants were placed in a moist chamber for 48 hours. Sap was expressed from inoculated and noninoculated leaves of both barleys. Conidia of *H. teres* were suspended in expressed crude sap or its filtrate that had passed through a 0.45 μm Millipore filter on

glass slides and incubated in a moist chamber for 9 hours. Then the fungus was killed, chlorophyll removed with 95% ethanol, and the germ tubes were measured.

The germ tubes produced by spores germinated in nonfiltered sap from inoculated seedlings of C.I. 4976 were significantly ($P = 0.05$) shorter (121 μm) than those produced in sap from similar noninoculated seedlings (181 μm) of the same line (Table 3). Although not statistically significant, germ-tube growth was less in nonfiltered sap from inoculated Trophy seedlings, than in nonfiltered sap from noninoculated Trophy seedlings.

The growth of germ tubes of spores germinated in sap filtrates from inoculated C.I. 4976 (132 μm) was significantly less ($P = 0.05$) than that of germ tubes in filtrates from inoculated Trophy (220 μm).

Tests for the presence of antifungal substances in a diffusate on leaves of barley inoculated with H. teres.—The technique used to obtain the exudates in these experiments is a modification of the water-diffusate methods described by Mizukami (2) and Müller (3). Ten- to 12-day-old seedlings of C.I. 4976 and Trophy were inoculated with a conidial suspension of *H. teres* (8,000 spores/ml). Check seedlings were sprayed with water. All plants were then placed in a moist chamber. The humidifier in this chamber was adjusted to maintain free water on the leaves but not to cause noticeable run off. After the plants were in the moist chamber for 24 hours, the water on the leaf surface of each treatment was collected separately.

In each of three experiments, conidia of *H. teres* were suspended in three drops of each diffusate samples on glass slides and placed in a moist chamber. After 9 hours, the fungus was killed and the germ tubes were measured for each treatment. The germ tubes produced in the diffusate sample from leaves of inoculated C.I. 4976 were significantly ($P = 0.05$) shorter (121 μm) than the mean length of germ tubes in diffusate samples from noninoculated C.I. 4976 (185 μm), inoculated Trophy (175 μm), and noninoculated Trophy (207 μm).

Effect of heat on the antifungal substance.—Sap was expressed from inoculated plants of C.I. 4976 and filtered through a 0.45 μm Millipore filter. Part of the sap filtrate was heated at 82 C for 4 minutes in a water bath and then cooled immediately. When the filtrate had cooled, conidia of *H. teres* were suspended in this, as well as in nonheated filtrate on glass slides, and placed in a moist chamber. Germ tubes of spores were measured after 9 hours. The length of germ tubes produced by spores germinated in heated sap filtrate from inoculated plants was significantly ($P = 0.01$) longer (254 μm) than those produced in nonheated sap filtrate (94 μm) from the same plants. These data indicate that the antifungal substance produced in the leaf tissue of C.I. 4976 as a result of invasion by *H. teres* is heat labile.

DISCUSSION.—The resistance of barley line C.I. 4976 to *H. teres* is characterized by a smaller number of macroscopically visible lesions, diminished development of visible lesions, and reduced sporulation. Although an antifungal substance was detected on inoculated leaves of C.I. 4976, it apparently is not induced early enough in the infection process, or in high enough concentration, to significantly restrict development of the fungus up to the point of penetration. However, after penetration, fungus growth was retarded within tissues of resistant varieties.

Bioassays of sap extracted from leaves of inoculated and noninoculated barleys indicate that the development of an antifungal substance is stimulated as a result of the host-parasite interaction (Table 3). Although an inhibitory substance is produced even in a susceptible barley (e.g. Trophy) as a result of invasion by *H. teres,* the potency of its antifungal activity was significantly less than that which occurred in extracts from C.I. 4976.

These experiments do not dispel the possibility that a toxic substance is produced in barley simply as a result of mechanical injury to tissue when sap is extracted from foliage. However, it appears that the penetration of the host by the fungus stimulates the release or formation of considerably greater concentrations of inhibitory substances over that which may be released as a result of mechanical injury done in the process of expressing the sap.

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