Chlorotic Lesion Response of Maize to *Cercospora zeae-maydis* and Its Effect on Gray Leaf Spot Disease

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ABSTRACT

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Chlorotic lesions (CL) developed on certain $F_{2:3}$ and $F_{3:4}$ families derived from a cross between a previously described CL-resistant maize inbred (NC250A) and a susceptible (S) lesion maize inbred (B73) in response to infection by *Cercospora zeae-maydis*. CL trait expression was consistent (displayed phenotype across locations at midepidemic assessment) on 12 of the 60 $F_{2:3}$ families studied. Heterogeneous (CL/S) lesion phenotypes were displayed by 36 families at midepidemic. Consistent, characteristic S lesions were exhibited by 12 $F_{2:3}$ families. Selected $F_{2:3}$ families representing each lesion type class produced $F_{3:4}$ progenies that predominantly displayed lesion phenotypes consistent with the parental class. Families exhibiting the CL trait at midepidemic assessment had significantly lower apparent infection rates, percent leaf area affected (PLAA), and area under disease progress curves based on PLAA and lesion area. Heterogeneous families tended to have intermediate mean gray leaf spot severity, whereas disease severity and progress were highest on homozygous S lesion-type families. Because the CL trait was associated with decreased gray leaf spot severity and progress, selection for this trait in a population derived from NC250A × B73 would decrease the impact of epidemics caused by *C. zeae-maydis*. Segregation analyses of midepidemic CL responses of $F_{2:3}$ and $F_{3:4}$ families during 1992 and 1993 suggested the presence of a major factor in inheritance of the CL response. Segregation ratios observed at a later assessment date during 1992 did not support monogenic inheritance.

Cercospora zeae-maydis Tehon & E.Y. Daniels (26) causes gray leaf spot disease in maize (*Zea mays* L.) (2,16). Increased levels of inoculum and gray leaf spot severity are associated with continuous corn production and conservation tillage (15,25). Both production practices have become more widely used in the U.S. Corn Belt, and gray leaf spot has become economically important in additional areas where it previously has been only a minor disease. This disease causes annual yield losses of 10 to 25% in endemic areas, although greater losses can occur (7). Germ plasm resistant to *C. zeae-maydis* is available (2,3,7,16), and its usage will likely remain the most effective and economical method of disease control.

Resistance to *C. zeae-maydis* is controlled quantitatively, and additive genetic effects are important for expression (2,7,8,13, 28,29). Gray leaf spot progress and severity also are influenced by lesion type (10). The resistant maize inbred NC250A displays resistance characterized by chlorotic lesions (CL) in response to *C. zeae-maydis* infection (10). The CL response causes a reduction in lesion size, delay in disease progress, and inhibition of fungal sporulation. Dominant allelic interaction for the CL response was observed in hybrids containing NC250A (10). Several quantitative trait loci (QTL) were associated with genetic resistance to *C. zeae-maydis* in $F_{2:3}$ maize NC250A × B73 (susceptible lesion inbred) progenies (3). Two QTL had significant dominance deviations, whereas three showed significant additive effects (3). Thus, the CL response is probably controlled by one or more of the gray leaf spot-resistance QTL identified previously. Oligogenic CL resistance displayed by maize genotypes resistant to *Exserohilum turcicum* (12) and *Bipolaris maydis* (5) has been transferred to susceptible, economically important inbred lines. Understanding the relationship between the CL response and host resistance to *C. zeae-maydis* is desirable to enhance disease assessment and selection for host resistance. Should CL resistance to gray leaf spot be controlled oligogenically, transferring the trait to susceptible genotypes may provide economical, effective disease control.

Our objectives were to (i) develop a family structure that would allow characterization of inheritance of the CL response in $F_{2:3}$ and $F_{3:4}$ families; (ii) assess the effect of the CL response to *C. zeae-maydis* on development of gray leaf spot; and (iii) evaluate phenotypic correlations for the resistance parameters to increase selection efficiency.

MATERIALS AND METHODS

1992 genetic material. In 1991, three random F_1 plants derived from a cross between single plants of maize inbreds NC250A and B73 were self-pollinated at the Ohio Agricultural Research and Development Center near Wooster. Ears were shelled individually, and equal numbers of seeds from each ear were composited. F_2

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kernels were planted at the USDA-ARS experimental farm in Isabella, Puerto Rico, during the winter of 1991 to 1992. Randomly selected plants were self-pollinated, and ears were dried and individually shelled. Sixty $F_{2:3}$ families and susceptible (S) lesion (susceptible) inbred B73, CL (resistant) inbreds NC250A and NC288, and fleck (F) (resistant) inbred Pa875 were assessed for reactions to *C. zeae-maydis* during 1992.

1993 genetic material. The 60 $F_{2:3}$ families and inbred checks described above were reassessed for reactions to *C. zeae-maydis*. Additionally, four families homogeneous for the CL trait, three families segregating for lesion type (CL/S), and two families homogeneous for the S lesion type, identified in the 1992 experiment, were selected for progeny testing. Remnant seed from the nine selected $F_{2:3}$ families was planted in Puerto Rico during the winter of 1992 to 1993. Plants were self-pollinated, and individual ears were identified, shelled, and packaged separately. Six $F_{3:4}$ families were planted to test each homogeneous CL and each homogeneous S type $F_{2:3}$ family. Twelve $F_{3:4}$ families were planted to test each CL/S segregating $F_{2:3}$ family. The nine parental $F_{2:3}$ families were included in the experiment as were NC250A, Pa875, and B73 inbred checks.

1992 field plots. On 3 June, seeds of the 60 $F_{2:3}$ families and 4 inbreds (divided into 3 sets of 20 randomly assigned families plus inbreds) were planted in separate experiments, using a randomized complete block design with two replications, near Wooster. Sets were assigned randomly within replicates. Experimental units were 1-row plots, 3.5 m long, with between-row spacing of 0.76 m. Fifteen kernels were hand-planted and subsequently thinned to twelve plants per row to reduce differences in disease spread within plots due to variable population density (6). Seeds of the same lines were hand-planted following the same procedure at Columbus on 27 May. $F_{2:3}$ family sets and replicates were identical but randomized independently at each location.

1993 field plots. On 27 May, seeds of the 60 $F_{2:3}$ families and inbreds (divided into 3 sets) as well as the 72 $F_{3:4}$ families, 9 $F_{2:3}$ parents, and 3 check inbreds were planted in separate experiments, using a randomized complete block design with two replications, near Wooster. Seeds were hand-planted. The experimental design was identical to 1992. The 60 $F_{2:3}$ family sets and replicates were identical to those in 1992 but were randomized independently at each location. Fertilizers and herbicides used during both years were applied according to standard recommendations (1).

Inoculum preparation. Portions of *C. zeae-maydis* that colonized air-dried leaf tissue collected the previous season were incubated in petri dishes lined with moist filter paper and placed under high relative humidity for 3 days to allow sporulation. Single conidia were picked from conidiophores with a sterile glass needle and placed on V8 juice agar in petri plates (16). Cultures were grown for 5 days at 28°C with 12 h of darkness and 12 h of fluorescent light (320 $\mu E\ m^{-2}\ s^{-1}$). Conidia were spread over the agar surface by placing 1 ml of sterile distilled water on the agar and streaking with a sterile glass rod. Cultures were maintained an additional 5 days as before. Fungal cultures were transferred to moistened autoclaved oat (*Avena sativa*) kernels in 2-liter flasks. Flasks were incubated for an additional 10 to 12 days and shaken every other day. Kernels colonized by the fungus were air-dried for 3 to 4 days at 22 to 24°C prior to use.

Inoculation. In 1992 and 1993, plants were inoculated by placing approximately 2 g of infested oat kernels into the whorl at the V6, V8, and V10 growth stages (20). Initial inoculation dates were 2 and 9 July 1992 at Columbus and Wooster, respectively. The initial inoculation date was 12 July 1993 at Wooster. Overhead sprinkler irrigation was applied for 1 h at dusk on each of 14 days after inoculation if rainfall was not predicted to occur during the evening. The inoculation procedure was the same in 1993 as in 1992; however, overhead sprinkler irrigation was applied for 20 min during each of 12 h on three consecutive nights (9 p.m. to 8 a.m.) each week from the date of first inoculation through the last disease assessment date.

Disease assessments. To document transition in lesion types over time, lesions were classified as CL, S, or F (10) on all plants within families and inbreds 48 (19 and 26 August), 57 (28 August and 4 September), and 72 (12 and 19 September) days after initial inoculations (DAI) at Columbus and Wooster, respectively, during 1992, and 57 (7 September) and 68 (18 September) DAI at Wooster during 1993. To evaluate disease progress over time, five plants in each plot were selected randomly and tagged, and ear leaves were rated for percent ear leaf area affected (PLAA) by the gray leaf spot assessment scales developed by Smith (23). During 1992, PLAA values were determined 35, 47, 61, and 80 DAI at Wooster and 39, 48, 57, and 72 DAI at Columbus. In 1993, PLAA values were determined 38, 49, 55, 61, 70, and 79 DAI at Wooster.

Two lesions on each of the five tagged plants per plot were marked during the first PLAA assessment, and their lengths and widths were measured. Relatively small lesions were selected for measurements so their increase in size could be monitored with age. Lesion measurements were recorded on each subsequent PLAA assessment date. Mean lesion areas were calculated for each family on each date.

Sporulation, expressed as the number of conidia per square millimeter of lesion tissue, was determined for the 72 $F_{3:4}$ families during the late season on two sampling dates approximately 10 days apart at Wooster during 1993. Three intact lesions from the leaf above the ear were excised and measured per genotype for each of the two replicates. The lesion tissues were placed on a wire-mesh screen in a glass petri dish with a water-saturated filter paper in the bottom. Dishes containing the lesions were maintained on a lab bench at 28°C with 12 h of darkness and 12 h of fluorescent light (320 $\mu E\ m^{-2}\ s^{-1}$). After 48 h, lesions were placed in 10-ml test tubes containing 2.5 ml of distilled water. Tubes were agitated for 15 s with a bench-top vortex mixer (Super-Mixer, Lab-Line Instruments, Melrose Park, IL). The spore suspension was pipetted onto a hemacytometer, and conidia counts were determined.

Statistical methods. Data analysis was based on the assumption that entries (within lesion type groups), sets, blocks, and environments were random. Analysis of variance (ANOVA) indicated that mean squares for both replicates (blocks) and sets were not significant, so we considered their treatment random. A combined analysis was performed for the NC250A × B73 $F_{2:3}$ experiment (19).

Apparent infection rates (r) were determined by calculating the slopes of regression lines representing the increase in disease severity over time, using the exponential model (4). The area under the disease progress curve (AUDPC) (22) was calculated for ear leaf PLAA and lesion area by the midpoint rule standardized by dividing the AUDPC by the number of days from the first to the last assessment for each observation (11). Differences in r, ear leaf PLAA, and AUDPC values were determined by ANOVA (24).

Single-df orthogonal contrasts were used to compare r, ear leaf PLAA, and AUDPC values for the CL, CL/S, and S type groups from the 60 $F_{2:3}$ progenies. Orthogonal contrasts were performed with data taken 57 DAI at Columbus during 1992 and 68 DAI at Wooster during 1993. Lesion type groups of $F_{3:4}$ progenies also were compared by single-df orthogonal contrasts in the same manner with data obtained 68 DAI during 1993. At these times, gray leaf spot lesions were present on the upper leaves of the progeny tested, and the susceptible check, B73, averaged approximately 50 PLAA, thus the epidemic appeared to be at its midpoint. The error terms used in among-group comparisons included variability among entries within a group, because it was assumed that entries within groups were random.

Segregation ratios for lesion responses between the 60 $F_{2:3}$ families at epidemic midpoint (57 DAI at Columbus during 1992 and 68 DAI at Wooster during 1993) were analyzed by a chi-square goodness-of-fit test for an expected 1 CL:2 CL/S:1 S ratio. Segregation ratios for lesion responses of $F_{2:3}$ families at late season (72 DAI) also were tested for the same expected segregation ratios. Chi-square goodness-of-fit tests were performed to test ratios be-

tween segregating $F_{3:4}$ families at epidemic midpoints at Wooster during 1993. Because the treatments consisted of a random set of genotypes, phenotypic correlations of PLAA, AUDPC for lesion area, and sporulation were derived from analysis of covariance, using the ANOVAs for each variable.

RESULTS

1992 disease development. Gray leaf spot began to spread within plots during mid-August in Columbus and late August near Wooster. Late-season PLAA and AUDPC based on PLAA did not differ across locations within a season (1992) for the 60 $F_{2:3}$ families (Table 1). AUDPC based on lesion area and r were significantly different across locations and seasons. Differences in disease levels between locations were likely due to earlier inoculation in Columbus and differences in precipitation patterns.

1993 disease development. Gray leaf spot developed slowly in plots near Wooster. Gray leaf spot severity was lower during 1993 than during 1992 at this location, but rate of disease increase was greater during 1993 than during 1992 due to rapid disease spread later in the season (Table 1).

Transition of lesion type. Lesion types expressed by the 60 $F_{2:3}$ families were classified during 1992 and 1993. Lesion class segregation ratios from early (48 DAI) or late (72 DAI) 1992 groupings differ from results based on the midepidemic (57 DAI) grouping because individual plant lesion responses changed over time (Table 2). Early in the epidemic, 31 families were classified as homogeneous for the CL response; 19 were reclassified during the epidemic; and 12 remained in the CL group at 72 DAI. Lesion type grouping for 57 of the 60 families studied in 1993 displayed identical phenotypes in 1992 at the first assessment date in each year (48 DAI, 1992; 57 DAI, 1993).

Nine $F_{2:3}$ families differing for early season lesion expression were selected during the 1992 season (Table 3). These selections and their progeny were examined in 1993. The $F_{3:4}$ families derived from the selected $F_{2:3}$ parents also displayed transitional lesion types. Thus, transition of lesion type occurred on some genotypes, whereas others tended to display a single lesion type throughout the assessment period.

Lesion type segregation analyses. Segregation of $F_{2:3}$ families at the midepidemic assessment (57 DAI) in 1992 (18 CL:27 CL/S:15 S) were consistent with a 1:2:1 monogenic segregation in the F_2 population from which the families were derived. Chi-square goodness-of-fit tests to compare the obtained ratios (57 DAI) with a 1:2:1 expected ratio were highly significant ($P = 0.64$). Segregation ratios obtained from late-season assessments (72 DAI, 1992) were not consistent with monogenic inheritance of the CL response. Classification of phenotypic segregation of $F_{2:3}$ families at midepidemic in 1993 (18 CL:28 CL/S:14 S) was nearly identical to that observed in 1992. Chi-square goodness-of-fit tests were again consistent with expected monogenic segregation ratios ($P = 0.65$). Late-season lesion phenotypes were not scored because of delayed disease onset in 1993. $F_{3:4}$ families derived from selected $F_{2:3}$ parents were classified for lesion types. All 557 plants within 24 $F_{3:4}$ families derived from 4 CL $F_{2:3}$ parental families were classified as CL (68 DAI). CL/S parents produced families segregating 1 CL:2 CL/S:1 S for lesion type (Table 4). Two S parental $F_{2:3}$ families produced 12 $F_{3:4}$ families consisting of a total of 269 susceptible and 11 CL plant phenotypes. The chi-square goodness-of-fit tests applied to observed versus expected ratios derived from

TABLE 1. Mean gray leaf spot development in 60 maize $F_{2:3}$ families derived from NC250A × B73 during 1992 and 1993 experiments at Wooster and Columbus, OH

Environment	r[a] (per day)	Final PLAA[b]	AUDPC PLAA[c]	AUDPC Lesion area[d]
1992, Wooster	0.160	42.2	7.8	18.4
1992, Columbus	0.084	39.7	8.7	27.1
1993, Wooster	0.291	20.8	3.2	11.1
$LSD_{0.05}$	0.048	19.5	4.4	5.3

[a] r = apparent infection rate or slopes of regression lines representing the increase in disease severity from 35 to 80 days after inoculation at Wooster during 1992, 39 to 72 days after inoculation at Columbus during 1992, and 38 to 79 days after inoculation at Wooster during 1993, using the exponential model.
[b] Mean percent ear leaf area affected (PLAA) based on a single late-season assessment on five individual plants per replication.
[c] Area under the disease progress curve (AUDPC) based on PLAA from four assessments from 35 to 80 days after inoculation at Wooster during 1992, 39 to 72 days after inoculation at Columbus during 1992, and 38 to 79 days after inoculation at Wooster during 1993.
[d] AUDPC based on lesion area from 35 to 80 days after inoculation at Wooster during 1992, 39 to 72 days after inoculation at Columbus during 1992, and 38 to 72 days after inoculation at Wooster during 1993.

TABLE 2. Transition from chlorotic to susceptible gray spot lesion type (CL and S, respectively) expression for NC250A × B73 maize $F_{2:3}$ families based on lesion type determined at various assessment times in Wooster and Columbus, OH, experiments during 1992 and in the Wooster experiment during 1993

Lesion type	DAI, 1992[a] 48	57	72	DAI, 1993[a] 57	68
CL	31	18	12	28	18
CL/S	17	27	23	20	28
S	12	15	25	12	14

[a] DAI = days after initial inoculation. Number of families classified as homogeneous for CL, heterogeneous for CL/S lesions, and homogeneous for S lesions.

TABLE 3. Transition of gray spot lesion type on nine selected NC250A × B73 maize $F_{2:3}$ families tested near Wooster, OH, during 1992 and 1993

Family	DAI, 1992[a] 48	57	72	DAI, 1993[a] 57	68
548-6	CL	CL	CL	CL	CL
550-20	CL	CL	CL/S	CL	CL
553-22	CL[b]	CL	CL/S	CL	CL
555-14	CL	CL	CL	CL	CL
550-24	CL/S	CL	CL/S	CL	CL/S
556-3	CL	CL/S	CL/S	CL	CL/S
550-16	CL/S	CL/S	CL/S	CL/S	S
553-1	CL/S	CL/S	S[c]	CL/S	S
553-2	S	S	S	S	S

[a] DAI = days after initial inoculation. Lesion types classified as homogeneous for chlorotic lesion (CL), heterogeneous for chlorotic and susceptible lesions (CL/S), and homogeneous for susceptible lesions (S).
[b] One plant was rated S; however the line was considered CL.
[c] One plant was rated CL; however the line was considered S.

TABLE 4. Progeny testing of three selected $F_{2:3}$ maize families segregating for gray spot lesion type with 36 NC250A × B73 $F_{3:4}$ families inoculated with *Cercospora zeae-maydis* during 1993

$F_{2:3}$ family[a]	$F_{3:4}$ families[b] No.	Response	No. of segregating families: Obs. $F_{3:4}$ pheno.[c] CL	CL/S	S	Ex. pheno. ratios[c] (CL:CL/S:S)	P
550-24	12	CL/S	3	5	4	1:2:1	0.90
556-3	12	CL/S	3	5	4	1:2:1	0.90
550-16	12	CL/S	1	7	4	1:2:1	0.65
Total	36	CL/S	7	17	12	1:2:1	0.87

[a] $F_{2:3}$ families from which 36 heterogeneous chlorotic/susceptible lesion type (CL/S) $F_{3:4}$ families were derived.
[b] CL = chlorotic lesion response; S = susceptible lesion type at midepidemic; and CL/S = segregating for CL and S 68 days after inoculation.
[c] Obs. = observed; pheno. = phenotype or phenotypic; and Ex. = expected.

TABLE 5. Mean squares obtained from analysis of variance for gray leaf spot severity on 60 NC250A × B73 maize $F_{2:3}$ families, grouped according to lesion response determined at a midepidemic assessment and combined from three environments in Ohio during 1992 and 1993[a]

Source[b]	df	r[c]	PLAA[d]	AUDPC	
				PLAA[e]	Lesion area[f]
Genotype	59	0.005**	1,476.0**	67.5**	33.7
Among groups	2	0.030**	16,317.0**	646.7**	168.9**
CL/S vs. CL or S	1	0.0001	16,352.5**	675.3**	30.9
CL vs. S	1	0.059**	16,281.5**	618.1**	306.9**
Within groups	57	0.004	955.3**	47.2**	28.9
CL	17	0.003	586.3	21.5	23.0
CL/S	26	0.004	1,371.6**	71.8**	34.4
S	14	0.004	630.2	32.6	25.9
G × E[g]	118	0.003	416.9	20.8	34.9
Error	177	0.002	287.6	15.1	33.5

[a] ** indicate significance at $P = 0.01$.
[b] CL = families homogeneous for the chlorotic lesion response; CL/S = families segregating for lesion type; and S = families homogeneous for the susceptible lesion type.
[c] r = apparent infection rate or slopes of regression lines representing the increase in disease severity from 35 to 80 days after inoculation, using the exponential model.
[d] Mean percent ear leaf area affected (PLAA) based on a single late-season assessment 80 days after inoculation on five individual plants per replication.
[e] Area under the disease progress curve (AUDPC) based on PLAA from four assessments from 35 to 80 days after inoculation.
[f] AUDPC based on lesion area from 35 to 80 days after inoculation.
[g] Genotype × environment.

TABLE 6. Gray leaf spot severity on 60 NC250A × B73 maize $F_{2:3}$ families and check inbreds combined from three environments in Ohio during 1992 to 1993

Entry[a]	No. of families	r[b]	PLAA[c]	AUDPC		Lesion type[f]
				PLAA[d]	Lesion area[e]	
$F_{2:3}$ (CL)	18	0.159	20.8	3.9	17.6	CL
$F_{2:3}$ (CL/S)	27	0.184	36.7	7.0	19.0	CL/S
$F_{2:3}$ (S)	15	0.191	45.8	9.0	20.2	S
NC250A		0.141	5.1	0.9	14.2	CL
NC288		0.175	7.4	1.4	17.5	CL
Pa875		0.159	3.9	0.9	4.7	F
B73		0.205	68.3	15.6	22.7	S
Mean		0.177	32.1	6.3	18.2	

[a] CL = $F_{2:3}$ families homogeneous for the chlorotic lesion response; CL/S = $F_{2:3}$ families segregating for lesion type; and S = $F_{2:3}$ families homogeneous for the susceptible lesion type.
[b] r = apparent infection rate or slopes of regression lines representing the increase in disease severity from 35 to 80 days after inoculation, using the exponential model.
[c] Mean percent ear leaf area affected (PLAA) based on a single late-season assessment 80 days after inoculation on five individual plants per replication.
[d] Area under the disease progress curve (AUDPC) based on PLAA from four assessments from 35 to 80 days after inoculation.
[e] AUDPC based on lesion area from 35 to 80 days after inoculation.
[f] Lesion type classified as CL = chlorotic; F = fleck; or S = susceptible.

midepidemic data of NC250A × B73 $F_{3:4}$ segregating families further supported the hypothesis of monogenic inheritance of the CL response to *C. zeae-maydis* at midepidemic (Table 4).

Effects of CL response on gray leaf spot. Results from our combined analysis involving four traits indicated that significant genotype × environment interaction did not occur for any trait, although differences were significant among lesion type groups for disease variables (Table 5). The heterogeneous, segregating CL/S group differed from the homogeneous resistant (CL) and homogeneous susceptible (S) groups evaluated in terms of PLAA and AUDPC based on PLAA. Disease severity of the CL group, for all traits measured, was significantly lower than the susceptible group (Table 6). PLAA and AUDPC based on PLAA differences within groups were significant (Table 5). Within the segregating group, only PLAA differences were significant. This was expected because both resistant and susceptible individuals comprised a segregating family. $F_{2:3}$ families within the segregating group had less PLAA and lower AUDPC based on PLAA than families displaying the S lesion response (Table 6).

Previous research showed that the CL trait reduced gray leaf spot severity on inbreds and hybrids. Disease data from families grouped according to lesion reaction occurring on a midepidemic date indicated that families homogeneous for the CL response inhibited infection rate, had lower PLAA values, and reduced AUDPC values for PLAA and lesion area (Table 6).

Interpretation of ANOVA results indicated that significant differences existed for r, PLAA, and AUDPC based on PLAA for the 1993 $F_{3:4}$ families (Tables 7 and 8). All sources, except within the CL/S S groups, differed for AUDPC based on lesion area enlargement (Table 7). Differences for conidia per square millimeter were significant only within the S group.

Phenotypic correlations. Midepidemic and late-season PLAA measurements were correlated with AUDPC based on lesion area in the NC250A × B73 $F_{2:3}$ and $F_{3:4}$ families (Table 9). Late-season PLAA measurement was not correlated with conidia per square millimeter.

DISCUSSION

CL that progressed to S lesions in response to *C. zeae-maydis* have been described on inbred lines (10). Lesion transition also occurred within partially inbred families during this study. Dominant allelic interaction regulating CL expression has been reported (3,8,10). In this study, many heterozygotes expressed CL at an early date. As the epidemic progressed, the CL expression may have been moderated, and some heterozygotes could then be reclassified as susceptible.

Gene activity for the CL response also may be influenced by light quantity or intensity because light affects cercosporin production by the pathogen (14). The environmental effect on the symptom response we observed may be analogous to expression of major gene CL resistance to *E. turcicum*, which was reported to be influenced by light (17) and temperature (17,27). In one study, temperature affected lesion expression by influencing a change in lesion type from resistant to susceptible over time (27). Another possibility is that an additional modifying factor or factors could have mitigated CL expression through epistasis (21).

We emphasized study of the CL trait with midepidemic assessment data because lesion type was readily observable at this time and comparison of genotypes displaying various responses to the pathogen could be made. Early assessments did not reveal differences in resistance responses and late assessments were undesirable because rate of disease progress in susceptible genotypes

TABLE 7. Summary of analysis of variance for gray leaf spot severity on 72 NC250A × B73 maize $F_{3:4}$ families representing chlorotic lesions, susceptible, and segregating phenotypic classifications for 1993

		Mean squares[b]				
				AUDPC		
Source[a]	df	r[c]	PLAA[d]	PLAA[e]	Lesion area[f]	Conidia[g]
Genotype	71	0.001**	543.4**	19.3**	18.4**	5,401.8
Among groups	2	0.001**	6,982.3**	275.1**	139.5**	999.5
CL/S vs. CL or S	1	0.001*	9,584.2**	352.0**	237.0**	1,071.6
CL vs. S	1	0.001*	4,380.4**	198.6**	42.0*	927.4
Within groups	69	0.001**	356.7**	11.8**	14.9**	5,529.4
CL	30	0.001**	201.2**	6.1**	19.7**	3,493.2
CL/S	18	0.001**	414.9**	10.2**	10.6	5,307.6
S	21	0.001**	529.1**	21.4**	11.6	8,628.5*
Error	71	0.0002	75.1	2.8	7.6	4352.7

[a] CL = $F_{3:4}$ families homogeneous for the chlorotic lesion response; CL/S = $F_{3:4}$ families segregating for lesion type; and S = $F_{3:4}$ families homogeneous for the susceptible lesion response.
[b] * and ** indicate significance at $P = 0.05$ and 0.01, respectively.
[c] r = apparent infection rate or slopes of regression lines representing the increase in disease severity from 35 to 80 days after inoculation, using the exponential model.
[d] Mean percent ear leaf area affected (PLAA) based on a single late-season assessment 80 days after inoculation on five individual plants per replication.
[e] Area under the disease progress curve (AUDPC) based on ear leaf PLAA from four assessments from 35 to 80 days after inoculation.
[f] AUDPC based on lesion area from 35 to 80 days after inoculation.
[g] Mean number of conidia × 10^4/mm^2 from two sample dates with two replicate samples of four lesions per date.

TABLE 8. Parameters representing gray leaf spot severity caused by *Cercospora zeae-maydis* on NC205A × B73 maize $F_{2:3}$ families, $F_{3:4}$ progeny, and check inbreds during 1993

				AUDPC			
Entry[a]	No. of families	r[b]	PLAA[c]	PLAA[d]	Lesion area[e]	No. of conidia[f]	Lesion type[g]
CL	31	0.279	14.5	2.5	11.9	104.3	CL
CL/S	19	0.304	29.0	5.0	12.6	110.8	CL/S
S	22	0.315	37.1	7.1	15.1	111.7	S
$Parents_{CL}$	4	0.273	12.4	2.1	12.1	128.7	CL
$Parents_{CL/S}$	3	0.289	16.8	3.0	14.6	89.2	CL/S
$Parents_{S}$	2	0.291	19.7	3.6	16.2	68.3	S
NC250A		0.236	2.1	0.4	7.3	53.9	CL
Pa875		0.240	2.3	0.4	5.1	73.6	F
B73		0.315	36.7	7.7	16.1	147.7	S
Mean		0.294	23.8	4.3	13.0	107.1	

[a] CL = families homogeneous for the chlorotic lesion response; CL/S = families segregating for lesion type; and S = families homogeneous for the susceptible lesion type.
[b] r = apparent infection rate or slopes of regression lines representing the increase in disease severity from 35 to 80 days after inoculation, using the exponential model.
[c] Mean percent ear leaf area affected (PLAA) based on a single late-season assessment 80 days after inoculation on five individual plants per replication.
[d] Area under the disease progress curve (AUDPC) based on ear leaf PLAA from four assessments from 35 to 80 days after inoculation.
[e] AUDPC based on lesion area from 35 to 80 days after inoculation.
[f] Mean number of conidia × 10^4/mm^2 from two sample dates with two replicate samples of four lesions per date.
[g] Lesion types classified as homogeneous for chlorotic lesions (CL), heterogeneous for chlorotic and susceptible lesions (CL/S), homogeneous for susceptible lesions (S), and homogeneous for fleck (F).

was slowed by limited leaf surface area available for infection and complications resulting from leaf senescence (4). In contrast, disease progress in resistant hosts may have reached maximum growth rates late in the season, resulting in artificially high rates of disease increase. This would have reduced differences between r and AUDPC between susceptible and resistant progenies.

Data showing inheritance of CL expression within segregating populations has not been reported previously. Our results indicated that CL expression may be similar to other typical qualitative traits, because location and season effects on classification were small. The ability to accurately group plants into CL/S categories by quick visual estimation also supported the hypothesis of qualitative inheritance for CL resistance to *C. zeae-maydis*. Chi-square analyses of midepiphytotic ratings of $F_{2:3}$ families and $F_{3:4}$ segregating families were consistent with the hypothesis that a single gene conferring the CL response was segregating in the expected ratios (9). Analysis of late-season ratings of $F_{2:3}$ families was not consistent with monogenic inheritance.

Another objective was to assess the impact of CL resistance on gray leaf spot in segregating progeny. Overall, our results indicate that the CL trait is heritable and reduces gray leaf spot severity and progress in heterogeneous progeny. Some CL genotypes have significantly greater disease than others, indicating that the factor or factors controlling CL are likely a subset of multiple genes affecting *C. zeae-maydis* infection and spread. The CL trait expressed by some maize inbred lines has little or no effect on gray leaf spot severity (10). This was not the case for NC250A, because CL is typically associated with reduced disease levels.

Expression of resistance to *C. zeae-maydis* also is influenced by environmental factors (3). Multiple trait measurements involving several plants on several dates at two locations during 2 years increased experimental precision. A researcher performing studies involving gray leaf spot resistance may want to consider incorporating these assessments. Additional precision also could be gained by increasing replication. Multiple measurements on developing lesions are needed to derive AUDPC based on lesion size. Multiple PLAA assessments also are necessary to calculate r. Practical considerations demand that disease estimation in the field be quick and effective. Previous results indicate that one late-season PLAA assessment may be sufficient to evaluate *C. zeae-maydis*

TABLE 9. Phenotypic correlations involving gray leaf spot severity variables for NC250A × B73 maize $F_{2:3}$ and $F_{3:4}$ progenies at Wooster, OH, from 1992 to 1993

Variables[a]	*n*	Phenotypic correlation[b]
80 DAI, 1992, PLAA, and lesion size AUDPC	60	0.87**
1992, *r*, and lesion size AUDPC	60	0.72**
61 DAI, 1992, PLAA, and lesion size AUDPC	60	0.62**
1992, *r*, and lesion size AUDPC	60	0.68**
79 DAI, 1993, PLAA, and lesion size AUDPC	72	0.44**
1993, *r*, and lesion size AUDPC	72	0.51**
79 DAI, 1993, PLAA, and conidia/mm^2	72	0.01

[a] DAI = days after initial inoculation; PLAA = percent leaf area affected; AUDPC = area under the disease progress curve; and *r* = apparent infection rate or slopes of regression lines representing the increase in disease severity from 35 to 80 days after inoculation, using the exponential model.
[b] ** indicate significance at $P = 0.01$.

resistance (8,18). Late-season lesion type assessment may be desirable to eliminate all but the most resistant CL genotypes. Phenotypic correlations between the late-season rating and the other integrated evaluations were highly significant. Thus, a single late-season PLAA rating also will be a suitable, indirect measure of AUDPC, although it cannot replace integrated assessments in certain studies (3). Highly resistant genotypes that produce the CL response can be determined at this time.

Gray leaf spot severity and progress within and among NC250A × B73 families displaying the CL response was significantly reduced. Thus, the CL trait affected PLAA, *r*, AUDPC based on PLAA, and AUDPC based on lesion area. Because the CL reaction inhibits disease development, selecting progeny displaying the trait will reduce gray leaf spot progress and severity in this cross, but conceivably not in others. The response can be exploited because selection for the NC250A-type CL response is simple and effective and will delay epiphytotics caused by *C. zeae-maydis*.

LITERATURE CITED

1. Anonymous. 1989. Corn production. Pages 31-34 in: Ohio Agronomy Guide. Ohio State Univ. Ext. Bull. 472.
2. Ayers, J. E., Johnson, M. W., Jr., and Hill, R. R., Jr. 1984. Identifying resistance to gray leaf spot. Pages 157-175 in: Proc. 39th Annu. Corn Sorghum Res. Conf. American Seed Trade Association, Washington, DC.
3. Bubeck, D. M., Goodman, M. M., Beavis, W. D., and Grant, D. 1993. Quantitative trait loci controlling resistance to gray leaf spot in maize. Crop Sci. 33:838-847.
4. Campbell, C. L., and Madden, L. V. 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York.
5. Craig, J., and Fajemisin, J. M. 1969. Inheritance of chlorotic lesion resistance to *Helminthosporium maydis* in maize. Plant Dis. Rep. 53:742-743.
6. de Nazareno, N. R. X., Lipps, P. E., and Madden, L. V. 1993. Effect of levels of corn residue on the epidemiology of gray leaf spot of corn in Ohio. Plant Dis. 77:67-70.
7. Donahue, P. J., Stromberg, E. L., and Myers, S. L. 1991. Inheritance of reaction to gray leaf spot in a diallel cross of 14 maize inbreds. Crop Sci. 31:926-931.
8. Elwinger, G. F., Johnson, M. W., Hill, R. R., Jr., and Ayers, J. E. 1990. Inheritance of resistance to gray leaf spot of corn. Crop Sci. 30:350-358.
9. Freppon, J. T. 1994. Inheritance of the chlorotic lesion response to gray leaf spot in maize. Ph.D. dissertation. The Ohio State University, Columbus.
10. Freppon, J. T., Lipps, P. E., and Pratt, R. C. 1994. Characterization of the chlorotic lesion response by maize to *Cercospora zeae-maydis*. Plant Dis. 78:945-949.
11. Fry, W. E. 1978. Quantification of general resistance of potato cultivars and fungicide effects for integrated control of potato late blight. Phytopathology 68:1650-1655.
12. Hooker, A. L. 1963. Inheritance of chlorotic lesion resistance to *Helminthosporium turcicum* in seedling corn. Phytopathology 53:660-662.
13. Huff, C. A., Ayers, J. E., and Hill, R. R., Jr. 1988. Inheritance of resistance in corn (*Zea mays*) to gray leaf spot. Phytopathology 78:790-794.
14. Jenns, A. E., Daub, M. E., and Upchurch, R. G. 1989. Regulation of cercosporin accumulation in culture by medium and temperature manipulation. Phytopathology 79:213-219.
15. Kingsland, G. C. 1963. Cercospora leaf blight of corn: A case history of a local epiphytotic in South Carolina. Plant Dis. Rep. 47:724-725.
16. Latterell, F. M., and Rossi, A. E. 1983. Gray leaf spot of corn: A disease on the move. Plant Dis. 67:842-847.
17. Leath, S., Thakur, R. P., and Leonard, K. J. 1990. Variation in expression of monogenic resistance in corn to *Exserohilum turcicum* race 3 under different temperature and light regimes. Phytopathology 80:309-313.
18. Lipps, P. E., and Pratt, R. C. 1991. Corn gray leaf spot research—1990. Ohio Agric. Res. Dev. Cent. Plant Pathol. Ser. 87.
19. McIntosh, M. S. 1983. Analysis of combined experiments. Agron. J. 75: 153-155.
20. Ritchie, S. W., Hanway, J. J., and Benson, G. O. 1989. How a corn plant develops. Iowa State Univ. Spec. Rep. 48.
21. Saghai-Maroof, M. A., Yu, Y. G., Xiang, Z. X., Stromberg, E. L., and Rufener, G. K. Identification of quantitative trait loci controlling resistance to gray leaf spot disease in maize. Theor. Appl. Genet. In press.
22. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.
23. Smith, K. L. 1989. Epidemiology of gray leaf spot of field corn (*Zea mays* L.) caused by *Cercospora zeae-maydis* Tehon & Daniels. Ph.D. dissertation. University of Maryland, College Park.
24. Steel, R. G. D., and Torrie, J. H. 1980. Principles and procedures of statistics: A biometrical approach. McGraw-Hill Book Co., New York.
25. Stromberg, E. L., and Donahue, P. J. 1986. Hybrid performance and yield losses associated with gray leaf spot disease. Pages 92-104 in: Proc. 41st Annu. Corn Sorghum Res. Conf. American Seed Trade Association, Washington, DC.
26. Tehon, L. R., and Daniels, E. Y. 1925. Notes on parasitic fungi of Illinois. Mycologia 71:240-249.
27. Thakur, R. P., Leonard, K. J., and Leath, S. 1989. Effects of temperature and light on virulence of *Exserohilum turcicum* on corn. Phytopathology 79:631-635.
28. Thompson, D. L., Bergquist, R. R., Payne, G. A., Bowman, D. T., and Goodman, M. M. 1987. Inheritance of resistance to gray leaf spot in maize. Crop Sci. 27:243-246.
29. Ulrich, J. F., Hawk, J. A., and Carroll, R. B. 1990. Diallel analysis of maize inbreds for resistance to gray leaf spot. Crop Sci. 30:1198-1200.