Receptivity, Incubation Period, and Lesion Size as Criteria for Screening Barley Genotypes for Resistance to *Pyrenophora teres*

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ABSTRACT

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Barley genotypes that limited the size of lesions caused by *Pyrenophora teres* did not always reduce receptivity (number of lesions per unit leaf area). Receptivity on five barley genotypes increased as the duration of leaf wetness (hours) was increased following inoculation of seedlings with spores of *P. teres*. The linear model $Y=\beta_0+\beta_1 X+E$ adequately described the relationship between the duration of leaf wetness (X) and increase in receptivity (Y) on five barley genotypes. Either the rate parameter (β_1) or relative receptivity (tested for a 15-hr leaf wetness duration period) could be used to quantify resistance that reduces receptivity. Cultivar Glenn had the highest receptivity and breeding line ND B112 the lowest. Lesion size was not greatly affected by increasing leaf wetness duration up to 24 hr, but lesion size had nearly doubled after 40 hr. Differences in incubation period were detected by determining the time (hours) within which 50% of the lesions appeared on each barley genotype. ND B112 had the greatest effect on delaying the appearance of lesions. These experiments suggest that plant breeders could increase the level of resistance to *P. teres* by utilizing barley genotypes that restrict lesion size and reduce receptivity and by making appropriate crosses and selections to combine these resistance components.

Net blotch, which is caused in barley by *Pyrenophora teres* (Died.) Drechsl. (imperfect stage, *Drechslera teres* Sacc.), occurs wherever barley (*Hordeum vulgare* L.) is grown. Net blotch was first described in the United States in the early 1900s and is believed to have been introduced into North America with barley (3). Infection causes considerable necrosis of foliage resulting in low yields and grain of poor quality (3,4). Net blotch is a major disease in the Red River Valley in the United States where 80% of the barley used by the malting and brewing industries is produced (10). Malting barley accounts for approximately one-fourth of the total barley production in the United States (10).

Resistance to *P. teres* is recognized by a reduction in lesion size. Several genes for resistance have been found among the USDA collection of barley (2,7). Upon transfer of these genes into new backgrounds, however, resistance expression is adversely affected (7). The six-row spring barley CI 11531 (breeding line ND B112) has been used extensively in crosses for the development of malting barley cultivars resistant to net blotch. Lesions are small on ND B112. In cultivars derived from crosses involving ND B112, however, it has not been possible to recover the full degree of ND B112's resistance. Still, attempts to develop resistant genotypes based on the evaluation of lesion size have been successful and are continuing (1,5).

Additional progress toward the development of genotypes resistant to *P. teres* might be made if genotypes can be identified that have the effect of reducing the rate of epidemics caused by *P. teres*. Host genotypes that reduce pathogen sporulation (i.e., reduce the number of spores per lesion or spores per unit lesion area), increase the latent period, or lower relative receptivity (the proportion of spores applied that result in lesions on one genotype relative to the number of lesions per unit leaf area on other genotypes) would slow the infection rate of *P. teres* and result in a lower disease proportion at crop maturity. Rate-limiting resistance has been considered desirable for other pathogens of small grains. The terms slow-mildewing and slow-rusting were coined to denote plant genotypes that have the effect of reducing the apparent infection rate of plant pathogen genotypes within a host population (9). In obligate parasite systems, latent period has been defined as the time from inoculation to the time 50% of the lesions (pustules or colonies) are visible (9,11). However, facultative parasites, such as *P. teres*, sporulate on necrotic tissue following moisture periods of sufficient duration and thus latent period, as defined above, would be dependent on sufficient periods of free moisture following the appearance of necrotic leaf areas. In such systems, the incubation period (visible necrosis) may provide a measurement that closely parallels the latent period.

In an earlier study, no differences in sporulation per unit lesion area or incubation period were found between barley cultivars Larker (susceptible, based on lesion size) and Glenn (moderately resistant, based on lesion size) (8). However, Glenn was significantly more receptive than Larker (8).

Our purpose was to determine the relative receptivities and incubation periods of several six-row spring barley genotypes and to determine if the duration of leaf wetness following inoculation affects lesion size or receptivity.

MATERIALS AND METHODS

Production of inoculum. A single isolate of *P. teres*, originally isolated from a barley field in Fargo, ND, was used for all experiments. Spores from 10-day-old cultures of *P. teres* grown on barley leaf agar (8) were harvested by adding approximately 20 ml of distilled water and gently dislodging the spores with a sterile, latex-tipped glass rod. The suspension was filtered through three layers of cheesecloth and adjusted to a concentration of 1,000 spores per milliliter. Gelatin-water (1%, w/w) was added (1:1, v/v) to the spore suspension to provide a final spore concentration of 500 spores per milliliter in 0.5% gelatin. A wetting agent, Tween-20 (polyoxyethylene sorbitan monolaurate), was added to the suspension at a rate of one drop per 250 ml.

Production of barley seedlings. Barley seedlings were produced by using the method of Berglund and Pederson (1). Each replication consisted of 10 seeds of each of five barley genotypes (Larker, Dickson, Glenn, Morex, and the breeding line ND B112) placed embryo end down, 1.5 cm apart along the top edge of moistened seed germination paper (38 lb, brown; Anchor Paper

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Co., St. Paul, MN). The bottom portion of the seed germination paper was folded over the seeds to secure them in place. The paper was rolled together with a waxed paper backing and secured with a rubber band. Barley genotypes were randomized within each seed roll. The seed rolls were placed upright in plastic trays and then placed inside a growth chamber maintained at 20 C. Distilled water was added and maintained at a depth of 2 cm in each tray.

Inoculation procedure. When seedlings were in the early two-leaf stage (approximately 10 days), the germination papers were unrolled and laid flat in a dew chamber. Seedlings were inoculated uniformly with an artist's air brush operated at 104 kPa at the rate of 50 ml of spore suspension per 300 plants (six seedling rolls). After seedlings were inoculated, they were left in the dew chamber for periods ranging from 6 to 40 hr at a temperature of 20 ± 1 C. A portable fan was used to dry the plants before they were returned to a growth chamber maintained at a constant temperature of 20 C and fluorescent light (350 $\mu E \cdot m^{-2} \cdot sec^{-1}$) for a 16-hr photoperiod. There were six replications (seed rolls) and the experiment was repeated twice.

Measurement of host response to infection. The number of lesions per unit leaf area (square millimeters) was recorded 5 days after inoculation. Leaf area of seedlings was determined with a LI-COR model 3000 leaf area meter (LI-COR, Inc., Lincoln, NE). Lesion size was measured 7 days after inoculation by averaging the lengths (millimeters) of 30 lesions for each cultivar in a replication and estimated visually by using a rating scale in which 1 = fleck and 9 = maximum lesion size.

Relative receptivity and incubation period of 12 barley genotypes. Seedlings were produced and inoculated as described above except all plants were removed from the dew chamber after a leaf wetness period of 15 hr following inoculation. To ascertain incubation period, the number of lesions per leaf surface were recorded 48, 72, 96, 120, and 144 hr after inoculation. Lesions were marked with an indelible pen at each assessment period and only the new visible lesions were counted during subsequent assessment periods. Data were transformed to probits (proportion of lesions visible) and the time (hours) in which 50% of the lesions were visible for each barley genotype was calculated from the linear regression coefficient and y-intercept by using the method described by Shaner (11). There were 25 plants per replication and six replications per experiment. The experiment was conducted three times.

RESULTS

Effect of leaf wetness duration on receptivity and lesion length. Receptivity of all barley genotypes increased as the period of leaf wetness was extended following inoculation with *P. teres* (Fig. 1). Receptivity did not increase on any of the barley genotypes after 30 hr of continuous leaf wetness. A linear regression model ($Y = \beta_0 + \beta_1 X + E$ in which E is the unexplained error term) adequately described the relationship between the duration of leaf wetness periods (X) and the number of lesions (Y) that developed on each genotype (Table 1). Regression coefficients for Glenn and Morex were significantly greater ($P \leq 0.05$) than for Larker, Dickson, and ND B112. The regression coefficient for ND B112 was significantly less ($P \leq 0.05$) than for the other cultivars. There were no significant differences among genotypes in relation to the point where the regression lines intercepted the x-axis (Table 1) which indicated that the minimum moist period for infection was the same for all cultivars.

Periods of leaf wetness longer than 9 hr and less than 24 hr did not significantly affect lesion size (Fig. 2). In general, the sizes of lesions, estimated visually or by measurement of their lengths, were

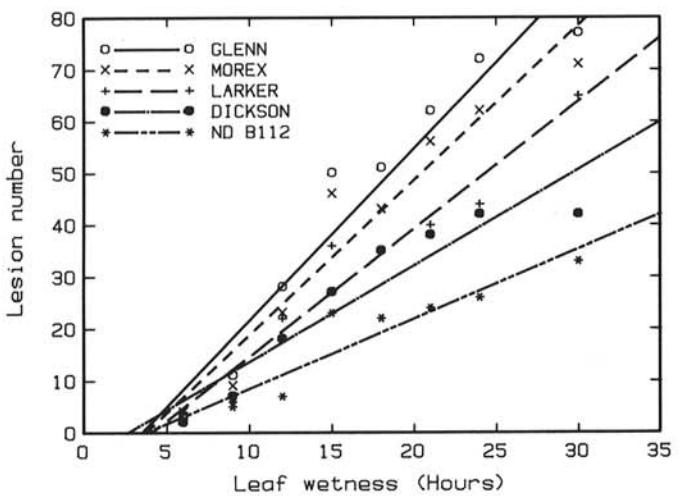

Fig. 1. Relationship between duration (hours) of leaf wetness following inoculation with *Pyrenophora teres* and lesion number for five barley genotypes.

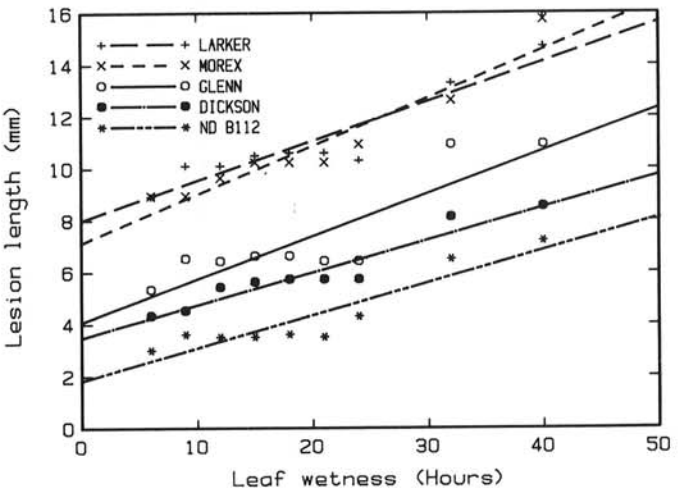

Fig. 2. Relationship between duration (hours) of leaf wetness following inoculation with *Pyrenophora teres* and lesion size for five barley genotypes.

TABLE 1. Regression of number of lesions developing on five barley genotypes on hours of leaf wetness (X) based on the linear model Y (genotype) = $\beta_0 + \beta_1 X + E$[a]

Regression equation	x-intercept	R^2 (%)[b]	F[c]
Y(Glenn) = −11.7 + 3.35 X	4.0	96.4	135.6**
Y(Morex) = −11.1 + 2.95 X	4.1	94.5	85.6**
Y(Larker) = −10.1 + 2.36 X	4.6	90.3	46.7**
Y(Dickson) = −4.9 + 1.85 X	2.8	86.4	31.8**
Y(ND B112) = −7.1 + 1.39 X	5.0	96.9	158.8**

[a] E is the unexplained error term.
[b] R^2 is the coefficient of determination.
[c] F-test significant at the 0.01 level (there is a linear relationship between X and Y).

TABLE 2. Regression of length (millimeters) of lesions caused by *Pyrenophora teres* on hours of barley leaf wetness based on the linear model Y (genotype) = $\beta_0 + \beta_1 X + E$

Regression equation[a]	R^2(%)[b]
Y(Larker) = 8.0 + 0.16 X[c]	86
Y(Morex) = 7.1 + 0.19 X	92
Y(Glenn) = 4.1 + 0.17 X	74
Y(Dickson) = 3.5 + 0.13 X	89
Y(ND B112) = 1.8 + 0.13 X	83

[a] All F-tests were significant at $P \leq 0.01$.
[b] R^2 is the coefficient of determination.
[c] β_1 parameters for the five barley genotypes were not significantly different, $P \leq 0.05$.

significantly smaller ($P \leq 0.05$) if the leaf wetness duration was 6 hr and significantly larger ($P \leq 0.05$) if leaf wetness duration was longer than 24 hr for all five genotypes. Lesion size nearly doubled after 40 hr of leaf wetness following inoculation (Fig. 2). Regression coefficients relating duration of leaf wetness period (X) to lesion length (Y) for each barley genotype were not significantly different (Table 2).

Relative infectivity and incubation period of 12 barley genotypes. Relative differences in receptivity among barley genotypes were detectable after a 15-hr postinoculation leaf wetness period. Glenn was the most receptive of the 12 barley genotypes and ND B112 was the least (Table 3). There was a strong linear relationship between relative receptivity (Table 2) and the regression coefficient (β_1) for cultivars (Table 1) relating hours of leaf wetness to lesion number (Fig. 3).

The effect of barley genotype on lesion size was determined visually by using a rating scale (range, 1 to 9) and by measuring lesion length (millimeters). With either rating method, lesions of *P. teres* were largest on Larker and smallest on Norbert and ND B112. Although lesion size (visual rating) was largest on Larker, five of the other 11 genotypes tested were significantly more receptive (Table 2). Both rating methods provided similar mean separations; however, the visual method was found to have a stronger relationship with relative receptivity ($R^2 = 68\%$) than did lesion length ($R^2 = 45\%$) (Fig. 4).

Barley genotype ND B112 had the longest incubation period (time in which 50% of the total lesion population becomes visible), but the range in T_{50} values among genotypes was 20 hr or less (Table 3). Incubation period was correlated ($P \leq 0.05$) with lesion size ($r = -0.63$), lesion length ($r = -0.59$), and relative receptivity ($r = -0.54$).

DISCUSSION

The disease proportion of barley genotypes inoculated with *P. teres* increases as the duration of leaf wetness is extended (1). The disease proportion is a function of both lesion size and lesion number:

lesion size (mm^2) × lesion number = diseased leaf area (mm^2)

and

diseased leaf area/total leaf area = disease proportion.

TABLE 3. Receptivity, lesion size, and incubation period (T_{50}) of barley genotypes inoculated with *Pyrenophora teres*

Genotype	Relative receptivity[v]	Lesion		Incubation[x] period (T_{50})
		Size[w]	Length (mm)	
Glenn	100 a[y]	6.0 bc	6.6 bc	61 a
Bumper	92 a	5.7 cd	6.0 bcd	64 ab
Morex	81 b	6.7 b	10.2 a	59 a
Hazen	78 b	6.5 b	7.5 b	63 ab
Park	76 b	6.0 bc	4.8 d	70 b
Larker	66 c	7.5 a	10.5 a	62 ab
Nordic	61 c	5.0 d	4.7 d	60 a
Robust	59 c	5.0 d	5.6 cd	58 a
Dickson	57 c	5.0 d	5.0 d	68 b
Beacon	51 d	5.5 d	7.0 b	65 ab
Norbert[z]	48 d	3.0 e	3.0 e	69 b
ND B112	36 e	3.0 e	3.6 e	78 c

[v] All values relative to those for Glenn after a 15-hr duration of leaf wetness following inoculation with spores of *P. teres*.
[w] Based on a visual rating scale of 1 to 9 in which 1 = fleck and 9 = maximum lesion size.
[x] The time (hours) in which 50% of the total number of lesions were visible, calculated from the linear regression coefficient and y-intercept of probits versus time.
[y] Values followed by the same letters are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).
[z] Two-rowed barley.

Therefore, selection of resistant lines based on reduced lesion size may not reduce disease proportion to the fullest extent possible if receptivity is enhanced, as in the case of Glenn barley. Glenn "appears" to be more resistant than Larker based on lesion size when in fact, the disease proportions are nearly identical. Sherwood et al (12) showed that overestimation of spotted areas (disease proportion) was directly proportional to the number and size of spots. Evaluating lesion size and receptivity in breeding programs rather than disease proportion could eliminate or reduce the error associated with visual screening for disease proportion. Our study showed that the increase in disease proportion is the result primarily of an increase in receptivity (lesion number) and not because of increased lesion size for postinoculation leaf wetness durations between 9 and 24 hr. Moreover, there was a significant cultivar effect on receptivity in response to increasing leaf wetness durations but little cultivar effect on lesion size since lesions on all cultivars increased in size at the same rate as the leaf wetness period was lengthened.

Keeling and Banttari (6) previously reported that the number of lesions on barley lines increased with increased leaf wetness duration. We found the linear model $Y_{\text{genotype}} = \beta_0 + \beta_1 X + E$ could be used to quantify and compare genotypic effects upon the rate lesions of *P. teres* appear in response to increasing leaf wetness periods. Because we were dealing with a population of lesions of *P.*

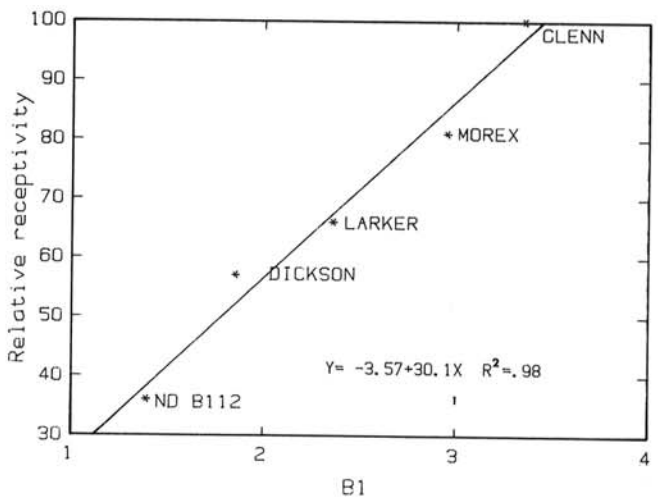

Fig. 3. Relationship between the regression coefficient relating hours of leaf wetness to lesion number (β_1) and relative receptivity of five barley cultivars to *Pyrenophora teres*.

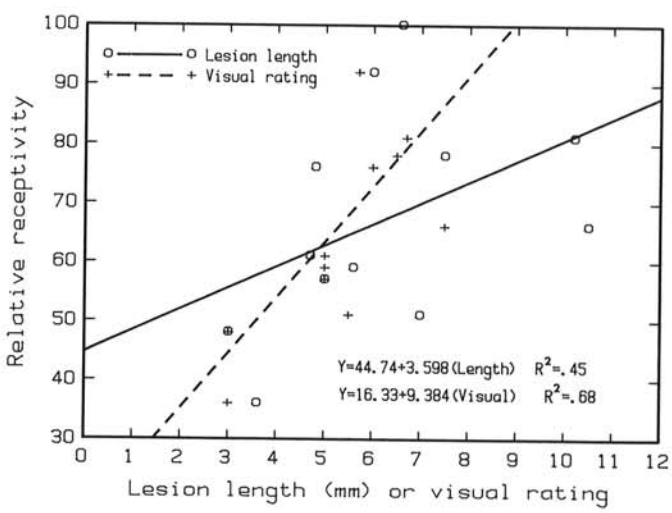

Fig. 4. Relationship between lesion size (visual rating or length) and relative receptivity of 12 barley cultivars to *Pyrenophora teres*.

teres on each barley genotype, and because approximately one-half of the potential number of lesions at 30 hr of leaf wetness had developed after 15 hr of leaf wetness, we chose 15 hr as the postinoculation leaf wetness duration period at which to measure relative receptivity among barley genotypes. Either the rate parameter (β_1) or relative receptivity after 15 hr of leaf wetness could be used to quantify genotypic effects upon lesion number, but the latter was easier to determine as well as being strongly related to β_1 ($R^2 = 98\%$).

All regression lines of the five barley genotypes intercepted the x-axis at approximately the same point (3–5 hr of leaf wetness duration), which indicates that there is little genotypic effect on the minimum number of leaf wetness hours required for infection. This agrees with the findings of Keeling and Banttari (6) who reported finding no differences among resistant and susceptible barley genotypes on the infection process (spore germination, germ tube growth, and host penetration).

Although the incubation period was longer on some genotypes than on others, it appears more feasible to select genotypes for resistance to *P. teres* based upon the ability of a genotype to restrict both lesion size and receptivity since these components were correlated with incubation period and were less labor intensive to perform.

Keeling and Banttari (6) reported finding differences in the number of spores produced in 3-mm-diameter samples of lesions on resistant versus susceptible barley genotypes. Reduced pathogen sporulation has been shown to be an important component of rate-reducing resistance (9). Selection of barley genotypes for reduced sporulation, in addition to reduced lesion size and receptivity, would further restrict epidemics caused by *P. teres*.

We have used a single isolate of *P. teres* to demonstrate the existence of genotypic effects upon resistance components in barley. All isolates collected to date in North Dakota and tested on several cultivars and lines have resulted in the same ranking of barley genotypes based on lesion size. The stability of receptivity as a resistance component in barley genotypes is imperative if this attribute is to be exploited. In an earlier study (8), we found that the receptivity of Larker and Glenn did not change after five serial transfers of *P. teres* maintained separately on either Larker or Glenn.

These methods can be used as a means of evaluating barley genotypes to identify components of resistance to *P. teres*. This information would aid plant breeders in making selections of adapted genotypes (for malting quality) for use as parents to combine resistance components and thereby increase the level of resistance and yet maintain malting quality standards. Such experiments are currently under way.

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