Survival of *Cercospora zeae-maydis* in Corn Residue in Ohio

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ABSTRACT

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Sporulation of *Cercospora zeae-maydis* was detected from lesions on pieces of leaf blades and sheaths kept on the soil surface or buried 5–10 cm beneath the soil surface during the winter and spring months of 1990 and 1991. Sporulation was not detected after May on buried infected tissues. The mean number of conidia per square millimeter of lesion varied from 2.1 to 5,430.6 on leaf blade tissues at two locations and from 5.9 to 104.6 on leaf sheath tissues on the soil surface at one location. However, the coefficient of variation among replications of each treatment was high. Samples of conidia from leaf blades in May 1990 and June 1991 had 50–80% germination. The lack of sporulation on infected tissues buried from December to May substantiates the benefit of tillage to reduce the amount of overwintering inoculum and verifies the potential for epidemic development posed by infected residue left on the soil surface.

The increase in severity in corn (*Zea mays* L.) of gray leaf spot, caused by *Cercospora zeae-maydis* Tehon & E.Y. Daniels, has been attributed to increases in area planted to reduced or no-tillage corn (4,9,12,14,17). First reported in Illinois in the early 1920s (18), *C. zeae-maydis* is now endemic in the corn-producing areas of the Mid-Atlantic and eastern corn belt regions (9,12). In Ohio, the disease has been most severe in the east-central part of the state (approximately 8,000 ha affected), with severity ranging from a few lesions per plant to nearly 100% of the leaf area affected by 6 wk after tasseling (11).

Boosalis et al (5) and Kirby (8) reviewed the role of crop debris left on the soil surface as a result of conservation tillage practices on plant diseases. They concluded that no-tillage may increase, decrease, or have no effect on plant disease intensity, depending on the pathogen. However, they did not consider gray leaf spot in their reports.

According to the guidelines of the Conservation Tillage Information Center (2), a conservation tillage practice leaves at least 30% of the soil surface covered with crop residue from the previous season. By 1990, because of agronomic benefits to the soil and restriction of erosion, 36.2% of the total field corn area in Ohio was under some form of conservation tillage practice: 21.9% no-till, 0.9% ridge-till, and 13.4% mulch-till. In Ohio, adoption of no-tillage was slow in the early 1970s, but in 1987 and 1990, 19.8 and 21.9%, respectively, of the cultivated corn area was under this system (1,2). In 1987, for instance, some counties (e.g., Muskingum County) had 100% of the field corn produced under conservation tillage practices (i.e., at least 30% of the soil surface was covered with crop residue).

C. zeae-maydis produces lesions on both the leaf blade and sheath (9). During the growing season, sporulation is abundant on those lesions under warm, foggy, or humid conditions (9). Therefore, if the pathogen survives through the winter in Ohio, infected tissue left on the soil surface could serve as the source of inoculum for gray leaf spot for the next season. Payne and Waldron (13) studied survival of *C. zeae-maydis* on infected corn debris. They concluded, on the basis of conidiophores on corn tissues, that the pathogen survived from November to May in tissue above ground at Fletcher and Raleigh, North Carolina. Below ground, however, the pathogen survived until February at Fletcher and until May at Raleigh. Similarly, Ureta (19) reported that overwinter survival of *C. zeae-maydis* until mid-April in Georgetown and Newark, Delaware, was limited to residues kept at 45 and 92 cm above ground, on the soil surface, or under corn debris but not buried 10 cm in soil. Ureta (19) also reported that buried pathogen did not survive past mid-March. In our laboratory, *C. zeae-maydis* could sporulate from leaf tissue stored indoors in paper bags (at room conditions) for 2 yr (*unpublished*). However, overwinter survival of *C. zeae-maydis* in the field in the U.S. corn belt states has not been studied.

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The objectives of this research were: 1) to determine the capacity of *C. zeae-maydis* to overwinter, under Ohio conditions, in infected tissue kept on the soil surface or buried and 2) to estimate the amount and variability in number of conidia produced in surviving lesions on overwintered tissues.

MATERIALS AND METHODS

General methodology was similar to that used by others (10,13). The study was conducted in Columbus during 1989–1990 and in Wooster during 1990–1991.

Columbus, 1989–1990. On 14 December 1989, leaf blades with gray leaf spot lesions from standing stalks were collected from a commercial cornfield in Coshocton County, Ohio, and 10 g of infected dried leaf tissue was compacted between metal hardware screens (0.62 square openings per square centimeter). On 20 December 1989, 30 screens were placed in a field near Columbus (Agronomy Farm, The Ohio State University). Half were held perpendicularly by upright stakes at the soil surface, and the other half were buried to a depth of 5–10 cm to simulate tillage. Enough infected tissue was prepared to allow retrieval of three replicate samples (hardware screens) per treatment on 20 January, 17 February, and 24 March and six replicates on 19 May 1990. The experimental design was a randomized complete block.

Samples were collected once a month (January to March and May), and leaf segments with 1,200–2,800 mm^2 of total lesion area per replicate were washed in a solution of water and 5.65% sodium hypochlorite (9:1, v/v). Washed segments were placed on a metal screen in 10-cm-diameter petri plates (three plates per replicate) lined with moistened filter paper. Lesion area was estimated by multiplying ruler-measured lesion length by width. The petri plates were placed inside a dew chamber, with 12 hr of light (46 $\mu E \cdot m^{-2} \cdot s^{-1}$, 26 ± 1 C) and 12 hr of dark (18 ± 1 C), for 6 days. After incubation, tissue segments were vortexed in 20 ml of distilled water plus Tween 20 (one drop in 500 ml of water), and a hemacytometer was used to estimate the conidia concentration on 30 subsamples per replicate. The average number of conidia per milliliter was multiplied by the volume of water used to dislodge the conidia and divided by the total lesion area to obtain an estimate of conidia per square millimeter of lesion. A hygrothermograph was placed inside the chamber to monitor temperature and relative humidity during incubation to ensure similar conditions for each sampling period.

In May 1990, conidial germination was estimated by placing several drops of the spore suspension on a glass slide inside a petri plate lined with moistened filter paper and kept for 2–3 hr at room temperature. Twenty conidia were counted randomly under a light microscope, and the number of germinated conidia was recorded. A conidium was considered germinated when the length of the germ tube was at least equal to the maximum width of the conidium.

Wooster, 1990–1991. On 16 October 1990, individual plants showing severe and distinct symptoms on both leaf blades and sheaths were collected before harvest from a commercial cornfield near West Lafayette, Ohio. Bundles of infected plants were stored in an unheated barn until December 1990, when both leaf blade and sheath segments were removed and cut into approximately 20-cm^2 pieces. Lesion area on the leaf blades was estimated as previously described. For leaf sheath tissues, lesion borders were traced with a felt-tipped marker. A nylon grid (25 square openings per square centimeter) was placed over the lesions and the number of squares within the lesion border was counted to estimate lesion area. A single layer of four leaf blade pieces with an average of 2,464 mm^2 of lesion area was stapled between two 15 × 15 cm nylon screens (25 square openings per square centimeter). A single layer of four to 10 leaf sheath pieces containing an average total of 3,145 mm^2 of lesion area was prepared in the same manner. Individual screen pairs with enclosed tissues constituted a replicate.

On 21 December 1990, the screen pairs were installed in the field near Wooster (Ohio Agricultural Research and Development Center). Half of the screens with either leaf blade or sheath tissues were held upright by stakes and the other half were buried as previously described. The experimental design was a randomized complete block with five replicates per retrieval date. Samples were retrieved on 7 March, 14 May, and 25 June 1991. In the laboratory, individual screens were washed under tap water to remove soil and dust. Tissues were washed in distilled water and placed on a metal screen inside 14.5-cm-diameter glass petri plates lined with moistened filter paper. The petri plates were randomly distributed inside a growth chamber with a constant temperature (26 ± 1 C) and 12 hr of light (86 $\mu E \cdot m^{-2} \cdot s^{-1}$) per day. A mister installed inside the chamber was set to deliver three periods of 3 hr of mist interspaced with 1.5 hr of no mist during the dark period to enhance water saturation. Four to 5 days after incubation, tissues were washed by rubbing the lesion surface with a finger in a known volume of distilled water. Samples were then vortexed and conidial concentration and number of conidia per square millimeter of lesion were estimated as described above. A thermistor and a printed-circuit wetness sensor (coated with white latex paint), connected to a microprocessor data logger registering temperature and water deposition every 5 min, were installed inside a separate petri plate to monitor the microenvironment during incubation.

In June 1991, conidial germination was estimated as previously described by randomly counting 50 conidia per sample.

For both years, air temperature (at 1.2 m above soil surface) and precipitation information from December to June were obtained from the Ohio Autoweather Network weather stations located no more than 2 km from each experiment site.

Statistical analysis. Data were analyzed for each year separately, using the Minitab Statistical Software, release 7.1 (Minitab Inc., State College, PA). Analysis of variance (ANOVA) (16) was used to determine the effects of treatment (surface or buried residue) and time (month) and their interaction on conidial production. Time was considered a repeated measure in the analysis. The least significant difference was calculated when a main effect or interaction was significant. The probability level employed for significance was $P \leq 0.05$. Plots of the predicted values vs. the residuals from ANOVA were assessed for homogeneity of the variances, and the original data were transformed to produce approximately constant variance.

RESULTS

The current and long-term monthly averages, hereafter called normal, of rainfall and air and soil temperatures for 1989–1990 and 1990–1991 are shown in Table 1. Rainfall was greater than the normal average during 1989–1990 because of above-normal precipitation in February, May, and June. Otherwise, the end of winter and beginning of spring were drier than normal. For 1990–1991, the total rainfall was less than normal, including during the period from January through June, which was much drier than normal.

Except for December 1989, the air temperature for winter 1989–1990 was higher than normal, but the temperature average for the period was close to the long-term average. During 1990–1991, air temperatures were generally higher than the long-term average. Soil temperatures also were higher, averaging 1.5 C above normal, during 1990–1991.

Conidia were produced throughout the experiment on tissue that remained on the soil surface but not on buried tissue (Table 2). By May in both years, buried tissues were almost completely decomposed (Fig. 1). In 1990, lesions on buried leaf blades sporulated up to mid-February, whereas in 1991, sporulation on buried tissues was detected up to March. During the 1990–1991 study, lesions on leaf blades sporulated substantially more than did lesions on leaf

sheaths, except for the June 1991 sampling time.

The residuals plot from the ANOVA of the original conidia counts indicated that variances increased with the mean; therefore, the analysis was repeated using data transformed as ln(conidia per square millimeter + 1). ANOVA indicated that treatment (position in the field) main effect was significant ($P < 0.05$), but the main effect of time and treatment × time interaction effect was not for the 1989–1990 experiment. For the 1990–1991 experiment, the treatment (tissue type and position in the field) and time and their interaction were significant ($P < 0.05$). This interaction was because conidial production decreased over time in tissues buried in the soil but increased and then decreased in tissues on the surface (Table 2).

The variability in conidia numbers was quite high. The overall coefficients of variation (CVs) of the original data were 117.4 and 100.8% for the 2 yr; for the log-transformed data, they were reduced to 74.8% for the 1989–1990 experiment and to 21.8% for the 1990–1991 experiment. When the variability for each treatment and time was assessed, the CV was generally highest for buried samples (*data not shown*).

In May 1990, 53% (SE = 11.2) of the conidia from blade tissues germinated. In June 1991, 77% (SE = 5.9) and 85% (SE = 5.0) of the conidia from sheath and blade tissues, respectively, germinated.

Table 1. Winter and spring monthly totals of rainfall and means of air and soil temperatures in two locations in Ohio during 1989–1991[x]

Location	Date	Rainfall (mm)		Temperature[y] (C)			
				Air		Soil	
		Total	Normal[z]	Mean	Normal[z]	Mean	Normal[z]
Columbus	Dec. 1989	44	78	−7.1	0.1	4.2	4.6
	Jan. 1990	66	85	2.8	−1.6	3.6	2.6
	Feb. 1990	131	69	3.1	−0.7	4.3	2.8
	Mar. 1990	34	97	7.4	4.9	7.8	5.9
	Apr. 1990	71	93	10.5	10.7	11.0	11.1
	May 1990	178	107	15.1	16.5	11.8	16.0
	June 1990	133	110	21.4	21.4	16.4	21.4
Combined		657	639	7.6	7.3	8.4	9.2
Wooster	Dec. 1990	156	75	1.7	−1.2	4.2	3.1
	Jan. 1991	41	77	−2.5	−3.0	1.2	1.0
	Feb. 1991	22	61	0.7	−2.4	2.0	1.6
	Mar. 1991	56	89	5.0	3.2	5.4	4.4
	Apr. 1991	76	85	11.7	9.0	11.4	9.6
	May 1991	82	100	19.6	14.9	19.4	16.1
	June 1991	42	101	22.0	20.0	23.8	21.1
Combined		475	588	8.3	5.8	9.6	8.1

[x] Adapted from the Ohio Autoweather Network Station reports published by the Department of Agricultural Engineering and the Statistics Laboratory from the Ohio Agricultural Research and Development Center, Wooster, and the Department of Geography, Miami University, Ohio.
[y] Air temperature at 1.2 m above the soil, soil temperature at a depth of 10 cm.
[z] Averages over 80 yr for rainfall and air temperature and over 10 yr for soil temperature.

Table 2. Recovery of *Cercospora zeae-maydis* conidia from lesions on corn leaf sheaths and blades maintained on the soil surface or buried during the winter and spring of 1989–1990 and 1990–1991 in two locations in Ohio

Location	Date[u]	Surface		Buried	
		Sheath[v]	Blade	Sheath[v]	Blade
Columbus	20 Dec. 1989[w]	...	8.4[x]		
	15 Jan. 1990	...	4.2	...	0.7
	12 Feb. 1990	...	11.4	...	1.3
	18 Mar. 1990	...	2.1	...	0.0
	14 May 1990	...	5.6	...	0.0
Mean[y]			5.8 a		0.5 b
Wooster	21 Dec. 1990[w]	5.9	5,430.6		
	7 Mar. 1991	13.9 d	29.6 c	0.9 e	10.1 d
	14 May 1991	104.6 b	356.8 a	0.0 f	0.0 f
	25 June 1991	85.5 b	145.9 b	0.0 f	0.0 f
Mean[z]		68.0	177.4	0.3	3.4

[u] Samples were removed from the field on the indicated date except for the first date.
[v] Leaf sheath samples were not tested during 1989–1990.
[w] December data represent the conidial production from tissues before samples were placed in the field and are not included in the analysis. Columbus data and Wooster data were analyzed separately. Analysis of variance was done on data transformed as ln(conidia per square millimeter of lesion + 1).
[x] Average of three and five replicates for conidia per square millimeter of lesion.
[y] Because of nonsignificant interaction of time and treatment ($P = 0.35$), comparison was restricted to main effect means for 1989–1990. Means are significantly different ($P = 0.05$) on the basis of analysis of variance.
[z] Because of significant interaction of time and treatment ($P < 0.001$), multiple comparisons were made on interaction means for 1990–1991. Values followed by the same letter are not significantly different ($P \geq 0.05$) on the basis of least significant difference.

DISCUSSION

In general, 1989–1990 was characterized by a warmer winter and a wetter spring than the long-term average, whereas 1990–1991 was warmer in the winter but drier most of the winter and spring months than the long-term average (Table 1). These conditions resulted in soil temperatures that deviated greatly from normal during certain months. Regardless of these different environmental conditions, the pathogen was able to sporulate, producing viable conidia from plant tissues on the soil surface (Table 2). *C. zeae-maydis* survived long enough on the surface to produce initial inoculum for leaf blight the following season. Our results suggest that buried infected tissues cannot act as a source of inoculum because sporulation ceased before May in both years. This is in disagreement with the results of Payne and Waldron (13), who reported that the fungus survived in buried residues until May at Raleigh, North Carolina.

The reduction in the number of conidia per square millimeter of lesion in buried tissue was partly a result of quicker decomposition of corn tissues below ground. For instance, a sample buried until May 1990 was almost completely decomposed, making it impossible to differentiate lesions on corn tissues inside the packets (Fig. 1A).

We feel the experimental procedure in the second year was an improvement over that in the first year because only one layer of tissue was pressed between screens, making it easier to handle individual tissue samples. By June 1991, the lesions on buried blade segments were almost completely decomposed and the total leaf tissue surrounding the lesions was reduced to less than 5% of the initial amount. Even though leaf sheaths were thicker than leaf blades, the amount of decomposition was similar in the buried samples. Weathering also occurred on the samples kept on the surface of the soil, but the lesions were still visible at the end of the experiments.

The lower number of conidia per square millimeter of lesions on blades for

1989–1990 compared with 1990–1991 could be partly explained by some aspects in the methodology used as well as the location of the study, the environment, and the source of the samples. The samples in 1989, but not those in 1990, were washed with 10% sodium chlorite before incubation to induce sporulation. The incubation temperature was kept constant (26 C) in the 1990–1991 experiment, whereas different temperatures for the light and dark periods (26 C and 18 C, respectively) were used in the 1989–1990 experiment. *C. zeae-maydis* requires high temperatures (>20 C) for maximum development (3,9,15). Even though the two hybrids used appeared equally susceptible, some genotype, year, or location interaction effects may have accounted for differences in the amount of nutritional reserves for the pathogen to survive.

Lesions on blades produced significantly more conidia per unit area than did lesions on sheaths, although this phenomenon was not shown to be repeatable. This difference was expected, considering that conidiophores emerge through stomatal openings (9) and leaf blades have more stomata per unit area than do leaf sheaths (7).

Conidial production was highly variable, as denoted by the high CV values. Part of this variability could be due to the difference in sporulation dependent on age of lesions, since samples contained an aggregate from young to senescent lesions. An increase in replicates from three to five gave some improvement in CV, but the values were still quite high, making it difficult to detect small differences between treatments and times. However, the qualitative differences between treatments and times were very consistent, making general conclusions possible.

In Ohio, if corn leaf sheath and blade residue is buried in the fall, the tissue will probably decompose by spring. Therefore, if the soil is tilled to bury infected residue in the fall, a 1-yr rotation away from corn may not be required to reduce the levels of gray leaf spot to below damaging. This differs from corn anthracnose (10), caused by *Colletotrichum graminicola* (Ces.) G.W. Wils., in which the fungus can survive in stalk tissues, which decompose slower than leaf tissues.

C. zeae-maydis is wind-dispersed, and if neighboring fields are heavily infected with the pathogen, burying the residue in the fall may not prevent reintroduction of the pathogen into the field. This was experimentally observed at Dresden, Ohio, in 1989 (6), where gray leaf spot gradients from a surface source of inoculum were not distinct. The gradients were masked, presumably by other sources of inoculum in addition to infested corn residue placed in the center of the experimental plots.

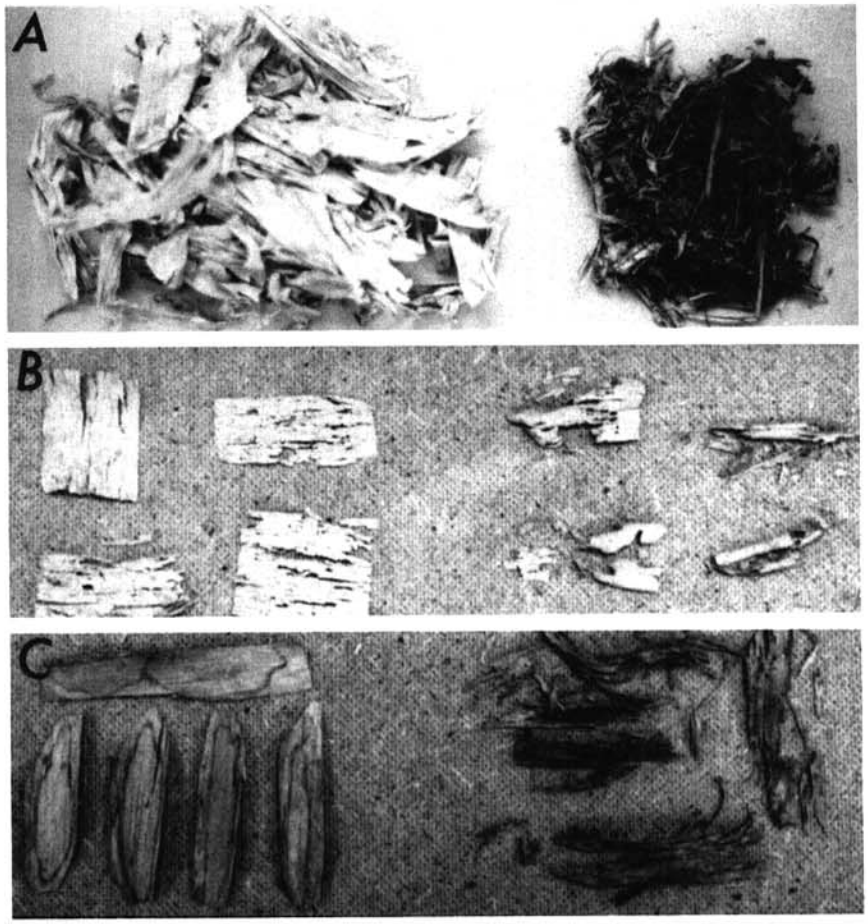

Fig. 1. Degree of degradation of corn residue infected with *Cercospora zeae-maydis* and remaining in the field from December to mid-May: **(A)** Leaf blade pieces (left) kept on the soil surface and (right) buried 5–10 cm in the soil during 1989–1990. **(B)** Leaf blade pieces and **(C)** leaf sheath pieces (left) kept on the soil surface and (right) buried 5–10 cm in the soil during 1990–1991.

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