Inheritance of Latent Period Length in Maize Infected with *Exserohilum turcicum*

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ABSTRACT

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Extended latent period length is an important component of partial resistance of maize to northern leaf blight (NLB) caused by *Exserohilum turcicum.* Latent period length is easily measured on seedling plants under both field and greenhouse conditions and has been shown to be a relatively stable trait over a range of temperature and light conditions. The inheritance of latent period length was studied in F_2 and backcross generations of crosses of the experimental inbred line 69-1 (highly resistant, long latent period) and Mo17 (intermediately resistant, intermediate latent period) with the highly susceptible inbred line A632. Studies were conducted under both winter greenhouse conditions and in the field. Differences in mean latent periods between the two parental inbred lines varied from 14.1 days (A632 × 69-1; 1993 field experiment) to 1.8 days (A632 × Mo17; 1993 greenhouse experiment). Analysis of generation means of log-transformed latent periods revealed that over 92% of the variation among generation means could be explained by additive gene action and that dominance and epistatic effects were negligible. Heritability and gene number estimates were in agreement with previously published estimates for partial resistance to NLB measured as reduced disease severity on adult plants. Selection on some sort of progeny mean basis would probably be the most efficient and rapid means of selection for increased latent period and could potentially be more effective than selection for reduced NLB severity after anthesis.

Additional keywords: corn, *Setosphaeria turcicum*

Northern leaf blight (NLB), caused by the fungus *Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs (teleomorph = *Setosphaeria turcicum* (Luttrell) K. J. Leonard & E. G. Suggs; synonym = *Helminthosporium turcicum* Pass.), is an important foliar disease of maize (*Zea mays* L.) that occurs worldwide virtually everywhere maize is grown (8,22,26). The disease is most prevalent and damaging when cool to moderate temperatures and moist conditions prevail during the growing season (8,22,26).

The disease can cause extensive defoliation during the grain-filling period, resulting in grain yield losses of 50% or more (8,18,19,30). Resistance in maize to NLB is generally classified as one of two types: major gene resistance conferred by the *Ht1, Ht2, Ht3,* or *HtN* genes that is race-specific, or partial resistance that is under polygenic control and is effective against all pathogen biotypes (6,7,8,9,10, 11,17,20,29). The *Ht1, Ht2,* and *Ht3* genes confer a "chlorotic lesion" type of reaction to the pathogen (7,9,10), whereas the *HtN* gene results in a delay of symptoms until after anthesis (6,21). Virulence to each of these genes has been demonstrated in populations of *E. turcicum* in the U.S. and elsewhere(14,16,24,27,28,31,32). Emphasis in commercial maize-breeding programs in the U.S. has been on exploiting partial resistance to NLB, although the *Ht1* gene was used extensively prior to the discovery of isolates of *E. turcicum* with corresponding virulence to it.

Partial resistance of maize to NLB is inherited in a polygenic manner with mostly additive gene action involved (11,13). Early mapping studies using reciprocal translocations indicated that up to 10 chromosome arms of maize may be involved (12). Partial resistance of maize to NLB is expressed as a reduction in development of the disease and percent leaf area infected, which in turn may result from expression of several possible components, including incubation period, latent period, lesion size, lesion expansion rate, and sporulation intensity (1,2,4,5,20,23,25). An increase in latent period length, measured as the length of time from inoculation to the formation of necrotic lesions, is the one component most related to disease development on adult plants and is expressed over a range of temperature and light conditions (1,4,25). In an attempt to map specific components of partial resistance to NLB in the commercially important inbred line Mo17 using reciprocal translocations, Brewster et al. (2) found that loci controlling latent or incubation period and lesion numbers often mapped to the same chromosome arms as loci governing percent disease severity on adult plants. Other components of resistance such as lesion size and sporulation intensity were highly variable and not associated with disease severity. A more recent attempt to map quantitative trait loci controlling partial resistance to NLB in Mo17 using restriction fragment length polymorphisms found up to five quantitative trait loci responsible for percent NLB severity, three of which were also associated with lesion numbers (5). Latent period length, however, was not measured in that study.

The objectives of this research were to determine the inheritance of latent period length in F_2 and backcross generations of crosses between inbred lines with long and short latent periods when inoculated with *E. turcicum.*

MATERIALS AND METHODS

The highly resistant experimental maize inbred line 69-1 derived from the BS19 synthetic (3,4), and the intermediately resistant public inbred line Mo17 (2,5) were crossed to the highly susceptible inbred line A632 to develop segregating populations to study the inheritance of latent period length. The resulting F_1 crosses were selfed and crossed to each parent inbred line to form the F_2 and backcross generations, respectively.

Seeds of the parental inbred lines, the F_1, F_2, and backcross generations were planted in greenhouse and field trials to determine the mode of inheritance of latent period length. Greenhouse trials consisted of randomized complete block designs with five replications and six treatments (generations). Seeds were planted ten per row in flats containing a commercial artificial potting medium (MetroMix 200, W. R. Grace, Inc., Milpitas, Calif.), with five rows per flat, three flats per block. Each block consisted of a single row each of the parental inbred lines and F_1, three rows of each of the backcross generations, and six rows of the F_2. Seedlings were not thinned, so the final number of plants per row varied. Flats were fertilized twice weekly with a solution of a commercial 20-20-20 (NPK) fertilizer (Peter's Professional Plant Food, W. R. Grace, Inc.).

Plants were inoculated 3 to 4 weeks after planting when they were at the four- to six-leaf stage. Approximately 0.1 ml of a 10^4 conidia per ml suspension of *E. turcicum* (race O; isolate Et10) was pipetted into the leaf whorl and flats were placed overnight (16 h) in a mist chamber. Inocu-

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lum was prepared by placing maize leaf tissue infected with Et10 and collected from previous greenhouse experiments into a sealed plastic container with moist paper toweling for 72 h to induce conidiation. Conidia were washed from the sporulated leaves with tap water to which Tween 20 (1 drop per 100 ml) had been added, and the resulting suspension adjusted to final concentration with the aid of a hemacytometer.

Beginning 7 days after inoculation, plants were inspected daily between 1300 and 1500 h for the presence of necrotic lesions, and the number of symptomatic plants in each row recorded. The latent period of individual plants was recorded as the number of days to necrotic lesion formation (4), because the fungus is capable of sporulation once the lesions become necrotic. Data were log transformed prior to analysis to equalize variances of nonsegregating generations (parents and F_1).

The field experiments were conducted during the summers of 1992 and 1993 at the Genetics Gardens research plots near Raleigh, N.C. Plots were planted 17 April 1992 and 15 April 1993. The experimental design consisted of a randomized complete block with four replications. Forty seeds were planted in each row; rows were 6 m long and spaced 0.9 m apart. Plots were not thinned and the final stand varied somewhat in each row. Each block consisted of one row each of the parent inbred lines and F_1, two rows of each backcross, and four rows of the F_2.

Plants were inoculated at the four- to six-leaf stage by placing 20 to 30 grains of a sorghum grain inoculum in the leaf whorl. The sorghum grain inoculum was produced by inoculating flasks of autoclaved, moistened sorghum seed with conidial suspensions prepared by washing conidia from 10-day-old lactose caseinate agar cultures of *E. turcicum* (Et10). The inoculated sorghum cultures were grown for 2 weeks at room temperature and then stored at 4°C until used.

Latent period length on individual plants was measured as described for the greenhouse experiments.

The mean latent periods of each generation in each block were analyzed by analysis of variance and generation mean analysis using a multiple regression approach. The variation among generation means was fitted to the model $Y = m + b_1a + b_2d$, where m = overall mean, a = additive genetic effects, d = dominance genetic effects; and b_1, b_2 = the relative contribution of these effects to the means of each generation. The significance of residual variation among generation means was tested to determine the importance of epistasis. Field and greenhouse tests of the 69-1 × A632 derived population were each repeated once, whereas the Mo17 × A632 population was tested in the field and greenhouse once. Pooled within-plot variances of generations were used to calculate heritabilities and estimate numbers of effective factors controlling log latent period length in F_2 populations. Broad-sense heritabilities were calculated as $h^2 = s^2_{F2} - s^2_{(P1,P2,F1;pooled)}/s^2_{F2}$ (15). Estimates of the minimum number of effective factors (k) controlling resistance in the F_2 population were estimated by $k = (P_1 - P_2)/8*[s^2F_2 - s^2_{(P1,P2,F1;pooled)}]$(15).

RESULTS AND DISCUSSION

The mean difference in latent period between parent lines varied from 1.8 days in the Mo17 × A632 population (greenhouse 1993) to 14.1 days in the 69-1 × A632 population (field, 1993) (Figs. 1 and 2). This difference was greater in the field experiments than under greenhouse conditions (11.3 versus 4.9, and 7.2 versus 1.8 days, in the 69-1 × A632 and Mo17 × A632 populations, respectively). The distribution of latent period length in segre-

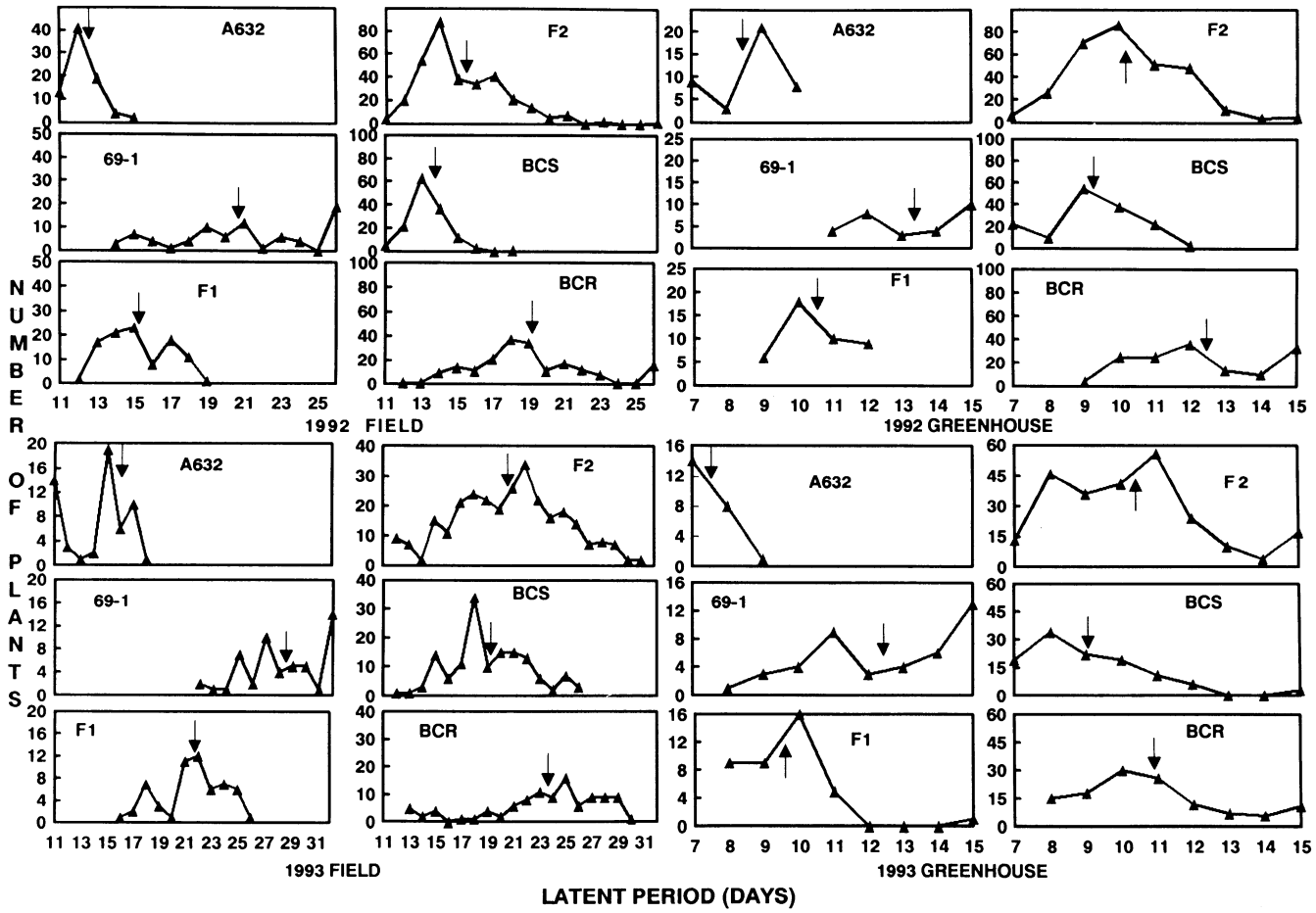

Fig. 1. Frequency distributions of latent period lengths (days to necrotic lesion formation) on individual plants of parental inbred lines 69-1 and A632, F_1, F_2, and backcross generations inoculated with *Exserohilum turcicum* in field and greenhouse trials. BCR and BCS represent the backcrosses of the F_1 to 69-1 and A632, respectively. Generation means are indicated by arrows.

gating generations was continuous without any obvious "breakpoints" for meaningful testing of Mendelian ratios (Figs. 1 and 2), so latent period length was treated as a quantitatively inherited character. Also, the variance of latent periods in the parental lines and F_1s was correlated with their means, so latent period data were log transformed, which normalized variances. The greater variability in latent period length among individual plants of the experimental inbred 69-1 was of particular concern since it was conceivable that some of this variability may have been a result of residual heterozygosity in this line at the F_6 level of inbreeding. However, this did not appear to be the case, as greenhouse tests of 10 individual ear selections of 69-1 and F_2s derived from 15 single F_1 plants did not significantly differ from each other(data not presented). In no instance did the mean of the F_1 generation deviate significantly from the midparent value, indicating the lack of dominance. The lack of any obvious dominance is further substantiated by generation mean analysis, in which dominance genetic effects were never statistically significant and additive effects accounted for 92 to 98% of the variation in latent period length among generation means in individual experiments (Table 1). In only one individual experiment (A632 × 69-1 population evaluated in the field in 1992) were residual (epistatic) effects significant, but only additive effects were significant in the combined analyses.

Estimates of broad-sense heritability for latent period length in F_2 generations ranged from 26 to 85% in individual trials, averaging 53% over all trials and populations (Table 2). Estimates of the number of effective factors governing latent period length averaged 6.4 and 3.8 in the A632 × 69-1 and A632 × Mo17 F_2 populations, respectively (Table 2). Estimates from all trials were between three and six genes with the exception of the 1992 field trial of A632 × 69-1, in which the estimate was highest (11.8) and the broad-sense heritability estimate lowest of all trials.

The preponderance of additive gene action conditioning latent period length in corn infected with *E. turcicum* is in agreement with previous studies of the inheritance of resistance to NLB, in which resistance was measured as disease severity on adult plants. The lack of significant dominance or epistatic effects means selection for increased latent period length should be straightforward with the performance of progeny predictable based upon the reactions of parents. Although it cannot be precluded that partial resistance to NLB in other sources of resistance is due to factors other than increased latent period, these data support previous studies that found latent period was the most important component (1,2,4,25).

With the exception of the 69-1 × A632 population tested in the field in 1992, broad-sense heritability estimates for latent period length in F_2s were in the medium to high range, indicating that selection for increased latent period length should result in rapid improvement. These estimates are similar to those reported by Hughes and Hooker (11) who measured partial resistance as reduced disease severity on adult (postanthesis) plants. Selection for increased latent period length, however, should be more efficient than selection for reduced disease severity, as selection can be made prior to anthesis, allowing selection of both pollen and seed parents. Latent period length can also be selected for in environments unconducive to severe NLB epidemics, as long as favorable conditions exist during the initial infection cycle. Furthermore, breeding materials may be evaluated as seedlings for latent period length in the greenhouse during the off season.

The estimates of the number of effective factors controlling latent period length are similar to those found in studies mapping genes for partial resistance (again with the exception of the 69-1 × A632 population in the field in 1992) (2,5). The relatively few genes or blocks of genes controlling latent period should allow for rapid gain from selection. The higher estimates of gene numbers in the 69-1 × A632 population versus Mo17 × A632 also indicate that the level of resistance is related to the number of resistance genes present, as 69-

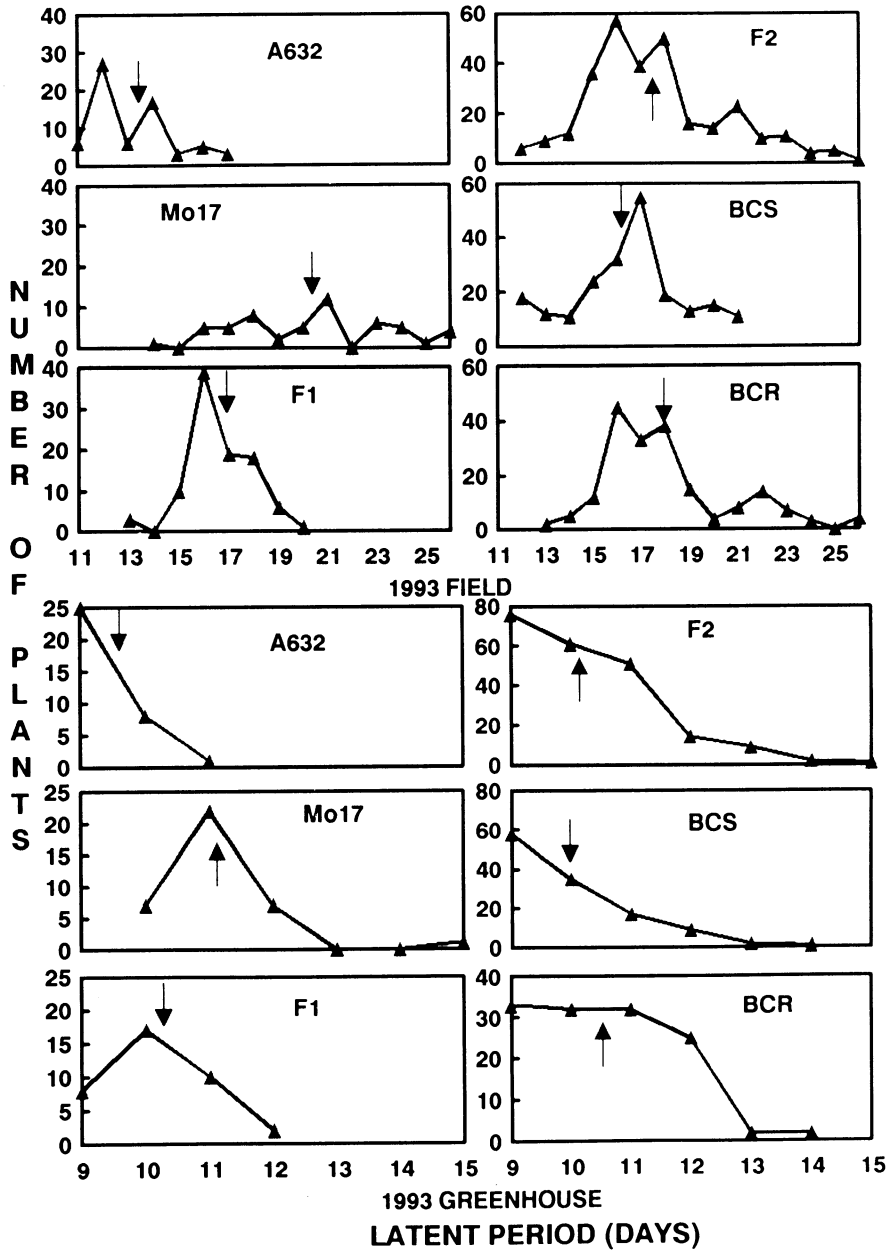

Fig. 2. Frequency distributions of latent period lengths (days to necrotic lesion formation) on individual plants of parental inbred lines Mo17 and A632, F_1, F_2, and backcross generations inoculated with *Exserohilum turcicum* in field and greenhouse trials. BCR and BCS represent the backcrosses of the F_1 to Mo17 and A632, respectively. Generation means are indicated by arrows.

Table 1. Generation mean analyses of latent period length (days to necrotic lesion formation) of parental inbred lines, F_1, F_2, and backcross generations in two populations following inoculation with race 0 of *Exserohilum turcicum*[a]

Trial	Source of variation	df	Mean squares: 69-1 × A632	Mean squares: Mo17 × A632
1992 Greenhouse	Generations	5	0.1635	. . .[b]
	Additive	1	0.7926***[c]	
	Dominance	1	0.0096 NS[d]	
	Residual	3	0.0051 NS	
	Reps	4	0.0056 NS	
	Error	20	0.0056	
1993 Greenhouse	Generations	5	0.1941	0.0185***
	Additive	1	0.9381***	0.0903***
	Dominance	1	0.0033 NS	0.0003 NS
	Residual	3	0.0097 NS	0.0006 NS
	Reps	4	0.0118 NS	0.0003 NS
	Error	20	0.0048	0.0011
1992 Field	Generations	5	0.1697	. . .[b]
	Additive	1	0.8210***	
	Dominance	1	0.0058 NS	
	Residual	3	0.0072*	
	Reps	3	0.0007 NS	
	Error	15	0.0020	
1993 Field	Generations	5	0.1907	0.0762
	Additive	1	0.9223***	0.3498***
	Dominance	1	0.0076 NS	0.0003 NS
	Residual	3	0.0079 NS	0.0103 NS
	Reps	3	0.0115 NS	0.0082 NS
	Error	15	0.0044	0.0041
Combined	Generations	5	0.6947	0.0873
	Additive	1	3.4579***[e]	0.3822**
	Dominance	1	0.0059 NS	0.0006 NS
	Residual	3	0.0032 NS	0.0179 NS
	Environments	3 (1)[f]	3.1149***	3.4939***
	Reps within environments	14 (7)	0.0076	0.0037
	Generations × environments	15 (5)	0.0078 NS	0.0138***
	Pooled error	70 (35)	0.0043	0.0024

[a] Data were log transformed prior to the analysis and fitted to the model $Y = m + b_1a + b_2d$, where Y = mean latent period of a given generation and b_1 and b_2 are the relative contribution of additive and dominance genetic effects, respectively.
[b] The Mo17 × A632 population was not evaluated in 1992.
[c] *,**,*** indicate the mean square is significant at the 0.05, 0.01, and 0.001 levels of probability, respectively.
[d] NS = not significant.
[e] The additive, dominance, residual genetic, and environment mean squares were tested with the generation × environment mean square.
[f] Numbers in parentheses are for the combined analysis of the Mo17 × A632 population.

Table 2. Estimates of broad sense heritability (h^2) and number of effective factors (k_1) for latent period length (log days) in two maize F_2 populations inoculated with race 0 of *Exserohilum turcicum*

	A632 × 69-1				A632 × Mo17	
	Field		Greenhouse		Field	Greenhouse
Estimate	1992	1993	1992	1993	1993	1993
h^2	0.26	0.56	0.45	0.51	0.85	0.56
k_1	11.8	4.7	5.8	3.3	4.4	3.2

1 has a much longer latent period than Mo17. Previous studies have also shown that the level of partial resistance to NLB is proportional to the number of resistance genes present in an inbred line (12,23).

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