Variability in Isolates of *Cercospora zeae-maydis*

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ABSTRACT

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The increased incidence and severity of gray leaf spot during the last several years prompted an evaluation of the variability present among 15 isolates of *Cercospora zeae-maydis* from several geographical regions of the eastern United States. Four susceptible corn hybrids were inoculated at the seven- to eight-leaf stage and placed in a greenhouse mist chamber. Two components of parasitic fitness, disease efficiency and lesion length were recorded 25 days after inoculation. Disease efficiency was measured as the percentage of lesions produced from a given amount of inoculum and lesion lengths were determined in millimeters. Significant differences in disease efficiency and lesion length occurred among isolates, while differences among hosts and host-by-isolate interactions were not significant. Lesion length was the only variable measured in the field. An analysis of variance over locations indicated a significant location-by-isolate and location-by-hybrid interaction. Orthogonal comparisons suggested the interactions were due primarily to three isolates and to one hybrid. Analysis of variance for each location demonstrated significant hybrid and isolate effects at both locations. The results of this study demonstrate the variability present in a collection of isolates of *C. zeae-maydis* and suggests that breeders should use more than one isolate when developing resistance to the pathogen.

Additional key words: corn, gray leaf spot, maize, *Zea mays*.

Gray leaf spot, which is caused by *Cercospora zeae-maydis* Tehon and Daniels (14), has been a relatively insignificant disease of corn (*Zea mays* L.). In recent years, it has increased in prevalence and severity in the eastern United States (from South Carolina to Pennsylvania, and west to Tennessee). Surveys conducted in Pennsylvania in 1983 and 1984 showed that the disease had progressed as far northward as the southcentral and southeastern regions of the state (2).

The increase in gray leaf spot incidence is believed to be associated with the increase of conservation-tillage farming practices (6,10). Conservation-tillage practices are used to prevent soil erosion, reduce energy costs, and conserve moisture during the growing season. These practices leave large amounts of crop debris on the soil surface in which *C. zeae-maydis* may overwinter as mycelium. The disease appears earlier in the growing season and is most severe in no-till, continuous-cropped corn (11). When disease development begins early in the season and favorable weather conditions such as warm temperatures and sustained periods of high humidity occur, yield losses of 20% can result (1,7).

The increased incidence and severity of gray leaf spot over the last 15 yr prompted an evaluation of the variability present among isolates of *C. zeae-maydis* from various regions of the eastern United States. A knowledge of pathogen variability can provide valuable information for breeding programs searching to identify, develop, and stabilize horizontal resistance which acts by reducing colonization and growth of the parasite and is considered to be race nonspecific (9). Isolate variability can be evaluated by examining components of parasitic fitness. Information on pathogen variability is also crucial to breeders attempting to use vertical resistance which acts by resisting the successful establishment of the parasite and is race specific (9).

In this research, disease efficiency (DE [the number of lesions produced from a given amount of inoculum]) and virulence (the relative ability to induce a given amount of disease on a particular host genotype under a given set of environmental conditions) were used to evaluate variation in isolates of *C. zeae-maydis* (9). Lesion length (LL) was used as an estimate of virulence. Two fitness parameters (DE and LL) were examined in greenhouse studies, and LL was examined in field studies.

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MATERIALS AND METHODS

Fifteen isolates of *C. zeae-maydis* were collected from areas of the eastern United States where gray leaf spot has been observed (Table 1). Leaf material was placed in a petri dish containing moistened filter paper to induce sporulation. Cultures were prepared by single-sporing from leaf material onto V-8 juice agar in petri plates (300 ml of V-8 vegetable juice, 700 ml of double-distilled water, 3 g of $CaCO_3$, and 17 g of flake agar) (3). After 14 days of incubation at 21 C under diurnal fluorescent light (12 hr of light and 12 hr of dark), sporulating plugs were transferred from dishes to test tubes containing 10 ml of V-8 juice agar/tube.

Inoculum was prepared by placing 10 ml of sterilized double-distilled water and 0.01 ml of a surfactant (Tween-20; polyoxyethylenesorbitan monolaurate) in 2-wk-old test tube cultures and agitating the tubes for approximately 1 min to dislodge the spores. The resulting spore suspension was poured into a petri dish containing V-8 juice agar, and after 5 min, the excess water was decanted from the dish. Dishes were maintained for 10 days under the conditions previously described.

Spores were harvested from each dish with 60 ml of double-distilled water and 0.05 ml of Tween-20 and strained through three layers of cheesecloth. Inoculum concentration was adjusted to approximately 5×10^4 conidia per milliliter.

Commercial corn hybrids Pioneer Brand 3184, PAG SX351, Cargill 967, and Jacques 8220 were selected for use in this study because, based on previous studies (1), they were among the hybrids most susceptible to gray leaf spot.

Greenhouse study. Inoculations with conidial suspensions were performed in the greenhouse by spraying the plants with a hand-held atomizer. Seeds of each hybrid were planted in 18-cm-diameter plastic pots in a sterilized potting mixture of soil, peat, and perlite (1:1:1, v/v) and thinned to one plant per pot prior to inoculation. Fifteen milliliters of inoculum from each isolate were applied to 7-wk-old corn seedlings (approximately at the seven- to eight-leaf stage). After inoculation, plants were placed in a mist

chamber and misted for 2 min at 10-min intervals from 2000 hours to 1000 hours for the duration of the experiment. This misting regime was a modification of one described by Beckman and Payne (4). The treatments were placed randomly in the chamber. The experimental design was a randomized complete block with four replications over time.

Numbers of lesions produced were recorded 25 days after inoculation and prior to the formation of secondary lesions. Disease efficiency was calculated as the percentage of lesions produced from the given inoculum concentration. The lengths (millimeters) of 20 lesions per isolate × hybrid combination, selected at random on inoculated leaves, were recorded and used to calculate the mean LL for each host × isolate combination. The data were subjected to analysis of variance procedures, and Duncan's (Bayesian) modified least significant difference test (15) was used to determine significant differences among isolates and hybrids.

Field study. The four hybrids used in the greenhouse experiment were planted in the field in May 1984. One experiment was located near University Park, PA, and the other near Landisville, PA.

Seed was sown in single-row plots 3.0 m long and spaced at a between-row width of 0.79 m. Each plot contained 10 plants (0.31-m within-row spacing). Sixty plots (15 isolates × 4 hybrids) were positioned randomly within each of three replications per location. The experimental design was a randomized complete block.

Inoculum concentrations were approximately 5×10^4 conidia per milliliter and prepared as previously described. Three plants (at the eight- to ten-leaf stage) in the middle of each row were inoculated at dusk with 35 ml of inoculum suspension delivered from a hand-held atomizer. All leaf surfaces surrounding the whorl were covered. LLs were recorded in millimeters at both locations 25 days after inoculation. A total of 10 lesions were selected at random on each of the inoculated plants for a total of 30 measurements of LL per isolate × hybrid combination per replication at each location. Average LL per experimental unit was determined prior to the analysis of variance.

The data were subjected to analysis of variance, and Duncan's (Bayesian) modified least significant difference test (15) was used to test for significant differences among isolates and hybrids. Orthogonal contrast techniques were used to determine the hybrids that contributed the most to the location × hybrid interaction. This was done by constructing a two-way table of location and hybrid means and subtracting the location mean from the mean of each hybrid. Then the contrast (1, −1) was computed on each hybrid. The total of the sum of squares of these contrasts equals the location × hybrid sum of squares. There is one degree of freedom associated with the contrast sum of squares for each hybrid. The error mean square is used in an *F*-test to determine significance for each hybrid. Hybrids with significant contrast mean squares were judged to be responsible for the significant location × hybrid interaction. The method permits immediate identification of the hybrids that contributed the most to the location × hybrid interaction (5). Similar calculations were used to analyze the location × isolate interaction.

Correlation analyses were used to examine the relationship between LL values at the two locations and between LL values of field studies with those of greenhouse studies.

RESULTS

Greenhouse study. There were significant differences in DE and LL among isolates of *C. zeae-maydis* (Table 2). Differences for these variables were not significant among hybrids and the isolate × host interaction was nonsignificant. Therefore, mean separation tests for isolates were performed on means across all hybrids. Disease efficiency varied among the 15 isolates, and isolates collected within a given locality also exhibited variability (Table 1). For example, DE of three isolates from Montgomery County, Virginia, ranged from 0.018 (MO-3) to 0.031% (MO-2). Lesion lengths among the 15 isolates ranged from 3.16 to 10.66 mm and, in general, the size of lesions was not related to the location from which the isolate was collected (Table 1). However, all of the Kentucky isolates produced lesions that were smaller than the average for all isolates.

Field study. There were significant differences among isolates and hybrids for LL when the data were analyzed across locations (Table 3) (12). Location × host and location × isolate interactions were significant; therefore, data from each location were subjected to separate analyses of variance (Table 4).

A significant source of variation in LL was attributed to hosts and isolates at both locations (Table 4). Lesion lengths of the 15 isolates were highly correlated ($r = 0.94$) between the two locations. There was a significant correlation ($r = 0.73$) of LL measurements for the field and greenhouse studies, although field measurements

TABLE 1. Mean disease efficiencies and lesion lengths determined 25 days after inoculation for 15 isolates of *Cercospora zeae-maydis*, after inoculation on four corn hybrids in the greenhouse

Isolate number	Location collected	Fitness attributes: Disease efficiency[x]	Fitness attributes: Lesion length[y]
JO-1	Johnson County, TN	0.023 bcd[z]	10.66 a
FR-2	Franklin County, PA	0.027 abc	10.50 ab
LE-1	Lebanon County, PA	0.024 abcd	8.94 bc
HE-1	Henderson County, NC	0.024 abcd	8.31 cd
MO-2	Montgomery County, VA	0.031 a	8.25 cd
HE-2	Henderson County, NC	0.026 abcd	7.25 de
MO-1	Montgomery County, VA	0.023 bcd	7.00 de
HU-1	Huntingdon County, PA	0.019 cd	6.94 def
FR-3	Franklin County, PA	0.025 abcd	6.31 ef
FR-4	Franklin County, PA	0.019 cd	6.13 ef
MO-3	Montgomery County, VA	0.018 d	5.38 fg
BR-2	Breathitt County, KY	0.023 bcd	4.19 gh
BR-1	Breathitt County, KY	0.028 ab	4.13 gh
FR-1	Franklin County, PA	0.025 abcd	3.94 gh
BR-3	Breathitt County, KY	0.021 bcd	3.16 h

[x] Values are expressed as the percentage of lesions produced from a given inoculum concentration of each isolate and are the average of all hybrids across four replications.

[y] Values are expressed in millimeters and are the average of all hybrids across four replications.

[z] Means followed by the same letter are not statistically different ($k = 100$; approximately $P = 0.05$) as determined by Duncan's (Bayesian) modified least significant difference test (15).

TABLE 2. Analysis of variance table for relative disease efficiencies and lesion lengths of 15 isolates of *Cercospora zeae-maydis* on four corn hybrids in the greenhouse

Source	df	Mean squares: Disease efficiency	Mean squares: Lesion length
Hybrid (H)	3	13,348.22	5.59
Isolate (I)	14	11,527.53**[a]	86.74**
H × I	42	4,124.58	4.05
Replications	3	37,470.66**	30.26**
Error	177	5,291.70	6.25

[a] Asterisks (**) denote significant difference at $P = 0.01$.

TABLE 3. Analysis of variance table for lesion lengths of 15 isolates of *Cercospora zeae-maydis* on four corn hybrids for locations combined

Source	df	Mean squares
Location (L)	1	3.08
Replications in L	4	13.29**[a]
Hybrid (H)	3	42.71**
Isolate (I)	14	150.71**
H × I	42	2.92
L × I	14	4.84**
L × H	3	8.44**
L × H × I	42	2.25
Error	236	2.19

[a] Asterisks (**) denote significant difference at $P = 0.01$.

TABLE 4. Analysis of variance table for lesion lengths of 15 isolates of *Cercospora zeae-maydis* on four corn hybrids at University Park, PA, and Landisville, PA

Source	df	Mean squares	
		University Park	Landisville
Hybrid (H)	3	34.52**[a]	16.63**
Isolate (I)	14	70.71**	84.84**
H × I	42	3.03	2.13
Replications	2	8.42**	18.16**
Error	118	2.11	2.27

[a] Asterisks (**) denote significant difference at $P = 0.01$.

TABLE 5. Mean lesion lengths determined 25 days after inoculation for 15 isolates of *Cercospora zeae-maydis* on four corn hybrids at University Park, PA, and Landisville, PA, and orthogonal contrast sum of squares

Isolate	Lesion length		Orthogonal contrast sum of squares[y]
	University Park	Landisville	
FR-2	9.08 a[z]	9.51 a[z]	0.38
MO-1	9.07 a	9.44 a	0.19
HE-2	8.98 a	9.55 a	0.87
FR-4	8.53 ab	7.28 cde	12.44*
HE-1	8.31 ab	7.82 bcd	2.77
FR-3	8.27 ab	8.18 bc	0.47
JO-1	7.54 bc	8.28 bc	1.82
HU-1	7.09 cd	8.79 ab	13.68*
MO-2	7.00 cd	6.90 de	0.51
LE-1	6.38 de	6.47 e	0.06
MO-3	5.33 ef	6.22 e	2.94
FR-1	5.15 f	6.48 e	7.80
BR-1	3.44 g	1.69 g	22.58**
BR-2	2.26 h	2.80 f	0.74
BR-3	1.98 h	1.78 fg	0.91
Mean	6.56	6.75	

[y] Contrast sum of squares for isolate $i =$

$$\left\{[(\overline{Y}_{i1} - \overline{\overline{Y}}_{11}) - (\overline{Y}_{i2} - \overline{\overline{Y}}_{12})]^2 \times rH\right\} / [(1)^2 + (-1)^2]$$

in which r = number of replications and H = number of hybrids.

[z] Values are expressed in millimeters and are the average of three replications. Means followed by the same letter are not statistically different ($k = 100$; approximately $P = 0.05$) as determined by Duncan's (Bayesian) modified significant different test (15). Asterisks (*) and (**) denote significant differences at $P = 0.05$ and 0.01, respectively.

were slightly lower (approximately 1 mm) than the greenhouse measurements (Tables 1 and 5).

LL measurements for the four hybrids were correlated between the two locations ($r = 0.71$). Pioneer Brand 3184 developed the largest lesions (Table 6) at both field locations and in the greenhouse. Lesions were consistently 1 mm longer on Pioneer Brand 3184 than on the other three hybrids in the field. A highly significant correlation ($r = 0.93$) was found between field LL data and greenhouse LL data for the hybrids.

Orthogonal contrast procedures, involving pairwise comparisons of the two locations for the four hybrids, demonstrated that LL differences between locations for Jacques 8220 were responsible for the significant hybrid × location interaction (Table 6). This hybrid exhibited the greatest deviation from the mean LLs at each location. Pairwise comparisons of the two locations for the 15 isolates showed that isolates HU-1, BR-1, and FR-4 contributed the largest amount of variation in LL between the two locations (Table 5). The location × host and location × isolate interactions were small compared to the variation among hosts and isolates (Table 3), and although statistically significant, may have been of little biological importance.

DISCUSSION

The results obtained from the greenhouse study on two fitness attributes (LL and DE) showed that variation occurred within 15 isolates of *C. zeae-maydis* obtained from different regions of the eastern United States. Field data comparing LLs of the same 15 isolates were similar to greenhouse findings.

In general, variation among isolates was not associated with the location from which the isolate was collected. However, the three isolates from Kentucky produced smaller lesions than most other isolates tested.

An isolate of a fungal pathogen is said to be more "fit" if it has the inherent ability to produce larger lesions, higher DE, and/or higher sporulation capacity on a specific host genotype relative to other isolates (9). This research has indicated that differences in fitness attributes exist among isolates of *C. zeae-maydis*.

Programs involved with developing resistance to gray leaf spot are in progress (1,8,13). Inbreds and hybrids differ in their level of resistance to the disease, but highly resistant lines have not been identified to date. Horizontal resistance appears to be a viable option for improving the level of gray leaf spot resistance in corn cultivars.

Studies that seek to identify variability between isolates of plant parasites have been conducted more frequently in recent years because of their obvious importance in disease management strategies using resistant cultivars. The information provided in

TABLE 6. Disease efficiencies and mean lesion lengths determined 25 days after inoculation with 15 isolates of *Cercospora zeae-maydis* in the greenhouse and in the field (University Park, PA, and Landisville, PA), and orthogonal contrast sum of squares

Hybrid	Greenhouse		Field lesion lengths		
	Disease efficiency[v]	Lesion length[w]	University Park	Landisville	Orthogonal contrast sum of squares[x]
Pioneer Brand 3184	0.021 a[y]	7.19 a[y]	7.70 a[z]	7.61 a[z]	1.76
Jacques 8220	0.026 a	6.55 a	6.76 b	6.22 b	11.99*
Cargill 967	0.024 a	6.64 a	5.74 c	6.45 b	6.08
Pag SX351	0.023 a	6.55 a	6.03 c	6.71 b	5.40
Mean	0.024	6.73	6.56	6.75	

[v] Values are expressed as percentages of lesions produced from a given inoculum concentration of each isolate and are the average of four replications.

[w] Values are expressed in millimeters and are the average of four replications.

[x] Contrast sum of squares for hybrid $i =$

$$\left\{[(\overline{Y}_{i1} - \overline{\overline{Y}}_{11}) - (\overline{Y}_{i2} - \overline{\overline{Y}}_{12})]^2 \times rI\right\} / [(1)^2 + (-1)^2]$$

in which r = no. of replications and I = no. of isolates.

[y] Means followed by the same letter are not statistically different ($k = 100$; approximately $P = 0.05$) as determined by Duncan's (Bayesian) modified least significant difference test (15).

[z] Values are expressed in millimeters and are the average of three replications. Means followed by the same letter are not statistically different ($k = 100$; approximately $P = 0.05$) as determined by Duncan's (Bayesian) modified significant difference test (15).

*Denotes significant difference at $P = 0.05$.

this study on the variability of *C. zeae-maydis* is relevant to the question of the stability of resistance to the pathogen. The data presented herein demonstrate that variability exists in populations of *C. zeae-maydis* and suggests that cultivars developed to reduce disease severity by limiting parameters such as DE and LL should be screened against a wide range of isolates.

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