Infection Cycle Components and Disease Progress of Gray Leaf Spot on Field Corn

C. E. RINGER, Research Associate, Soil Microbial Systems Laboratory, USDA-ARS, Beltsville, MD 20705-2350, and A. P. GRYBAUSKAS, Associate Professor, Department of Botany, University of Maryland, College Park 20742-5815

ABSTRACT

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The epidemiology of gray leaf spot disease (GLS) on field corn (*Zea mays*) was studied following artificial inoculation with the fungus, *Cercospora zeae-maydis*. Studies were based on relative differences in selected infection cycle components between a susceptible and a moderately resistant corn hybrid. Area under the disease progress curve, apparent infection rate, number of lesions, and sporulation capacity were lower and latent period was longer on the moderately resistant hybrid than on the susceptible hybrid. Based on comparisons of disease progress curves, infection cycle components, and rainfall patterns, it is postulated that rainfall and sporulation during early infection cycles have a significant effect on development of GLS. Management strategies aimed at decreasing levels of inoculum during early infection cycles are suggested as an effective means of reducing the severity of GLS.

Gray leaf spot (GLS), caused by *Cercospora zeae-maydis* Tehon & Daniels (27), is a prevalent and severe foliar disease of field corn (*Zea mays* L.) and one of the major yield-limiting diseases of corn in the United States. Losses due to reduced grain fill and lodging can be considerable in seasons favorable for disease development (6,10,24,25).

Gray leaf spot is a polycyclic disease, yet it is sensitive to agronomic practices, such as conventional tillage, that reduce primary inoculum. The pathogen overwinters in corn debris (4), and it has been demonstrated that greater severities of GLS occur in conservation- than in conventional-tillage fields (5).

Since chemical control of foliar pathogens of corn is usually uneconomical, except for seed producers, and a return to conventional tillage practices in many regions could be environmentally and economically unsound, resistant hybrids are one of the most effective means of managing this disease (14,24). Although there are genotypes with immunity and high levels of resistance to GLS (6,7, 12–14,29), a high level of resistance has not been incorporated into a commercial hybrid. There are increasing numbers of commercial hybrids with moderate levels of resistance to GLS (1,19,25,26). The resistance to GLS appears to be quantitatively inherited, and is expressed as rate-reducing resistance (21). Quantitative resistance to disease in plants is primarily expressed as resistance that reduces infection efficiency, extends latent period, and reduces sporulation (15,18). Since the expression of resistance to GLS is partial in most available genotypes and epidemics increase at a relatively low rate, it is unclear which components of the infection cycle should be emphasized in breeding for resistance to GLS. For example, only examining the reduced sporulation component, Leonard and Mundt (15) suggest that selection for reduced sporulation would be more effective in reducing rate of disease increase for diseases that increase at slow rather than high rates.

The objectives of this study were to identify the infection cycle components involved in the differential disease response between a moderately resistant hybrid and a susceptible hybrid and to study the effects of differences in these components on GLS epidemic development. An understanding of the relative importance of infection cycle components to GLS epidemic development can optimize the development of resistant hybrids and provide useful information for development of management strategies, simulation models, and disease forecasting.

MATERIALS AND METHODS

Experiments were conducted in 1988, 1989, and 1990 at the Beltsville unit of the Maryland Agricultural Experiment Station Central Maryland Research and Education Center, an area with little endemic GLS. A new field was selected each year, and plants were artificially inoculated, so that a known amount of inoculum could be introduced at a specific time. Fields used in 1988 and 1989 were planted with corn the preceding year, and the field used in 1990 was planted with wheat the preceding year.

Hybrids. Pioneer Brand 3184, a highly susceptible corn hybrid, and Pioneer Brand 3192, a moderately resistant hybrid, were selected based on performance in field trials in a naturally infested area near Keedysville, MD. These hybrids represented extremes in available resistance in commercial hybrids at the time

First author is former graduate student, Department of Botany, University of Maryland, College Park 20742-5815

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the study began (24,28). Leaf development and anthesis occurred several days earlier for the moderately resistant hybrid than for the susceptible hybrid, depending on environmental conditions, but both hybrids reached maturity at approximately 120 days or 2,860 growing degree units to black layer.

Field plots. Each experimental unit consisted of four rows 9.15 m long and 0.76 m apart and a border row of the same hybrid planted on either side of each experimental unit. Plots were separated at the ends by 1.83-m-wide unplanted alleys. Soil was tilled with a moldboard plow and disked in the early spring. The insecticide carbofuran was applied in furrow at planting at the rate of 1.7 kg a.i./ha in 1988 and 1.1 kg a.i./ha in 1989 and 1990. The following herbicides were applied at planting at the following rates: atrazine at 1.9, 1.6, and 1.7 kg a.i./ha in 1988, 1989, and 1990, respectively; metolachlor at 2.2 kg a.i./ha in 1988 and 1989; cyanazine at 2.0 kg a.i./ha in 1989, and alachlor at 2.8 kg a.i./ha in 1990. Seed was planted on 28, 31, and 24 May in 1988, 1989, and 1990, respectively, at the rate of 74,100 seeds/ha. After emergence, plants were thinned to a density of 51,900 plants/ha. Plots were irrigated overhead for 24 hr, beginning 36 hr before inoculation, in 1988 and 1990 to obtain humidity levels conducive to infection and, in 1988, to assure plant survival.

Inoculum. Senesced, diseased leaves were collected from a site near Keedysville, Maryland in 1987 and from the experimental plots in Beltsville in 1988 and 1989. The leaves were stored in the laboratory, and diseased tissue from the leaves was incubated in moist chambers for 48 hr at room temperature to produce conidia. Cultures of *C. zeae-maydis* were started on V8-juice agar (2) with conidia from the preceding year's diseased tissue and incubated in the dark at room temperature. Six plugs of 10- to 14-day-old sporulating colonies were aseptically transferred from V8-juice agar to a test tube containing 10 ml of a sterile 0.1% solution of Tween 80 and distilled water. The test tubes were shaken to dislodge conidia, and 0.3 ml of the resulting conidial suspension was transferred and spread on the surface of V8-juice agar in petri dishes. After 7–10 days of incubation in the dark at room temperature, 10 ml of a 0.1% solution of Tween 80 and distilled water was added to the petri dishes, and conidia were dislodged with a glass rod. The resulting conidial suspensions were strained through two layers of cheesecloth, combined, and brought to a final volume with distilled water. Conidial concentration was estimated using a hemacytometer.

Inoculation. Spore suspensions were sprayed over the tops of all plants in each experimental unit with a CO_2-pressurized sprayer delivering 0.25 L to each row. Spore concentrations were 1.5×10^4 spores per milliliter in 1988 and 1989 and 2.2×10^4 spores per milliliter in 1990. Plants were inoculated at dusk on 8 July 1988, 11 July 1989, and 6 July 1990. Time from planting to inoculation was 41 days in 1988 and 1989 and 43 days in 1990. All plants within an individual experiment were at either Growth Stage 5 or 6 (11) at time of inoculation.

Disease assessment. One week after inoculation and every day thereafter, plants were examined for the presence of symptoms and sporulation. After the appearance of disease symptoms, three leaves from each of three randomly selected plants were sampled from the experimental unit of each plot. Leaves were numbered 1 through 20 from the bottom to the top of the corn plant. Positions of sampled leaves ranged between Leaf 9 and Leaf 14. Leaf area was estimated using the formula, width $\times$ length $\times$ 0.7 (30). Leaves were evaluated for disease severity, number of lesions, latent period, and sporulation capacity. Time of assessment was recorded as number of days after inoculation (DAI). Sampled leaves were stored in the cold room at 4 C for 1–4 wk while lesion counts and disease severity assessments were made. Disease severity was assessed every 7–11 days after first symptoms appeared until plant senescence. Disease severities between 1 and 50% were estimated using standard area diagrams developed by Smith (24). Disease severities below 1% were calculated from measurements of the length and width of every lesion on each sampled leaf and the estimated leaf area.

Infection cycle components. All lesions on each sampled leaf were counted every 3–6 days after initial symptom appearance until 23 DAI in 1988 and 1989 and 24 DAI in 1990 when lesions began to coalesce. Classification of lesions as either sporulating or nonsporulating was done on the same day as leaf sampling every 3–5 days between 14 and 28 DAI. The upper surface of randomly selected necrotic lesions was viewed at 70$\times$ magnification with the aid of a dissecting microscope. In the first few days, when necrotic lesions were sparse, all lesions were assessed for the presence or absence of sporulation; later assessments were limited to five randomly selected necrotic lesions per leaf. Latent period (LP50) was defined as the number of days from inoculation until 50% of the sampled lesions sporulated. LP50 was estimated by regressing percent sporulating lesions on time ($Y = a + bt$) and then solving for number of days to 50% sporulating lesions; or $t = (0.5 - a)/b$, where t = number of days after inoculation, Y = percent sporulating lesions, b = slope of the regression line, and a = intercept of regression line. To assess sporulation capacity, randomly selected necrotic lesions, ranging in size from 1 to 56 mm^2, were washed and excised from leaves. In 1988, sporulation capacity was assessed on leaf samples collected 14 and 44 DAI and, in 1990, on leaf samples collected 17, 28, and 38 DAI. Lesion dimensions were measured in two perpendicular directions and then the excised lesions were incubated in moist chambers at 27 C. After 48 hr, conidia were washed from the upper surface of lesions with a 0.1% solution of Tween 80 and distilled water, collected on 2.5-cm-diameter cellulose-membrane filters with pore diameters of 1.2 μm, stained with a 0.02% solution of aniline blue, and counted using a compound microscope. Sporulation capacity was calculated from three and four lesions per replication per hybrid in 1988 and 1990, respectively.

Experimental design and analyses. Hybrids were randomized in a complete block design with 10 replications. Both the Gompertz (3) and logistic models were fitted to all disease severity curves using linearized forms of the equations and linear regression analysis. Slopes of the regression functions were compared using a general linear method as described by Neter and Wasserman (17). Significant differences between hybrids in disease severity and infection cycle components were based on the F test using the ANOVA procedure of SAS or, for data sets with missing data, the GLM

Table 1. Gray leaf spot disease assessments on the moderately resistant corn hybrid Pioneer Brand 3192 and the susceptible corn hybrid Pioneer Brand 3184

	Final disease severity (%)[a]		AUDPC[b]		Apparent infection rate[c]		R^2[d]	
Year	3192	3184	3192	3184	3192	3184	3192	3184
1988	17*[e]	30	245*	374	0.042*	0.047	0.78	0.66
1989	17*	38	171*	478	0.043*	0.063	0.82	0.86
1990	18*	38	300*	675	0.032*	0.041	0.83	0.82

[a] Disease severity is percentage of leaf tissue showing symptoms of gray leaf spot at 53, 49, and 68 days after inoculation in 1988, 1989, and 1990, respectively.
[b] Area under disease progress curve.
[c] Infection rate calculated by regressing the Gompertz transformation of disease severity on time in days after inoculation.
[d] Coefficient of determination for regression model from which apparent infection rate is derived.
[e] * indicates that means for Pioneer Brand 3192 and 3184 are significantly different at $P \leq 0.05$, based on the general linear method for comparing regression equations. Data are means of 10 replications.

procedure of SAS (SAS Institute, Cary, NC [22]). Data were transformed when appropriate to avoid violating assumptions of ANOVA.

RESULTS

Disease development. Disease symptoms first appeared on both hybrids 14 days after inoculation (DAI) each year. Lesion development on both hybrids was typical of lesions initiated under natural conditions (14). Other corn diseases were not significant; leaf rust was less than 1% of leaf area. The Gompertz model fit better to the data of all 3 yr than did the logistic model, based on F tests, examination of residual plots, and coefficients of determination (R^2), and was therefore used to estimate rates of disease increase. Final disease severity, area under the disease progress curve (23), and apparent infection rate were significantly lower for the moderately resistant hybrid than for the susceptible hybrid each year (Table 1).

Infection cycle components. Number of lesions that developed from the primary inoculum was significantly lower on the moderately resistant hybrid than on the susceptible hybrid in 1989 and 1990 (Table 2). In 1988, lesion numbers were very low, and no differences between hybrids were observed. The first sporulating lesions were observed on the susceptible hybrid 14 DAI each year, whereas, on the moderately resistant hybrid, the first sporulating lesions were observed 14 DAI in 1988 and 1989 and 17 DAI in 1990. Sporulation occurred only within necrotic tissue. Early sporulating lesions were often as small as 1 mm^2, with only one sporulating conidiophore. As lesions enlarged and matured, sporulating conidiophores became abundant, only to decline in number as lesions aged. A significantly lower percentage of lesions sporulated, once sporulation commenced, on the moderately resistant hybrid than on the susceptible hybrid in 1988 and 1989 (Table 2). On the first day of sporulation in 1990, low levels of sporulation occurred on the susceptible hybrid, but no sporulation was detected on the moderately resistant hybrid. Percent sporulating lesions was dramatically greater for both hybrids in 1988 than in 1989 or 1990 (Table 2). In 1988, 50% of the lesions sporulated so early that insufficient data points were available to perform statistical calculations of LP50. LP50 for 1988 was therefore estimated by interpolation. Differences between the hybrids in LP50 were significant for 1989 and 1990 (Table 2). Sporulation capacity was significantly lower for the moderately resistant hybrid than for the susceptible hybrid on the two sampling dates in 1988 and 28 DAI in 1990 (Table 3). Sporulation capacity among samples was quite variable. The coefficients of variation were as high as 136 and 162% in 1988 and 1990, respectively. Log-transformation of the data reduced coefficients of variation to a high of 36 and 21% in 1988 and 1990, respectively.

DISCUSSION

Under environmental conditions favorable for disease development, small differences in number of lesions, latent period, and sporulation contributed to significant and sometimes large differences between the two hybrids in disease severity and rate of disease progress. In order to speculate on the relative importance of primary and secondary infection cycles in GLS epidemics, it was necessary to estimate a time for first lesion appearance in the secondary infection cycle. Since sporulating lesions were first observed in the field 14 DAI for the susceptible hybrid in each year, 28 DAI was chosen as the day that a maximum number of lesions from primary infections and a minimum number of lesions from secondary infections would occur, assuming comparable environmental conditions.

The moderately resistant hybrid had fewer lesions per unit leaf area than the susceptible hybrid during the first infection cycle in each year. This was likely due, in part, to a lower infection efficiency for *C. zeae-maydis* on the moderately resistant hybrid than on the susceptible hybrid. Lesions on the moderately resistant hybrid reached LP50 later than lesions on the susceptible hybrid and produced fewer spores per unit lesion area. Like de Nazareno et al (5) in their study of *C. zeae-maydis* survival in debris, we encountered high variability in sporulation between samples, which we also attribute, in part, to differences in sporulation among lesions of varying maturity. Similarly, differences between hybrids in pathogen sporulation could be explained in part by differences between hybrids in average lesion maturity.

Gwinn et al (8) found no differences between hybrids of varying resistance in either sensitivity to cercosporin, a toxin produced by *C. zeae-maydis,* or penetration of stomata on inoculated leaf disks. They speculated that relative resistance among hybrids may involve differences in pathogen growth within host tissue. Additionally, Beckman and Payne (2) reported that plant genotype had little effect on symptom expression

Table 2. Components of the infection cycle of gray leaf spot on the moderately resistant corn hybrid Pioneer Brand 3192 and the susceptible corn hybrid Pioneer Brand 3184

	Number of lesions[a]		Sporulating lesions 14 DAI (%)		Latent period (LP50)[b] (days)	
Year	3192	3184	3192	3184	3192	3184
1988	0.13	0.15	31.0*	51.4	16.0	14.0
1989	1.01*[c]	1.80	0.5*	6.2	20.2*	19.2
1990	0.14*	0.30	0.0	2.5	21.9*	18.3

[a]Number of lesions per cm^2 leaf area 23 days after inoculation (DAI) in 1988 and 1989 and 24 DAI in 1990.
[b]LP50 is the number of days from inoculation until 50% of the sampled lesions sporulated.
[c]* indicates that means for Pioneer Brand 3192 and 3184 are significantly different at $P \leq 0.05$. Data are means of 10 replications.

Table 3. Sporulation capacity of *Cercospora zeae-maydis* on the moderately resistant corn hybrid Pioneer Brand 3192 and the susceptible corn hybrid Pioneer Brand 3184

	Number of conidia per mm^2 lesion area[a]			
	1988		1990	
Days after inoculation	3192	3184	3192	3184
14	59.5*[b]	211.2	...	...
17	...	...	141.0	219.8
28	...	...	131.5*	215.9
38	...	...	11.7	12.2
44	0.9*	1.7	...	...

[a]Data are back-transformed from ln (conidia per square millimeter of lesion + 1) and based on 10 replications.
[b]* indicates that means for Pioneer Brand 3192 and 3184 are significantly different at $P \leq 0.05$. Data are means of 10 replications.

Table 4. Rainfall at Beltsville, Maryland, for selected periods

	Total rainfall (mm)		
	1988	1989	1990
Planting to inoculation	15	229	138
Planting to 49 DAI[a]	238	366	415
Inoculation to 49 DAI	223	136	277
Inoculation to 28 DAI[b]	135	103	133
14 to 28 DAI[c]	108	39	22
28 to 49 DAI	88	33	144

[a]Days after inoculation. 49 DAI is latest date for disease comparison of the three years.
[b]28 DAI is estimated time of initial sporulation from lesions formed in the secondary infection cycle.
[c]14 DAI is time of initial sporulation from lesions formed in the primary infection cycle.

on plants in the greenhouse. We conducted supplemental tests using corn plants inoculated and incubated in the greenhouse to determine whether or not events occurring prior to host penetration played a role in differential disease development among hybrids. No meaningful differences were observed between the moderately resistant and susceptible hybrids in either germination of conidia or the percentage of conidia with at least one germ tube forming an appressorium over a stoma. Furthermore, upon examining the upper surface of leaves from the 1988 field experiment, we found a significantly greater number of stomata per unit leaf area on the moderately resistant hybrid than on the susceptible hybrid. It is therefore likely that the differences between hybrids in number of lesions in our study reflect differential growth of *C. zeae-maydis* after penetrating host tissue.

Development of GLS has long been thought to be highly dependent on environmental conditions such as extended periods of high relative humidity or leaf wetness (1,2,14,16,20) and seasons with high rainfall (9,14,24). In our study, 1988 was very dry until mid-July. Only 15 mm of rain fell in the time period between planting and inoculation in 1988 (Table 4). Low rainfall during this period may have been a contributing factor affecting the relatively low number of lesions in the first infection cycle for the two hybrids in 1988. Conversely, high rainfall during the same time period in 1989 created environmental conditions contributing to the relatively high number of primary lesions for the two hybrids and a relatively high level of disease severity for the susceptible hybrid. Total rainfall between planting and inoculation for the 3 yr appeared to correspond to the ranking between years in number of lesions in the first infection cycle. Seasonal rainfall, measured either between planting and 49 DAI or between inoculation and 49 DAI (Table 4) did not correspond with the ranking between years in disease severity at 49 DAI (Figs. 1 and 2), the latest date for which direct comparisons of the 3 yr could be made.

With the beginning of secondary disease symptoms after 28 DAI in 1988, an abrupt increase in the rate of disease development occurred (Figs. 1 and 2). The magnitude of this increase in rate of disease progress may have been related to the effects of varying environmental conditions on components of the infection cycle. Average air temperatures from 28 to 49 DAI were slightly higher in 1988 than in 1989 or 1990. Mean minimum air temperatures were 17.7, 18.0, and 18.0 C, and mean maximum air temperatures were 29.6, 27.3, and 26.3 C for the time period in 1988, 1989, and 1990, respectively. However, during the 2 wk between initiation of sporulation in the primary infection cycle and sporulation in the secondary infection cycle (14–28 DAI), rainfall occurred earliest and in greatest amounts in 1988 (Table 4). LP50 for the primary infection cycle for both hybrids was the shortest in 1988, when rainfall for the period (14–28 DAI) was greatest. Greater numbers of sporulating lesions in combination with greater rainfall and humidity between 14 and 28 DAI could have caused the abrupt increase in the rate of disease development after 28 DAI in 1988. Rupe et al (20) have also observed that periods of prolonged leaf wetness and high relative humidity were frequent during the 2 wk preceding large increases in GLS severity. The lowest disease severity at 49 DAI and lowest apparent infection rate occurred in 1990 for both hybrids. Total seasonal rainfall was highest in this year; however, over one-half of the rainfall recorded between inoculation and 49 DAI in 1990 occurred in the last 3 wk, 28–49 DAI. Total rainfall between inoculation and 28 DAI was similar for the 3 yr. Major differences in rainfall between years occurred between the time periods planting to inoculation and 14–28 DAI. Relatively high levels of rainfall between planting and the primary infection cycle (1989) or relatively high levels of rainfall with sporulation during the primary infection cycle (1988) may have been more important than total seasonal rainfall in influencing differences in disease severity and rate of disease progress.

Due to the relatively long latent period for GLS (LP50 = 14–19 days for the susceptible hybrid in the field) and

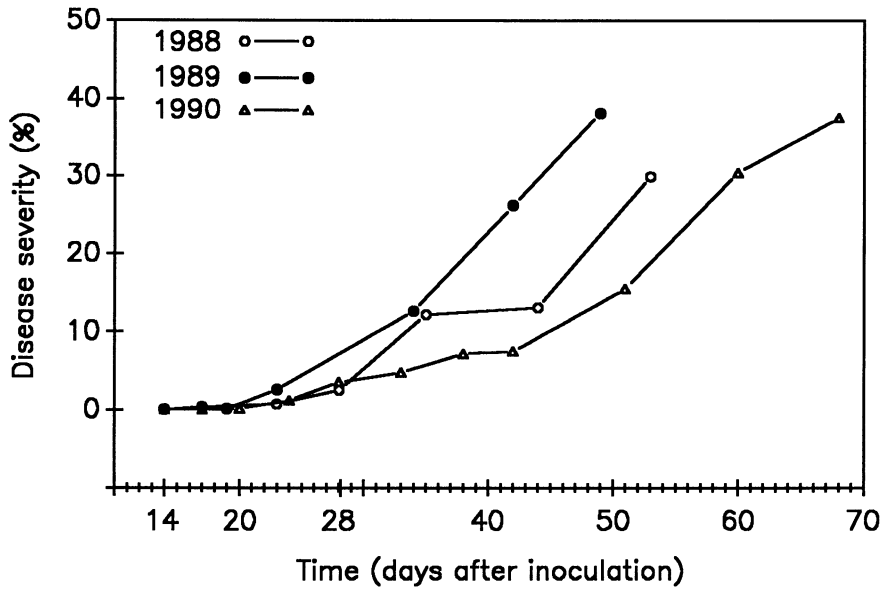

Fig. 1. Disease progress curves for gray leaf spot on the susceptible corn hybrid Pioneer Brand 3184 at Beltsville, MD, in 1988, 1989, and 1990.

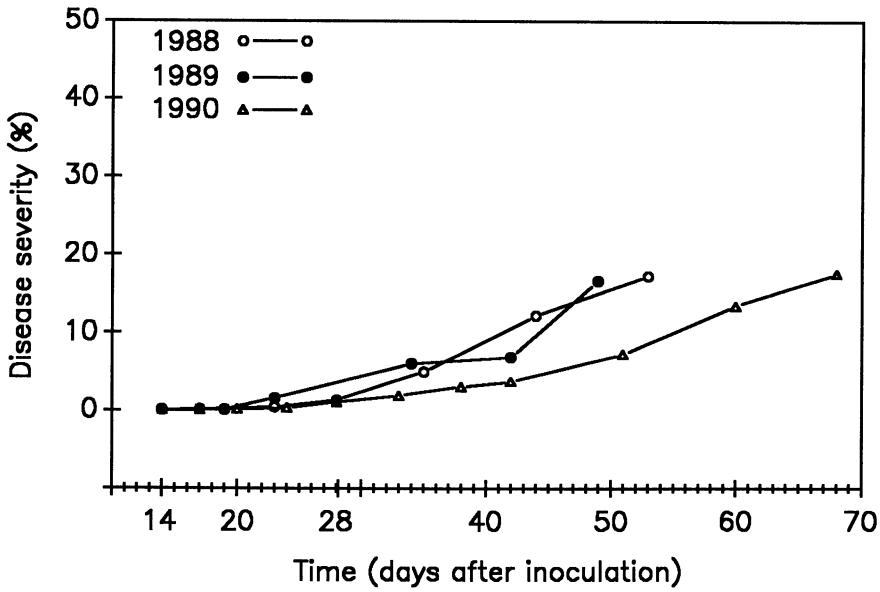

Fig. 2. Disease progress curves for gray leaf spot on the moderately resistant corn hybrid Pioneer Brand 3192 at Beltsville, MD, in 1988, 1989, and 1990.

limited number of infection cycles, differences between hybrids or years in numbers of infection cycles may play a less important role in determining levels of GLS than amount of inoculum that can be generated during early infection cycles. A calculation of the potential number of infection cycles using the shortest and longest LP50s in the study, assuming the LP50s to be the same length in subsequent infection cycles, results in a difference of only one infection cycle in the time period between inoculation and plant maturity. It is generally perceived that GLS is a "late-season" disease. Perhaps, when levels of inoculum or rainfall are low during early infection cycles, high levels of GLS do not occur until late in the season due to the relatively long latent period and low number of infection cycles. As the number of infection cycles decreases, the effects of infection cycle components other than latent period become relatively more important in reducing the apparent infection rate (15). Reducing inoculum by decreasing the size of lesions, number of spores per lesion area, and infection efficiency of *C. zeae-maydis* through breeding would therefore appear to be effective means of reducing GLS epidemics. Based on 1988 results, however, additional research should be conducted to determine the relative importance of latent period and other infection cycle components and their effect on apparent infection rate under conditions in which the environment is highly conducive to disease development during the primary infection cycle.

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