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

N. R. X. de NAZARENO, Former Graduate Student, P. E. LIPPS, Associate Professor, and L. V. MADDEN, Professor, Department of Plant Pathology, Ohio Agricultural Research and Development Center (OARDC), The Ohio State University, Wooster 44691

**ABSTRACT**


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 conidiospores 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.

---

Portion of thesis submitted by the first author in partial fulfillment of the requirements of the Ph.D. degree. Present address of first author: IAPAR, C. Postal: 2301, 80001 Curitiba, Paraná, Brasil.

Accepted for publication 24 January 1992 (submitted for electronic processing).

© 1992 The American Phytopathological Society

560 Plant Disease/Vol. 76 No. 6
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

**Columbus, 1989–1990.** On 14 December
1989, leaf blades with gray leaf spot
lesions from standing stalks were col-
lected 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 (Agron-
omy 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 mm² of total
lesion area per replicate were washed in
a solution of water and 5.65% sodium
hypochlorite (9:1, v/v). Washed seg-
ments 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 μE·m⁻²·s⁻¹, 26 ± 1 C) and 12 hr
of dark (18 ± 1 C), for 6 days. After
incubation, tissue segments were vor-
texed 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 tem-
perature. 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 from a com-
mercial cornfield near West Lafayette, Ohio. Bundles of infected plants were stored in an
unheated barn until December 1990, when
both leaf blade and sheath seg-
ments were removed and cut into
approximately 20-cm² 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 mm² 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 mm²
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 Devel-
opment 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 μE·m⁻²·s⁻¹) per day. A mister
installed inside the chamber was set to
deliver three periods of 3 hr of mist
interspersed with 1.5 hr of no mist during
the dark period to enhance water
saturation. Four to 5 days after incuba-
tion, tissues were washed by rubbing the
lesion surface with a finger in a known
volume of distilled water. Samples were
then vortexed to achieve uniform concen-
tration and number of conidia per square
millimeter of lesion were estimated as
described above. A thermostor 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 Auto-
weather Network weather stations
located no more than 2 km from each
experiment site.

**Statistical analysis.** Data were ana-
yzed 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* ≤ 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 pro-
duce 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 the period from Jan-
uary 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 tempera-
tures also were higher, averaging 1.5 C

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 sub-
stantially 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 \(\times\) 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 higher 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.

**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 a 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

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

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>Rainfall (mm)</th>
<th>Temperature(^2) (C)</th>
<th>Soil</th>
<th>Air</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Normal(^1)</td>
<td>Mean</td>
<td>Normal(^1)</td>
</tr>
<tr>
<td>Columbus</td>
<td>Dec. 1989</td>
<td>44</td>
<td>78</td>
<td>-7.1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Jan. 1990</td>
<td>66</td>
<td>85</td>
<td>2.8</td>
<td>-1.6</td>
</tr>
<tr>
<td></td>
<td>Feb. 1990</td>
<td>131</td>
<td>69</td>
<td>3.1</td>
<td>-0.7</td>
</tr>
<tr>
<td></td>
<td>Mar. 1990</td>
<td>34</td>
<td>97</td>
<td>7.4</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>Apr. 1990</td>
<td>71</td>
<td>93</td>
<td>10.5</td>
<td>10.7</td>
</tr>
<tr>
<td></td>
<td>May 1990</td>
<td>178</td>
<td>107</td>
<td>15.1</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>June 1990</td>
<td>133</td>
<td>110</td>
<td>21.4</td>
<td>21.4</td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td>657</td>
<td>639</td>
<td>7.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Wooster</td>
<td>Dec. 1990</td>
<td>156</td>
<td>75</td>
<td>1.7</td>
<td>-1.2</td>
</tr>
<tr>
<td></td>
<td>Jan. 1991</td>
<td>41</td>
<td>77</td>
<td>-2.5</td>
<td>-3.0</td>
</tr>
<tr>
<td></td>
<td>Feb. 1991</td>
<td>22</td>
<td>61</td>
<td>0.7</td>
<td>-2.4</td>
</tr>
<tr>
<td></td>
<td>Mar. 1991</td>
<td>56</td>
<td>89</td>
<td>5.0</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Apr. 1991</td>
<td>76</td>
<td>85</td>
<td>11.7</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>May 1991</td>
<td>82</td>
<td>100</td>
<td>19.6</td>
<td>14.9</td>
</tr>
<tr>
<td></td>
<td>June 1991</td>
<td>42</td>
<td>101</td>
<td>22.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td>475</td>
<td>588</td>
<td>8.3</td>
<td>5.8</td>
</tr>
</tbody>
</table>

\(^{1}\)Adapted from the Ohio Autowather 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.

\(^{2}\)Air temperature at 1.2 m above the soil, soil temperature at a depth of 10 cm.

\(^{3}\)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

<table>
<thead>
<tr>
<th>Location</th>
<th>Date (^{*})</th>
<th>Surface</th>
<th>Buried</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sheath(^{*})</td>
<td>Blade</td>
</tr>
<tr>
<td>Columbus</td>
<td>20 Dec. 1989</td>
<td>8.4(^{*})</td>
<td>8.4(^{*})</td>
</tr>
<tr>
<td></td>
<td>15 Jan. 1990</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>12 Feb. 1990</td>
<td>11.4</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>18 Mar. 1990</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>14 May 1990</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>Mean(^{*})</td>
<td>5.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Wooster</td>
<td>21 Dec. 1990</td>
<td>5.9</td>
<td>5.436</td>
</tr>
<tr>
<td></td>
<td>7 Mar. 1991</td>
<td>13.9 d</td>
<td>29.6</td>
</tr>
<tr>
<td></td>
<td>14 May 1991</td>
<td>104.6 b</td>
<td>356.8 a</td>
</tr>
<tr>
<td></td>
<td>25 June 1991</td>
<td>85.5 b</td>
<td>145.9 b</td>
</tr>
<tr>
<td></td>
<td>Mean(^{*})</td>
<td>68.0</td>
<td>177.4</td>
</tr>
</tbody>
</table>

\(^{*}\)Samples were removed from the field on the indicated date except for the first date.

\(^{1}\)Leaf sheath samples were not tested during 1989–1990.

\(^{2}\)Decomposition 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).

\(^{3}\)Average of three and five replicates for conidia per square millimeter of lesion.

\(^{4}\)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.

\(^{5}\)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.

562 Plant Disease/Vol. 76 No. 6
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 re-introduction 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.

**ACKNOWLEDGMENTS**

Salaries and research support provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center and The Ohio State University and by IAPAR and C.N.Pq./Brasil. Manuscript No. 210-91.

**LITERATURE CITED**


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.