Lack of Close Correlation of Stalk-Rot Reactions of Corn Inbreds Inoculated with Diplodia maydis and Colletotrichum graminicola

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ABSTRACT

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Stalk-rot reactions of 99 corn inbreds inoculated with *Colletotrichum graminicola* or *Diplodia maydis* were not closely correlated. Because of this, selection for stalk-rot resistance on the basis of reactions to *D. maydis* alone may no longer be suitable in areas where *C. graminicola* is potentially damaging. Methods of inoculation and reactions of some widely-used inbreds to the two pathogens are presented.

Additional key words: resistance, anthracnose, *Zea mays,* maize.

The incidence of stalk rot of corn (*Zea mays* L.) caused by *Colletotrichum graminicola* (Ces.) Wils. has increased in the United States during the past several years (3, 7, 9, 10, 11). This increase is of concern since most corn inbreds and hybrids have not been selected for resistance to this pathogen. Corn breeders usually select for stalk-rot resistance on the basis of natural infection by *Diplodia maydis* (Berk.) Sacc. [Syn. *D. zeae* (Schw.) Lev.] and *Gibberella zeae* (Schw.) Petch (either or both), or on the basis of reaction to *D. maydis* following artificial inoculation. *Diplodia maydis* usually is used for resistance testing because inoculum is easy to produce, the disease develops rapidly, and resistance to *D. maydis* is reported to be correlated with resistance to *G. zeae* (5). These two pathogens are considered to be the most important stalk-rotting organisms in the United States Corn Belt (2). Therefore, it is important to identify inbred lines which are resistant to *C. graminicola* and to determine whether this resistance is correlated with resistance to *D. maydis*.

MATERIALS AND METHODS

Ninety-nine dent-corn inbred lines, which are used as parental lines of corn-belt hybrids, were evaluated for stalk-rot reaction. Lines were divided into two maturity groups (early and late) so that all lines were about the same physiological maturity when inoculated. Two replications in a completely randomized plot design were planted on 7 May at the Agronomy South Farm, Urbana, IL.

Diplodia maydis was cultured at room temperature on moistened, sterile oats in 1-liter flasks. After incubation for 5 to 6 weeks the oat substrate was placed in cheesecloth bags and kneaded in distilled water to release conidia. *Colletotrichum graminicola* was cultured on oatmeal agar plates for 2 to 3 weeks at room temperature and conidia were washed with distilled water from the culture surface. The concentrations of conidial suspensions of both organisms were estimated with a hemacytometer and adjusted to 2×10^5 conidia/ml by dilution with distilled water.

Inoculations were made using a 50-ml Vaco Pistol Grip Rubber Plunger Syringe (Ideal Instruments, Inc., Chicago, Illinois) fitted with a 2.3-mm diameter (11-gauge) stainless-steel needle. Needles were cut to 7-9 cm in length, hammered shut at the tip, and the tips were ground to a sharp point. Small holes were drilled in both sides of the needles about 1 cm from the tips. These altered needles could be punched into corn stalks without becoming clogged with stalk tissue.

Early-maturing inbreds were inoculated 73 days and late-maturing 87 to 89 days after planting. Time of inoculation was about 5 to 15 days after the full silk stage. About 2 ml of inoculum was injected into the first elongated internode above the brace roots. Half of the plants in plots inoculated with *C. graminicola* were inoculated by the stalk-injection method, and half by placing 2 ml of the conidia suspension behind the leaf sheath below the ear.

Ratings for stalk-rot reaction were made 30 to 32 days after inoculation. Plants inoculated with *D. maydis* were split lengthwise through the inoculated internode and rated as follows: 1 = 0-25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100% of the inoculated internode discolored, 5 = discoloration beyond the inoculated internode, and 6 = plant prematurely killed (6). Plants inoculated with *C. graminicola* were rated on the basis of number of discolored internodes because usually more than one internode was infected.

Ratings for individual plants were averaged to obtain plot scores, and Fisher's least significant difference (FLSD) values, for making comparisons between means, were calculated on the basis of plot scores (1). Correlation values (r) for reaction to *C. graminicola* and *D. maydis*

were based on the overall means for each inbred line.

RESULTS

The reaction of inbred lines to *D. maydis* and *C. graminicola* was quite different. Usually several internodes of plants inoculated with *C. graminicola* were discolored internally and had black roughened streaks on the stalk surface. In contrast, discoloration from *D. maydis* usually was restricted to the inoculated internode or at most to two internodes.

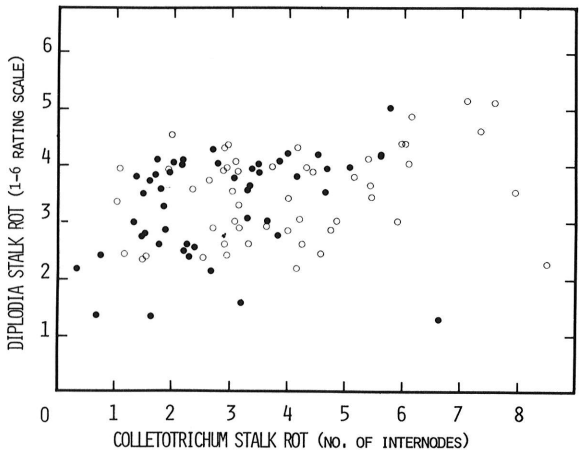

Fig. 1. Scatter diagram of co-ordinate reactions of 48 early-maturing (●) (r = 0.32) and 51 late-maturing (o) (r = 0.26) inbred corn lines after inoculation with *Colletotrichum graminicola* or *Diplodia maydis*. Ratings of reactions to the respective pathogens were made about 30 days after inoculation by injection of spore suspensions (2×10^5 conidia per milliliter) into the first elongated internode above the brace roots. See text for details of the respective rating scales.

Although previous workers have reported that stalk infections under natural conditions may originate from behind the leaf sheath (8, 11), only a few stalks of plants inoculated with *C. graminicola* behind the leaf sheath were discolored internally and these infections usually were associated with corn-borer tunnels. Thus, artificial inoculation by this method was not reliable for inducing stalk rot and results from this method of inoculation were discarded.

Stalk-rot reaction of the inbreds to the two fungi were not considered closely correlated in either the early-maturing (r = 0.32) or late-maturing (r = 0.26) inbred groups. Usually any correlation coefficient smaller than 0.7 is unreliable in predicting characters determined largely by heredity (4). The lack of close correlation is obvious from the diagram of co-ordinate reactions of inbreds tested (Fig. 1). If the scatter diagram (Fig. 1) was divided arbitrarily into quadrants with inbred reactions below 3 to *D. maydis* and below 4 to *C. graminicola* being considered resistant, 26 inbreds would be resistant to both organisms, 27 inbreds susceptible to both, seven resistant to *D. maydis* and susceptible to *C. graminicola,* and 39 susceptible to *D. maydis* and resistant to *C. graminicola.* However, if stalk-rot reactions to *C. graminicola* were rated on the same scale as reactions to *D. maydis,* only three out of the ninety-nine inbreds would be rated three or less (75% or less of the inoculated internode discolored) and considered resistant.

Messiaen et al. (8) indicated that in their tests, reactions to *C. graminicola* and *G. zeae* were correlated. They

TABLE 1. Stalk-rot reactions[a] of widely used, early-maturing and late-maturing corn inbred lines after inoculation with *Colletotrichum graminicola* or *Diplodia maydis*

Early-maturing inbred lines			Late-maturing inbred lines		
Inbred	*C. graminicola*	*D. maydis*	Inbred	*C. graminicola*	*D. maydis*
A554	2.17	4.09	33-16N	4.37	3.85
A619	3.59	3.83	B14A	4.88	2.89
A632	2.67	2.13	B37	6.03	4.27
A634	3.33	3.01	B57	5.40	3.47
A635	3.17	3.77	B68	4.11	2.15
C123	6.59[b]	1.17	B73	6.17	4.70
Oh43	0.73	2.42	C103	3.33	2.59
SD15	5.67	4.09	CI44	4.41[b]	3.79
Va26	0.76	1.33	CI66	2.30	3.55
W59E	1.97	3.83	H49	4.25	2.51
W64A	1.50	2.77	H84	2.89	3.91
W117	1.70	3.83	H93	7.17	4.96
W153R	5.86	4.82	H95	1.90	3.91
W182B	5.67	4.08	Mo17	1.47	2.30
			N28	4.00	2.76
			Pa762	2.88	2.59
			T232	3.63	2.83
FLSD[c]$_{0.05}$	1.30	0.62		1.26	0.62
FLSD$_{0.01}$	1.74	0.83		1.68	0.83

[a] *Colletotrichum graminicola* ratings were based on average number of discolored internodes of six inoculated plants per row in two replications, and those for *D. maydis* were based on average rating (scale 1 to 6) of percentage discoloration of the first elongated internode above the brace roots of 10 plants per row in two replications.

[b] Premature plant kill.

[c] Abbreviation FLSD = Fisher's least significant difference calculated on the basis of 48 early- and 51 late-maturing inbreds.

assumed that reactions to *C. graminicola* and *D. maydis* also would be correlated, because of Hooker's report that stalk-rot reactions to *G. zeae* and *D. maydis* were correlated (5). Based on the data presented herein, however, the correlation between stalk-rot reactions to the organisms is quite low.

The lack of correlation and the reaction of certain inbreds suggest that the plant characteristics responsible for resistance to *D. maydis* do not necessarily confer resistance to *C. graminicola*. For example, inbreds such as FR2A and FR2B, which have a similar genetic background and reaction to *D. maydis* (2.3 and 2.1, respectively), differ greatly in their reaction to *C. graminicola* (2.5 and 8.5, respectively). The inbred with the greatest difference of reaction to the two fungi was C123, which was resistant to *D. maydis* but highly susceptible to *C. graminicola* (Table 1).

Stalk-rot reactions of inbred lines that were used in the production of a million or more pounds of hybrid seed in 1974 (12) indicate various levels of resistance to the two organisms in the most-used parental inbreds (Table 1). Certain related inbreds had similar reactions to the two organisms. Inbred line Oh43 and the related inbred Va26 were resistant to both organisms. Other Oh43-related lines such as A619, H95, and Pa762 were slightly more susceptible. Inbred line B37 and its related line H93, as well as other related lines not included in the table (FR4A, FA4C, H93), were susceptible to both organisms. Another B37-related line, H84, was more resistant to *C. graminicola*. Lines related to B14 such as B14A, A632, A634, and A635 had intermediate reactions to both organisms (Table 1). Since some widely used parental inbred lines have good resistance to *C. graminicola* or *D. maydis* or to both, resistant hybrids can be produced for areas where either or both is a serious problem.

These data suggest that selection for stalk-rot resistance on the basis of reactions to *D. maydis* alone is no longer suitable in areas where anthracnose is potentially important. Indications are that the plant characteristics responsible for resistance to *D. maydis* do not necessarily confer resistance to *C. graminicola*.

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