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Influence of Inoculum From Buried and Surface Corn Residues on The Incidence of Corn Anthracnose

P. E. Lipps

Associate professor, Department of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster 44691.

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ABSTRACT

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Field experiments were conducted to determine the relative importance of buried and surface corn residues as sources of inoculum for anthracnose of corn. Naturally-infected corn residues or oat-kernel cultures of *Colletotrichum graminicola* were used to simulate inoculum sources. When oat-kernel inoculum was planted with seeds of different hybrids (C123 × FRB73, A632 × H95, B73 × Mo17, B73 × OH545, and FRB73 × FR16; not all were used each year during 2 yr of testing at two locations each year) no difference ($P = 0.05$) in the incidence of anthracnose stalk rot was detected between inoculated and uninoculated treatments, except for the very susceptible hybrid C123 × FRB73. During the second year, removal of leaves above the ears 2 wk after pollination to induce photosynthetic stress, did not influence the incidence of anthracnose stalk rot. Also, burying corn residues below seeds of A632 × H95 did not affect the incidence of anthracnose stalk rot. However, the incidence of anthracnose leaf blight and stalk rot was greater ($P = 0.05$) in treatments with surface residues than in treatments without residues. In an additional test, the incidence of anthracnose leaf blight and stalk rot was negatively correlated ($P = 0.01$) with distance from the residue area. These results indicate that surface corn residues, not buried residues, are an important source of inoculum for corn anthracnose.

Additional key words: reduced tillage, *Zea mays*.

Colletotrichum graminicola (Ces.) G. W. Wils., the cause of anthracnose of corn (*Zea mays* L.), survives overwinter in residues of the previous corn crop (3,4,6). Results from inoculation tests with conidial suspensions (2,8) and observations that the disease often occurs in reduced-tillage fields with residues left on the soil surface (2,4,9) indicate that conidia are the inoculum for the leaf blight phase.

The source of inoculum for the stalk rot phase of anthracnose may be from several different sources. Conidia produced in leaf lesions could be washed behind the leaf sheath by rain water and subsequently germinate and infect the stalk (9). Conidia produced on residues left on the soil surface could also be a source of inoculum since studies in North Carolina (4) and Ohio (3) indicate that acervuli may be produced on residues throughout the growing season. However, these possibilities do not account for reports of fields with severe stalk rot and very little leaf blight or severe stalk rot in the absence of previous-crop residues on the soil surface (2,9).

Inoculum for anthracnose stalk rot could possibly originate from buried residues (9). In studies to determine the survival capabilities of *C. graminicola*, the fungus remained viable until planting time in the spring in residues buried by fall plowing (3). However, the level of sporulation was significantly lower on buried residues than on residues left on the soil surface (3). If *C. graminicola* remained viable in buried corn tissues, the fungus could invade seedling roots from residues and later advance into the stalk (9). A seedling root rot caused by *C. graminicola* has been reported (7) but infected seed, not infested residues, was the source of inoculum. The possibility of infected kernels serving as a source of inoculum for stalk rot has not been investigated.

In Ohio, anthracnose is one of the most common diseases in commercial corn fields (3). The importance of corn residues as a source of inoculum for leaf blight and stalk rot has been difficult to determine because of high levels of disease in control (uninoculated) plots. This natural level of disease may indicate that inoculum for leaf blight and stalk rot originates from both local and distant sources.

The purpose of this investigation was to determine the relative importance of buried and surface residues as potential local sources of inoculum for anthracnose of corn.

MATERIALS AND METHODS

Plot location. Plots were located at Wooster, OH, on Wooster silt loam and at South Charleston, OH, on Crosby silt loam. Corn had not been planted in the plots at Wooster for at least 7 yr or at South Charleston for 3 yr. All plots were visually examined for presence of corn residues, and none were detected before or after plowing or during seed bed preparation. All plots were fertilized with 135 kg of phosphorus, 135 kg of potassium, and 280 kg of nitrogen per hectare. Weeds were controlled in all plots by preplant-incorporation of 2.2 kg of metolachlor (Dual) plus 3.4 kg of cyanazine (Bladex) per hectare. All plots, except where noted, were planted by hand and experimental units consisted of single rows, 7.5 m long, with 75 cm between rows and an in-row seed spacing of 18 cm.

Inoculum. Oat-kernel inoculum was prepared by growing *C. graminicola* at 22–24 C on moistened sterile oats (1 L of oats plus 0.6 L of deionized distilled water in 3-L flasks, autoclaved on two successive days, then inoculated with a culture of *C. graminicola*). After complete colonization, oat kernels were air-dried and mixed. Oat-kernel inoculum was used to simulate buried corn residues in the soil. At planting, 4-8 cm^3 of oat inoculum was placed in the furrow surrounding each untreated seed.

Corn-residue inoculum consisted of intact corn stalks of Pioneer Brand 3780 (Pioneer Hi-Bred International, Inc., Tipton, IN) collected after harvest the year preceding their use. Corn stalks had >25% of the rind surface covered with black-streak symptoms of anthracnose. Stalks were stripped of leaves, tied in loose bundles and stored overwinter in an unheated building prior to their use in the spring.

Buried oat-kernel inoculum. In 1981, the experimental design at the Wooster and South Charleston locations was a split plot with six blocks. Whole plots were seed planted with oat-kernel inoculum and seed planted alone served as the control. Subplots were different hybrids. Five hybrids were planted at Wooster (C123 × FRB73, A632 × H95, B73 × Mo17, B73 × OH545, and FRB × FR16) and four were planted at South Charleston (A632 × H95, B73 × Mo17, B73 × OH545, and FRB73 × FR16). In 1983, the experimental design at both locations was a split-split plot with four blocks. Whole plots were hybrids (A632 × H95, B73 × Mo17, and FRB73 × FR16), subplots were leaf removal above the ear leaf of alternate plants 2 wk after pollination to induce photosynthetic stress (1), and subsubplots were seed planted with oat-kernel inoculum, with autoclaved oat kernels, or without oat kernels. Plots were planted at Wooster on 5 May 1981 and 12 May 1983 and at South Charleston on 16 May 1981 and 13 May 1983. The percentage of plants with the black-streak symptom on the stalk surface plus internal discoloration of the pith typical of anthracnose stalk rot (2,9) was determined at Wooster on 13 October 1981 and 19 October 1983 and at South Charleston on 16 October 1981 and 25 October 1983.

Seedling root infection was determined by digging 60 plants (12 of each hybrid) randomly within different blocks from the Wooster plot 3 wk after planting with and without oat-kernel inoculum in 1981. Three coronal roots and the subcrown mesocotyl of each plant were washed with tap water, surface sterilized in 0.5% sodium hypochlorite for 15 sec, then rinsed three times in sterile distilled water. Tissues were pressed between paper towels to remove excess moisture and placed on 2% water agar containing streptomycin sulfate at 300 μg/ml. After 3–5 days at 23–26 C under continuous light (1,400 lux), tissues were examined microscopically (×20) for development of acervuli of *C. graminicola*.

Root infection was again determined by sampling groups of maturing plants that developed from seeds planted either with or without oat-kernel inoculum. Twenty plants of each hybrid per treatment were dug randomly from different blocks from the Wooster plot on 10 September 1981. Root systems were washed with tap water and three root segments (2–3 cm long) that had grown into the oat-kernel inoculum and a section (1 cm^3) from the pith area of the lower crown was excised from each plant. Tissues were surface sterilized, placed on 2% water agar, and examined for development of acervuli as described previously.

Buried corn-residue inoculum. Corn-residue inoculum, prepared as previously described, was broken into 15-cm-long pieces and placed in the seed furrow 5 cm below the soil surface at Wooster on 4 May 1981. Single rows of 100 seeds of hybrid A632 × H95 were planted 1 cm above the corn residue and covered with soil. Control plots were planted similarly, but without buried corn-residue inoculum. Paired control and infested plots were separated by two rows of corn in each of six blocks. The percentage of plants with black-streak symptoms on the stalk surface and internal pith discoloration was determined on 17 October 1981.

Surface corn residue. To determine the effect of surface corn residues on the incidence of anthracnose leaf blight and stalk rot, larger plots were needed to prevent spread of the inoculum. Seeds (Pioneer Brand 3780) were planted at Wooster on 8 June 1983 in four blocks consisting of three 12-row plots each. Rows were 15 m long with row and seed spacing as in the previous plots. On 13 June, corn-residue inoculum was placed in an area 5-20 cm wide on both sides of the center two rows in two of the three 12-row plots of each block. Corn residues remained in one of the two plots from 13 June to 10 October. Residue was taken out of the other plot on 13 July. The third 12-row plot did not receive corn residue and served as the control. All treatments were randomized in the four blocks. The percentage of plants with leaf lesions and the number of leaves with lesions per plant in the center two rows of each plot was determined on 13 July and 23 August (35 and 76 days after planting, respectively). The percentage of plants with black-streak symptoms on the stalk surface plus internal pith discoloration was determined 10 October.

Distance from surface corn residue. To determine the effects of distance from surface corn-residue on the incidence of anthracnose leaf blight and stalk rot, seeds of Pioneer Brand 3780 were planted in a single block on 26 April 1982 at South Charleston. The block consisted of 80 rows, 60 m long, with row and seed spacing as before. On 15 May, corn-residue inoculum was placed on the soil surface in the center of the block, in an area 5-20 cm wide on both sides of four rows for a length of 6 m. This central four-row area was the only area that received residue inoculum. The plot was marked off by placing stakes every 0.75 m within and across rows to a distance of 6 m from the edge of the corn-residue area. The number of leaves with lesions per plant and the number of plants with lesions at each distance from the residue area was recorded on 15 July (50 days after planting). The percentage of plants with black-streak symptoms on the stalk surface plus internal pith discoloration at each distance from the residue area was determined on 4 October. Individual plant data were combined at each distance into four means representing plants in a north, south (within rows), east and west (across rows) direction from the corn-residue area. The relationship between disease incidence data (mean percentage of plants with leaf lesions, mean number of leaves per plant with lesions, and the mean percentage of plants with stalk rot) and distance from the corn residue inoculum was determined with linear regression analysis and correlation.

RESULTS

Buried oat-kernel inoculum. In 1981, the incidence of anthracnose stalk rot was greater in corn hybrids at South Charleston than at Wooster (Table 1). At Wooster, corn hybrids A632 × H95 and C123 × FR873 had a higher incidence of anthracnose stalk rot than the three other hybrids tested. However, only C123 × FRB73 had significantly ($P = 0.05$) more stalk rot when seeds were planted with oat-kernel inoculum than did the uninfested control. At South Charleston, a high incidence of anthracnose stalk rot (44–64%) occurred in the four hybrids tested. No statistical differences were detected among hybrids tested or between infested and uninfested controls of each hybrid.

In 1983, the incidence of anthracnose stalk rot was about the same at the South Charleston and Wooster locations (Table 2). No statistical differences in the incidence of anthracnose stalk rot occurred among hybrids (B73 × Mo17, A632 × H95, and FRB73 × FR16), between leaf removed and not removed treatment or among the inoculation treatments (seeds planted with oat-kernel inoculum, autoclaved oats, or no oats).

C. graminicola infected corn seedlings within 3 wk after planting seeds with oat-kernel inoculum. Of the seedlings assayed from each of the hybrids tested at Wooster in 1981, acervuli with pink spore masses, which are characteristic of *C. graminicola*, developed on eight, six, nine, seven, and seven of the 12 plants sampled from infested plots of A632×H95, FRB73×FR16, B73×OH545, B73×Mo17, and C123×FRB73, respectively. Acervuli developed both on coronal roots and on the sub-crown mesocotyl of inoculated seedlings, but none developed on tissues of uninoculated seedlings of any hybrid. Of the 20 plants sampled on 10 September from each hybrid, one plant each of A632×H95 and FRB73×FR16, and two plants of C123 × FRB73, developed acervuli on roots that had grown through the oat kernel inoculum. Acervuli did not develop on roots of uninoculated plants of any hybrid.

TABLE 1. Effect of planting oat-kernel inoculum of *Colletotrichum graminicola* with seed of different corn hybrids on the incidence of anthracnose stalk rot at two locations in Ohio in 1981

	Stalk rot incidence[x] (%)			
	Wooster		South Charleston	
Hybrid	Inoculated[y]	Uninoculated	Inoculated	Uninoculated
A632 × H95	30	34	58	53
FRB73 × FR16	8	6	53	64
B73 × OH545	6	3	44	51
B73 × Mo17	8	9	53	61
C123 × FRB73	48	26	...	...
LSD ($P = 0.05$)	11.3		NS[z]	

[x] Stalk rot incidence determined by examining stalk surfaces for black-streak symptoms and the pith for discoloration on 13 October at Wooster, OH, and on 16 October at South Charleston, OH.
[y] Plots were infested by placing 4–8 cm^3 of oat kernels colonized by *C. graminicola* in the furrow surrounding the seed at planting.
[z] NS indicates interaction means not significantly different ($P = 0.05$) according to Fisher's least significant difference test.

Buried corn-residue inoculum. No difference ($P = 0.05$) in the percentage of plants with anthracnose stalk rot was detected between plants developing from seeds planted above buried corn residues (40%) or planted without residue inoculum (46%) at Wooster in 1981. Anthracnose leaf blight occurred in both infested and uninfested treatments early in the season on the lower three to four leaves with less than 1% of the leaf area affected. Leaf blight did not advance above the fourth leaf on any of the test plants during the remainder of the season.

Surface corn-residue. All plants in plots with surface corn-residue inoculum developed lesions on leaves (Table 3). By 13 July, some plants had discrete lesions on the first through the fourth leaf with up to 30% of the surface covered with lesions on the lowermost leaves. By 23 August, no increase in the number of leaves with lesions occurred, however, many of the first and some of the second leaves on infected plants had died and the percentage of leaf area affected on the remainder of the leaves had remained about the same (25–40%). No difference in the number of leaves with lesions per plant was detected between treatments with corn residue remaining in the field throughout the season and those with the corn residue retrieved from plots on 13 July. No lesions developed on any of the leaves of plants in the no-residue treatment by 13 July or 23 August.

A moderate level of anthracnose stalk rot occurred in plots that received corn-residue inoculum. The percentage of plants with stalk rot in plots with residue all season (33%) was not statistically different ($P = 0.05$) from plots that had corn residue from 13 June to 13 July (20%). The control no-residue treatment (2%) had a

TABLE 2. Effect of planting oat-kernel inoculum of *Colletotrichum graminicola* with seed of different corn hybrids and leaf-removal stress on the incidence of anthracnose stalk rot in different hybrids at two locations in Ohio in 1983

	Stalk rot incidence[w] (%) in hybrid:					
	B73 × Mo17		A632 × H95		FRB73 × FR16	
Location and inoculation treatment[x]	Leaf-removal stress[y]	Control	Leaf-removal stress[y]	Control	Leaf-removal stress[y]	Control
Wooster						
Oat-kernel inoculum	6	7	17	2	4	8
Autoclaved oats	5	7	5	3	2	5
No oats	12	11	17	9	3	2
LSD ($P = 0.05$) = NS[z]						
South Charleston						
Oat-kernel inoculum	12	7	12	4	4	8
Autoclaved oats	11	6	0	9	9	10
No oats	8	7	15	6	0	11
LSD ($P = 0.05$) = NS[z]						

[w] Stalk rot incidence determined by examining stalk surfaces for black-streak symptoms and the pith for discoloration on 19 October at Wooster and 25 October at South Charleston.
[x] Plots were infested by placed 4–8 cm^3 of oat kernels colonized by *C. graminicola* in the furrow surrounding the seed at planting time and control plots consisted of 4–8 cm^3 of autoclaved oats placed in the furrow surrounding the seed or no oats.
[y] All leaves above the ear were removed 2 wk after pollination to induce photosynthetic stress (1).
[z] NS indicates interaction means not significantly different ($P = 0.05$) according to the Fisher's least significant difference test.

TABLE 3. Effect of surface corn-residues infested with *Colletotrichum graminicola* on the incidence of anthracnose leaf blight and stalk rot at Wooster, OH in 1983

		13 July		23 August		10 October
Treatment[v]	Length of time residues remained in plots[w]	Leaf blight incidence[x] (%)	No. leaves with lesions per plant	Leaf blight incidence[x] (%)	Leaves with lesions per plant	Stalk rot incidence[y] (%)
Corn residue	13 June–10 October	100 [z]	2.9 a	100 a	2.4 a	33 a
Corn residue	13 June–13 July	100 a	3.1 a	100 a	2.1 a	20 a
No residue		0 b	0.0 b	0 b	0.0 b	2 b

[v] Corn-residue inoculum consisted of intact stalks of corn collected after harvest the year preceding their use.
[w] Corn-residue inoculum placed in plots on 13 June (seedling emergence) in both treatments but removed from the second treatment on 13 July.
[x] Leaf blight incidence determined as the percentage of plants with discrete lesions occurring on at least one leaf; dead leaves were not counted.
[y] Stalk rot incidence determined by examining stalk surfaces for black-streak symptoms and the pith for discoloration on 10 October.
[z] Numbers in a single column followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

significantly ($P = 0.05$) lower percentage of plants with stalk rot than did treatments with surface corn-residue inoculum.

Distance from surface corn-residues. Anthracnose leaf blight developed on the lower three to four leaves of plants in the corn-residue inoculum area by 15 July. No new lesions were detected on leaves above the fourth leaf on plants after this date. The percentage of plants with leaf lesions (Fig. 1A) and the number of leaves with lesions per plant (Fig. 1B) both were negatively correlated ($P < 0.01$) with distance from the residue area ($r = -0.79$ and -0.88, respectively). Means for the percentage of plants with leaf lesions and the number of leaves with lesions per plant at various distances from the residue area fit linear regression equations ($P < 0.01$) (Fig. 1A and B) with coefficients of determination of $r^2 = 0.63$ and 0.78, respectively.

The incidence of anthracnose stalk rot also decreased with increased distance from the corn-residue area (Fig. 1C). The percentage of plants with blackened rind surfaces and internal discoloration was negatively correlated ($P < 0.01$) with distance from the corn-residue area ($r = -0.62$). Considerable variation occurred in the percentage of plants with stalk rot at each distance from the corn-residue area and resulted in a poorer fit of the regression equation ($r^2 = 0.38$).

DISCUSSION

In North Carolina, Naylor and Leonard (4) reported that sporulation of *C. graminicola* on corn stalks buried in the soil in November was severely reduced by December and could not be detected in February. In Indiana, Vizvary and Warren (6) reported similar results with corn stalks buried in the fall and assayed for sporulation in the spring. In Ohio (3), sporulation from corn stalks buried in December was reduced to very low levels by mid-April. Results of these studies indicate that *C. graminicola* has very poor competitive saprophytic ability and is rapidly replaced by more competitive microorganisms in corn residues. Thus, if buried residues were a source of inoculum for stalk rot then root or crown infection must occur during the early part of the growing season (3,4,6).

Field observations indicate that inoculum for the stalk rot phase of anthracnose may originate in infested corn residue buried in the soil (2,9). Results of studies in this investigation with buried oat-kernel cultures and naturally infested corn-residue probably reflect the inability of *C. graminicola* to compete for food reserves in buried residues (6). Infections that resulted from seedling roots growing through oat-kernel inoculum could be detected within 3 wk after planting; by the end of the season, however, *C. graminicola* could be recovered from roots of only a few mature plants. There was no significant increase in the level of anthracnose stalk rot when seeds of different hybrids were planted with oat-kernel inoculum during 2 yr of testing at two locations per year (Tables 1 and 2). The exception to this was with the highly susceptible hybrid C123 × FRB73 (9). Trimming the upper leaves to induce photosynthetic stress on plants in the reproductive phase of growth has been reported to increase the incidence and severity of stalk rot diseases (1). However, there was no increase in the incidence of anthracnose stalk rot when the leaves above the ears were cut from plants 2 wk after pollination (Table 2). Buried corn-residue as a source of inoculum in plots did not increase incidence of stalk rot. The inability of *C. graminicola* to survive in residues in soil and the failure of artificial inoculum (buried oat kernels and corn residues) to act as potential sources of inoculum have led to the conclusion that the primary source of inoculum for anthracnose stalk rot does not originate from buried residues.

Results of studies with corn-residue inoculum on the soil surface indicate that inoculum for anthracnose originates from above-ground sources. Within 1 mo after placement of corn-residue inoculum in the field at seedling emergence, 100% of the plants developed leaf blight symptoms and no symptoms developed on plants in the uninfested plots (Table 3). When surface corn-residues were placed in the center of a field of corn, the incidence of anthracnose leaf blight was highest (100% plants infected) in the area with residues and the incidence decreased with increasing distance from the residue area (Fig. 1A and B). These results clearly demonstrate that surface corn-residues are a primary source of inoculum for the leaf blight phase of anthracnose.

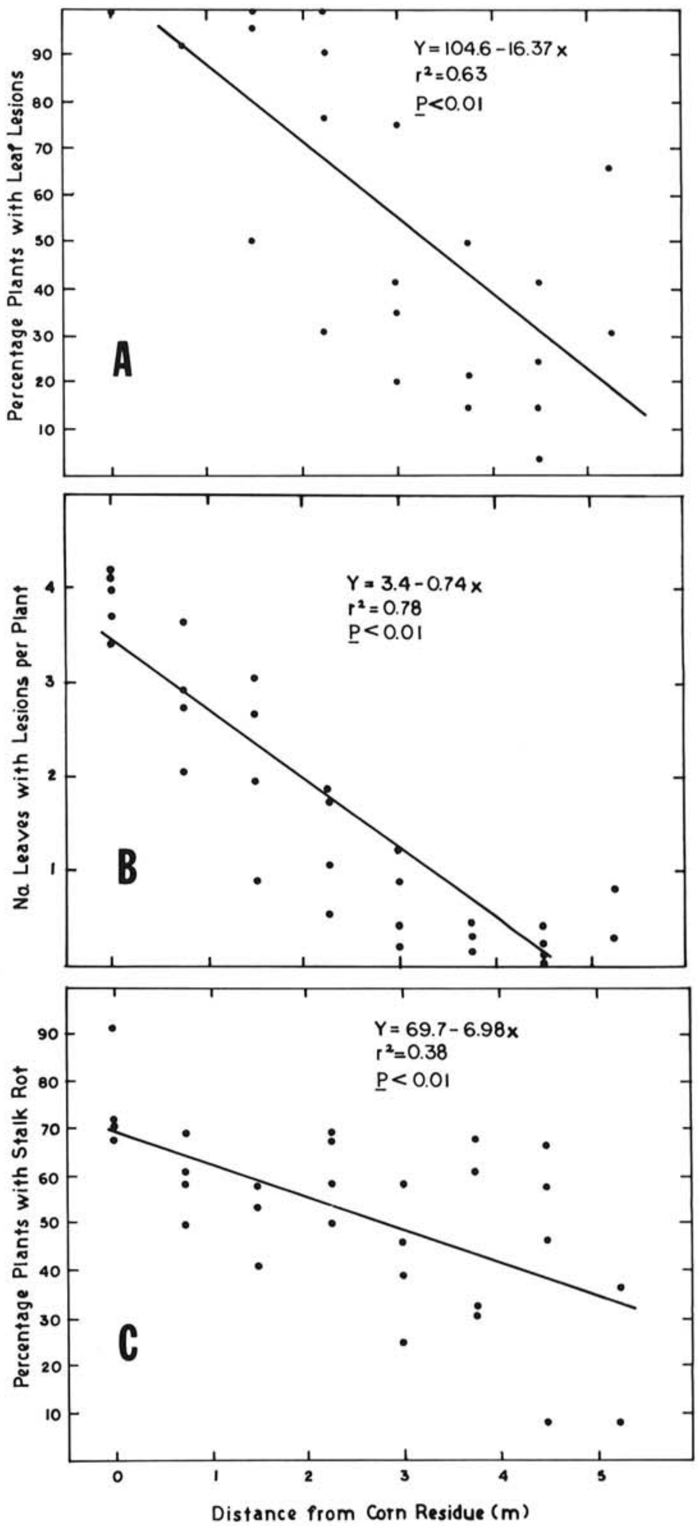

Fig. 1. Relationship between the incidence (Y) of corn anthrancose caused by *Colletotrichum graminicola* and the distance (x) of plants from surface corn-residue inoculum. **A,** Percentage of corn plants with leaf lesions; **B,** number of leaves with lesions per plant; and **C,** percentage of plants with stalk rot. Leaf blight and stalk rot incidence recorded 15 July and 4 October 1982, respectively, at South Charleston, OH. The four data points at each distance represent means from plants in a north, south, east and west direction from an area with surface corn-residue infested with *C. graminicola*. Means with the same value are represented by a single data point.

The exact source of inoculum for the stalk rot phase of anthracnose has not been determined. Results reported here indicate that conidia produced on corn residues or on infected leaf tissue may be potential sources of inoculum. In studies with surface corn-residue, the incidence of stalk rot was higher in the corn-residue plots than in the nonresidue plot (Table 3) and the incidence of stalk rot decreased with increased distance from the residue area ($r = -0.62$) (Fig. 1C). This information agrees with observations that crop rotation and clean plowing to bury corn residues reduces the incidence of anthracnose (2,4,9). Although these cultural practices may reduce the incidence of leaf blight, they may not always effectively control the stalk rot phase (9). In trials conducted at South Charleston in 1981 (Table 1) and at Wooster (buried corn-residue study) a high incidence of anthracnose stalk rot occurred in uninfested plots (51–64% and 46%, respectively). The South Charleston plot and the Wooster plot had not been planted to corn for 3 and 7 yr, respectively, and no corn residues were present in the plot at planting time. This indicates that distant sources of inoculum may be important in the epidemiology of anthracnose.

Recommendations for control of anthracnose should be based on information on both local and distant inoculum sources. Local sources of inoculum could include splash-disseminated conidia produced on crop residues left on the soil surface within the field or in adjacent fields (2,4,9); rain-splashed conidia produced in lesions on weed hosts within the field or adjacent to it (8), and conidia produced on the surface of leaves and washed by rain onto the surface of stalks or other leaves (9). Long-distance dispersal of inoculum could explain observations of leaf blight and stalk rot occurring in fields without a local source of inoculum (2,9). In this case, wind-blown, dry particulate matter consisting of desiccated conidia embedded in the mucilagenous spore matrix, as suggested by Nicholson and Moraes (5), or desiccated conidia associated with acervuli in dry leaf fragments could serve as inoculum. More research is needed to determine the importance of distant inoculum sources in the epidemiology of anthracnose.

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