Variation in Pathogenicity, Virulence, and Aggressiveness of *Colletotrichum graminicola* on Corn

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ABSTRACT

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Twelve isolates of *Colletotrichum graminicola* from corn and two from sorghum differed in pathogenicity, virulence, and aggressiveness following inoculation of stalks of three corn inbreds and two sorghum cultivars. Isolates were pathogenic only on the host species from which they were isolated. Of the 12 isolates from corn, one was not pathogenic. Variation in virulence ranged from virulence on all three corn inbreds to virulence on only the very susceptible inbred, C123. Aggressiveness, measured by the ability to cause premature death of the inbred C123, also varied among isolates. In general, isolates that caused the most discoloration of stalk pith were the most aggressive. Since inbred × isolate interactions were significant, results of studies on breeding for resistance and yield loss potential of anthracnose stalk rot could be greatly affected by the isolate used in the study.

Additional key words: *Sorghum bicolor, Zea mays.*

The incidence and severity of anthracnose stalk rot of corn (*Zea mays* L.) caused by *Colletotrichum graminicola* (Ces.) Wilson have increased in the United States during the past 25 yr. In 1961, Williams and Willis (18) isolated *C. graminicola* from 50% of rotted cornstalks collected near Wooster, OH. In 1963, Dale (1) reported that corn anthracnose was common in Arkansas. During the 1970s, corn anthracnose was recognized as a problem in North Carolina (5), Indiana (14), Kentucky (15), and Illinois (3). Yield losses from both the leaf-blight (12,19) and stalk-rot (8,11,17) phases of the disease have been reported.

In the leaf-blight phase of anthracnose, isolates of *C. graminicola* differ in both pathogenicity and virulence. Isolates from corn may or may not be pathogenic on other hosts, such as sorghum (*Sorghum bicolor* (L.) Moench) (1,7). Wheeler et al (15) found isolates from corn that were highly virulent on a sorghum cultivar that was resistant to an isolate from sorghum. Nicholson and Warren (10) found different responses of corn inbreds following inoculation with three isolates from corn. Forgey et al (2) suggested the possibility of eight physiological races, using 10 isolates from corn and 10 corn inbreds.

Variability of pathogenicity and virulence of *C. graminicola* isolates in the stalk-rot phase of anthracnose is not well documented. Nicholson et al (9) found isolates from corn that varied in ability to colonize and macerate corn pith tissue; however, further evidence of isolate variability is not available. The purpose of this study was to determine the pathogenicity, virulence, and aggressiveness of isolates of *C. graminicola* following inoculations of corn and sorghum stalks in the field.

MATERIALS AND METHODS

Three corn inbreds (C123, B73, and R177) that are susceptible, intermediate, and resistant, respectively, and two susceptible cultivars of sorghum (Black Spanish broomcorn and Rex sweet sorghum) were selected based on their reaction to stalk rot caused by *C. graminicola* (16,17). The experiment included three replications of a 5 × 15 factorial treatment design arranged in a split plot with the five cultivars or inbreds as whole plots and 14 isolates of *C. graminicola* and a control treatment as subplots. Plots were planted at the Agronomy/Plant Pathology South Farm, Urbana, IL, on 8 May 1979 and 1 May 1980. Each subplot contained one row of 12 plants spaced 30 cm in the row and 90 cm between rows.

The 14 *C. graminicola* isolates used in this study were obtained from stalks or leaves of diseased plants from eight midwestern and eastern states and included 12 isolated from corn and 2 from sorghum. All isolates were maintained on oatmeal agar and had a similar conidial size (5 × 30 μm) and colony characteristics except C6, which had sparse growth.

Inocula were obtained by culturing the isolates on oatmeal agar for 3–4 wk at room temperature. Conidia were removed by washing the culture surface with distilled water. Concentrations of conidial suspensions were estimated with a hemacytometer and

TABLE 1. Pathogenicity and virulence of 14 *Colletotrichum graminicola* isolates based on disease reaction following inoculation of three corn inbreds and two sorghum cultivars planted in 1979 and 1980

	Disease reaction[y]																									
	C1[z]		C2		C3		C4		C5		C6		C7		C8		C9		C10		C11		C12		S1		S2	
Host	79	80	79	80	79	80	79	80	79	80	79	80	79	80	79	80	79	80	79	80	79	80	79	80	79	80
C123	P	P	P	P	···	P	···	P	P	P	N	N	P	···	···	P	P	P	P	P	P	P	P	P	N	N	N	N
B73	P	P	P	P	···	N	···	N	P	P	N	N	P	···	···	N	P	N	P	N	N	N	P	N	N	N	N	N
R177	P	P	P	P	···	N	···	N	P	N	N	N	P	···	···	N	P	N	P	N	N	N	P	P	N	N	N	N
Black Spanish broomcorn	N	N	N	N	···	N	···	N	N	N	N	N	N	···	···	N	N	N	N	N	N	N	N	N	N	P	P	P
Rex sweet sorghum	N	N	N	N	···	N	···	N	N	N	N	N	N	···	···	N	N	N	N	N	N	N	N	N	P	N	P	P

[y] Based on amount of stalk pith discoloration: P = pathogenic, total number of internodes or number of internodes with >75% discoloration significantly greater ($P = 0.05$) than that of the water-injected control according to Fisher's least significant difference; N = nonpathogenic, no significant difference.
[z] Isolates designated C were from corn, those designated S were from sorghum. C1–C4 were from Illinois, C5 and S2 from Indiana, C6 from Iowa, C7 and C8 from Pennsylvania, C9 and C10 from Virginia, C11 from North Carolina, C12 from Tennessee, and S1 from Mississippi.

adjusted to 2×10^5 conidia per milliliter by dilution with distilled water. Plants were inoculated 7–15 days following anthesis by injecting 2 ml of the spore suspension into the first elongated internode using a 50-ml rubber syringe (Vaco Pistol Grip; Ideal Instruments, Inc., Chicago, IL) fitted with a stainless-steel needle similar to that described by Koehler (4). For the control treatment, distilled water was injected instead of the conidial suspension.

Two distinct evaluations of disease caused by the various isolates were measured, and pathogenicity, virulence, and aggressiveness of each isolate were determined from these measurements. The number of internodes with discoloration and the number of internodes 75% or more discolored were recorded after stalks had been split at 25 and 41 days after inoculation in 1979 and 1980, respectively. Twelve individual plants per subplot were rated to obtain subplot averages. An isolate was considered pathogenic on corn or sorghum if the number of internodes with discoloration or the number of internodes 75% or more discolored was significantly greater than the water-injected control. Virulence determinations of the pathogenic isolates (i.e., within host species) were based on which inbreds or cultivars had discoloration significantly greater than the water-injected control.

The numbers of dead plants in plots of the corn inbreds and sorghum cultivars were also recorded at 14 and 21 days after inoculation in 1979 and at 21, 28, 34, and 41 days after inoculation in 1980. Percentage of dead plants in each plot was considered a measure of disease incidence. Area under the disease progress curve (AUDPC) for disease incidence was calculated from these measurements in 1980 using the formula presented by Tooley and Grau (13). Aggressiveness of the isolates was determined by using the time and incidence of plant death in plots of the inbred C123 following inoculation as an indication of disease severity. Inbred C123 was used because it was the only one wherein the number of prematurely killed plants was significantly greater than in the water-injected control. These measurements were chosen because the magnitude of yield loss caused by stalk rots is determined by the time and amount of premature plant death (11).

RESULTS

Main effects of isolates, hosts, and isolate × host interaction were highly significant for number of discolored internodes, number of internodes 75% or more discolored, and percentage of prematurely killed plants at all dates. In general, *C. graminicola* isolates were pathogenic on the crop from which they were isolated (Table 1). The amount of stalk discoloration of corn inbreds inoculated with isolates from sorghum and of sorghum cultivars inoculated with isolates from corn was not significantly different from the water-injected control (Table 2). Both isolates from sorghum were pathogenic on sorghum cultivars. Inoculations with isolates S1 and S2 in 1979 resulted in significantly greater discoloration than the control. In 1980, the S1 isolate caused a significant reaction on broomcorn but not on sweet sorghum. All isolates from corn were pathogenic on corn in both years except for C6, which did not cause stalk discoloration significantly greater than the control in either year on corn or sorghum. This was the isolate that grew abnormally in culture.

TABLE 2. Classification of 14 isolates of *Colletotrichum graminicola* from corn and sorghum in relation to pathogenicity on corn and sorghum, virulence on three corn inbreds, and aggressiveness on corn inbred C123

Pathogenicity[x]	Virulence[y]	Aggressiveness[z]
Sorghum	C123	High
S1, S2	C1, C2, C3, C4, C5, C7, C8, C9, C10, C11, C12	C1, C2, C3, C5, C7
Corn	B73 and R177	Moderate
C1, C2, C3, C4, C5, C7, C8, C9, C10, C11, C12	C1, C2, C5, C7, C9, C10, C12	C9, C10, C11, C12
		Low
		C4, C8

[x] Based on ability of isolate to cause pith discoloration significantly greater than on water-injected control in any corn or sorghum genotype (Table 1).
[y] Based on ability of isolate to cause pith discoloration significantly greater than on water-injected control in corn inbreds C123, B73, and R177 (Table 1).
[z] Based on ability of isolate to cause premature death of inoculated plants of corn inbred C123 (Table 3).

Variation in virulence of the 11 isolates pathogenic on corn ranged from virulent on all three corn inbreds to only on the susceptible inbred C123 (Table 2). Three isolates (C1, C2, C7) caused significant stalk discoloration on all inbreds each year in which they were tested. Four isolates (C3, C4, C8, C11) caused significant stalk discoloration only on C123. The remaining four isolates (C5, C9, C10, C12) caused significant discoloration in all three inbreds in 1979 but caused differential reactions among inbreds in 1980.

Premature death of inoculated C123 plants varied among isolates. In 1979, isolates C1, C2, and C7 caused significantly greater premature death of C123 at both 14 and 21 days following inoculations than the control (Table 3). Isolates C5, C9, and C11 increased premature death of C123, but only at 21 days after inoculation. In 1980, isolates C1, C2, C3, and C5 caused significant amounts of premature death of C123 at all four sampling dates. Isolates C10 and C12 caused similar results only on the second and last dates, respectively. Other isolates virulent on C123 (C4 and C8) did not cause significant amounts of premature death in either year.

In 1980, the incidence of prematurely killed plants of C123, as expressed by AUDPC values, was significantly different among isolates. Those isolates with values significantly higher than the water-injected control included C1, C2, C3, C5, and C12.

DISCUSSION

Isolates of *C. graminicola* could be separated into groups on the basis of pathogenicity, virulence, and aggressiveness (Table 2). Within the corn isolates, differences were observed for virulence and aggressiveness; however, we do not believe the groups should be used to differentiate races. The amount of discoloration and time of plant death are quantitative measures of disease reaction that may not conform to race differentiation. Additionally, differences in resistance of inbreds in this study are based on quantitative rather than qualitative resistance. Resistance to the leaf-blight and stalk-rot phases of anthracnose is apparently controlled by separate genetic systems (6); thus, a classification of *C. graminicola* isolates or possible races could be separate for each phase of the disease.

Variation in the ability of isolates to produce discoloration of stalk tissue and premature plant death would have a definite effect on a breeding program for disease resistance. The significant inbred × isolate interaction implies that ranking of the inbreds could change depending on which isolate was used. Isolates such as C3, C4, C7, and C10 were all virulent on the inbred C123 but were avirulent on B73 and R177. If these isolates were used in a breeding program, the most susceptible genotypes would be identified, but genotypes with the susceptibility of B73 (17) would be classified as resistant.

Virulence of some isolates may decrease as they are maintained in culture. In this study, isolates C5, C9, and C12 all caused a significant amount of internode discoloration in all three corn inbreds in 1979, but they failed to do so with at least one of the inbreds in 1980. Virulence should be monitored with isolates used repeatedly in a breeding program.

The variation in ability of isolates to cause premature plant death is important to yield loss studies for *C. graminicola* since much of the potential effect on yield is the result of the premature death of plants (11). If an isolate does not cause premature plant death, significant yield losses may not result. Dale (1) inoculated several corn hybrids with *C. graminicola* and did not find an effect on yield. Inoculations with isolates that cause premature plant death does cause significant yield loss (8,17).

Variation in the production of pectic enzymes may be related to the ability of isolates to cause premature plant death. Nicholson et al (9) found that isolates varied in their ability to produce pectic enzymes in living pith tissue. They also found that those isolates that colonized the greatest amount of pith tissue also produced the largest amounts of pectic enzymes. We found that isolates that spread and caused the greatest amounts of internode discoloration were usually those that caused the greatest incidence of premature plant death. This may indicate that enzyme production could determine the aggressiveness of an isolate and its potential to cause a loss in yield.

The variation in virulence and aggressiveness of isolates of *C. graminicola* found in this study of the stalk-rot phase of anthracnose on corn has also been reported in the leaf-blight phase of the disease (2,10,15). The increased occurrence of more virulent isolates with higher levels of aggressiveness may be one possible explanation for the increased incidence and severity of corn anthracnose during the past 25 yr.

TABLE 3. Aggressiveness of 14 *Colletotrichum graminicola* isolates based on incidence of prematurely killed plants and AUDPC values following inoculation of corn inbred C123

	Percentage incidence of dead plants[x]						
	1979		1980				
Isolate	14 days[y]	21 days	21 days	28 days	34 days	41 days	AUDPC[z]
C1	70*	100*	84*	97*	100*	100*	1,928.5*
C2	64*	97*	64*	89*	100*	100*	1,801.5*
C3	···	···	34*	82*	100*	100*	1,652.3*
C4	···	···	13	29	39	83	770.0
C5	11	72*	25*	58*	88*	96*	1,371.3*
C6	0	26	0	8	29	59	445.4
C7	63*	91*	···	···	···	···	···
C8	···	···	0	24	70	91	927.2
C9	31	78*	2	5	16	87	448.1
C10	8	48	11	43*	64	93	1,055.3
C11	42	72*	8	30	62	91	938.3
C12	29	57	6	37	77	100*	1,117.0*
S1	5	25	4	10	28	79	534.6
S2	8	51	···	···	···	···	···
Water	0	22	3	16	41	85	676.2

[x] Values significantly larger ($P = 0.05$) than those for water-injected control according to Fisher's least significant difference.

[y] Number of days following inoculation.

[z] Area under the disease progress curve (AUDPC) = $\sum_{i=1}^{n-1} [(X_{i+1} + X_i)/2]$ $[t_{i+1} - t_i]$ in which X_i = incidence of dead plants at ith observation, t_i = time (days) of ith observation, and n = total number of observations. * = Values significantly larger ($P = 0.05$) than those for water-injected control according to Fisher's least significant difference.

LITERATURE CITED

1. Dale, J. L. 1963. Corn anthracnose. Plant Dis. Rep. 47:245-249.
2. Forgey, W. M., Blanco, M. H., and Loegering, W. Q. 1978. Differences in pathological capabilities and host specificity of *Colletotrichum graminicola* on *Zea mays*. Plant Dis. Rep. 62:573-576.
3. Hooker, A. L., and White, D. G. 1976. Prevalence of corn stalk rot fungi in Illinois. Plant Dis. Rep. 60:1032-1034.
4. Koehler, B. 1960. Corn stalk rots in Illinois. Ill. Agric. Exp. Stn. Bull. 658. 90 pp.
5. Leonard, K. J. 1974. Foliar pathogens of corn in North Carolina. Plant Dis. Rep. 58:532-534.
6. Lim, S. M., and White, D. G. 1978. Estimates of heterosis and combining ability for resistance of maize to *Colletotrichum graminicola*. Phytopathology 68:1336-1342.
7. Messiaen, C. M. 1955. Sur quelques anthracnose des plants cultivees. Ann. Epiphyt. 6:285-299.
8. Natti, T. A. 1981. Yield loss studies with anthracnose stalk rot of corn. Ph.D. thesis. University of Illinois, Urbana. 74 pp.
9. Nicholson, R. L., Turpin, C. A., and Warren, H. L. 1976. Role of pectolytic enzymes in susceptibility of living maize pith to *Colletotrichum graminicola*. Phytopathol. Z. 87:324-326.
10. Nicholson, R. L., and Warren, H. L. 1976. Criteria for evaluation of resistance to maize anthracnose. Phytopathology 66:86-90.
11. Perkins, J. M., and Hooker, A. L. 1978. Effects of anthracnose stalk rot on corn yields in Illinois. Plant Dis. Rep. 63:26-30.
12. Smith, D. R. 1976. Yield reduction in dent corn caused by *Colletotrichum graminicola*. Plant Dis. Rep. 60:967-970.
13. Tooley, P. W., and Grau, C. R. 1984. Field characterization of rate-reducing resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean. Phytopathology 74:1201-1208.
14. Warren, H. L., Nicholson, R. L., Ullstrup, A. J., and Sharvelle, E. G. 1973. Observations of *Colletotrichum graminicola* on sweet corn in Indiana. Plant Dis. Rep. 57:143-144.
15. Wheeler, H., Politis, D. J., and Poneleit, C. G. 1974. Pathogenicity, host range, and distribution of *Colletotrichum graminicola* on corn. Phytopathology 64:293-296.
16. White, D. G. 1977. Lack of close correlation of stalk-rot reactions of corn inbreds inoculated with *Diplodia maydis* and *Colletotrichum graminicola*. Phytopathology 67:105-107.
17. White, D. G., Yanney, J., and Natti, T. A. 1979. Anthracnose stalk rot. Proc. Annu. Corn Sorghum Res. Conf. 34:1-15.
18. Williams, L. E., and Willis, G. M. 1963. Disease of corn caused by *Colletotrichum graminicolum*. Phytopathology 53:364-365.
19. Zuber, M. S., Ainsworth, T. C., Blanco, M. H., and Darrah, L. L. 1981. Effects of anthracnose leaf blight on stalk rind strength and yield in F_1 single crosses in maize. Plant Dis. 65:719-722.