Effect of Second-Generation European Corn Borers on Resistance of Maize to *Diplodia maydis*

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ABSTRACT

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A 2-yr study was conducted to determine the effects of an infestation by second-generation European corn borers (ECB), *Ostrinia nubilalis*, on resistance of maize, *Zea mays*, to stalk rot (SR) caused by *Diplodia maydis*. SR infection had no effect on the ECB. SR damage was lowest in plots kept free of ECB with an insecticide and highest under high levels of infestation by ECB. Maize genotypes resistant to second-generation ECB also were resistant to SR. In genotypes resistant to SR but susceptible to ECB, resistance to SR broke down under high levels of infestation by ECB.

Additional key words: insect resistance.

Stalk rots (SR) of maize, *Zea mays* L., have been known for more than 75 yr and are considered the most destructive diseases of maize in the world (4,17). Some may be caused by several species of fungi and bacteria, occasionally becoming epidemic over wide areas (4). Koehler and Boewe (13) considered *Diplodia maydis* (Berk.) Sacc. (syn: *D. zeae*) the most common cause of SR of maize in the Corn Belt of the United States. This pathogen, however, is no longer the prevalent SR organism in part (Illinois) of the Corn Belt (9). Yield losses caused by all SRs in the United States frequently are greater than 7.5% (4,17). In 1978, maize production in the United States was over 7×10^9 bushels (19); SR may, therefore, cost American farmers more than $1 billion per year.

Factors that influence SR development include weather conditions, soil fertility, and maize genotypes (12). Incidence of SR was greater when maize plants were damaged by hail (12,18), spider mites (1), grasshoppers (12), chinch bugs (8), or European corn borers (ECB), *Ostrinia nubilalis* (Hübner) (2,3,12,15).

The first report of an association between SR and the ECB was that of Christensen and Schneider (3), who noted that the severity of SR was higher when maize plants were infested by the ECB and that maize genotypes usually free from SR were susceptible when infested by the ECB. Chiang and Wilcoxson (2) demonstrated that the presence of ECB larvae in artificially produced tunnels increased the incidence of SR but that artificial inoculation of tunnels with SR organisms had no effect on the ECB.

Control of both SR and the ECB by host-plant resistance has obvious advantages. Because an infestation by the ECB has been observed to increase the incidence of SR, it is important to understand the effects of host-plant resistance to one pest on the other. Therefore, our study was designed to determine the interactions of SR caused by *D. maydis* and the ECB and to determine the effects of host-plant resistance on these interactions.

MATERIALS AND METHODS

We used a split-plot experimental design with four replicates. Whole-plot treatments consisted of 10 maize genotypes (four plant introductions and six inbred lines) of known reaction to *D. maydis* or the ECB. Subplots consisted of the following six treatments: free of ECB (treated with the insecticide Sevin) and with natural levels of SR; natural levels of ECB and SR; free of ECB (insecticide treated) and inoculated with *D. maydis*; artificially infested with ECB egg masses and with natural levels of SR; natural levels of ECB and inoculated with *D. maydis*; and artificially infested with ECB egg masses and inoculated with *D. maydis*. Thus, subplots were a 3×2 factorial arrangement of treatments (three levels of ECB and two levels of SR).

The genotypes of maize were planted in single-row plots consisting of three three-plant hills, with 100 cm between hills and rows. Guard hills were planted between all plots to prevent ECB larvae from migrating between plots. Planting dates were 8 May 1979 at the Plant Introduction Station Farm, Ames, IA, and 25 April 1980 at the Iowa State University Research Farm, Ankeny, IA.

Cultures of *D. maydis* were grown on sterilized oat kernels for approximately 1 mo. A spore suspension (approximately 10^6 spores per milliliter) was made by blending the infected oats in a food blender for 15 sec, straining the mixture through a double layer of cheesecloth, and adding tap water to make the appropriate spore dilution.

The SR inoculum was placed in a brass inoculator. This inoculator was a hollow tube, 45×3 cm, with a hollow stainless-steel needle, 15×3 mm, mounted on one end. The needle had a hole on one side for the inoculum to flow through. Approximately 0.5 ml of the spore suspension was injected into the second internode of each plant 7 days after anthesis (12).

Plots were infested with 12 ECB egg masses (~300 eggs) per plant in six applications of two masses spaced 1 day apart. Infestations were made during anthesis by using techniques described by Guthrie et al (6).

Two criteria were used to evaluate damage caused by ECB: the first, a visual rating (1 = no damage, to 9 = extensive damage to sheath-collar tissue) was made on a plot basis (7); and the second, measurement of damage in the stalk (individual plant basis) was determined by dissecting the stalks and counting cavities (by measuring their accumulative length in centimeters). Guthrie et al (7) showed high genotypic and phenotypic correlations between these two techniques.

SR damage (individual plant basis) was recorded as the number of internodes, or fraction thereof, that were discolored.

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ECB and SR data were taken 60–70 days after anthesis. The data from individual plots were averaged to obtain a plot mean for each genotype and treatment in each replication.

RESULTS AND DISCUSSION

Differences among maize genotypes and the effects of the six combinations of SR-ECB treatments were highly significant for ECB sheath-collar feeding ratings, ECB cavity counts, and SR damage (Table 1).

The effects of the six combinations of SR-ECB treatments were separated into the effects of the three levels of ECB infestation, the two levels of SR infection, and the interaction of the two. As expected, levels of ECB infestation had a highly significant effect on ECB sheath-collar feeding ratings, the number of stalk cavities, and SR damage. The SR and ECB interaction did not affect either sheath-collar feeding ratings or the numbers of stalk cavities. This interaction, however, had a significant effect on SR damage. Amounts of SR infection in the maize genotypes had no significant effect on the ECB, but amounts of ECB infestation had a highly significant effect on SR damage (Table 1).

The SR-ECB × genotype interaction was highly significant for ECB sheath-collar feeding ratings, number of stalk cavities, and SR damage (Table 1), indicating that the six combinations of SR-ECB treatments did not affect all genotypes equally. These interactions were separated into SR × genotype, ECB × genotype, and SR × ECB × genotype interactions.

The SR × genotype interaction did not significantly affect either sheath-collar feeding ratings or numbers of stalk cavities. Thus, resistance or susceptibility of maize genotypes to SR had no effect on the ECB. However, genotypes differed significantly in their relative reactions to SR infection.

The ECB infestation × genotype interaction was highly significant for ECB sheath-collar feeding ratings and numbers of stalk cavities because four of the 10 genotypes were moderately to highly resistant to the ECB. The ECB infestation × genotype interaction also was highly significant for SR damage; ie, resistance or susceptibility of maize genotypes to the ECB had a highly significant effect on the severity of SR damage.

Genotypes of maize known to be highly resistant to sheath-collar feeding by second-generation ECB are: PI 162927, PI 186209 (10), B52 (5), and B86 (16). In our study (Table 2), genotypes that rated 1–4 for sheath-collar feeding damage or had 0–20 cm damage in the stalk were considered resistant; genotypes that rated 5–6 for sheath-collar damage or had 21–30 cm of damage in the stalk were considered intermediate in resistance; and genotypes that rated 7–9 for sheath-collar damage or had more than 30 cm of damage in the stalk were considered susceptible. Genotypes known to be resistant or susceptible to second-generation ECB showed comparable reactions in our study (Table 2).

Genotypes with 0–1.5 internodes rotted were considered resistant to SR (Table 2); genotypes with 1.6–2.5 internodes rotted were considered moderately resistant; genotypes with 2.6–3.0 internodes rotted were considered moderately susceptible; and genotypes with more than three internodes rotted were considered susceptible.

In almost all genotypes, SR damage was lowest in plots kept free of second-generation ECB with an insecticide, even when inoculated with *D. maydis* (Table 2). SR damage within genotypes was highest in plots artificially infested with ECB, in both *D. maydis*-inoculated and in uninoculated plots, showing that an infestation (entrance holes) by second-generation ECB increased SR damage.

Genotypes of maize (PI 171916, A257, A295, and CI187-2) susceptible to both second-generation ECB and SR, in general, had little SR damage in plots free of ECB, but had high SR damage in plots artificially infested with ECB (Table 2). The four maize genotypes (PI 162927, PI 186209, B52, and B86) that were resistant to the ECB also had a good level of resistance to SR, even when artificially infested with ECB egg masses and inoculated with *D. maydis*. Thus, resistance to second-generation ECB also may contribute to SR resistance. PI 172333 and B14A were resistant or moderately resistant to SR when inoculated and kept free of second-generation ECB with an insecticide, but were highly susceptible to SR when artificially infested with ECB egg masses in both *D. maydis*-inoculated and uninoculated plots.

Simple correlation coefficients were computed to determine more specifically the relationship between ECB infestation and incidence of SR. Correlations between ECB sheath-collar feeding ratings and number of stalk cavities were highly significant (r in 1979 = 0.87, r in 1980 = 0.91). This is in agreement with results obtained by Guthrie et al (7). Of more importance, however, is the extremely high degree of association between levels of ECB infestation and SR damage (sheath-collar feeding and SR damage r for 1979 = 0.73, r for 1980 = 0.83; number of cavities and SR damage r for 1979 = 0.81, r for 1980 = 0.85). High SR damage in plots of susceptible genotypes of maize (A295, CI187-2, PI 172333, and B14A) infested with ECBs and not inoculated with *D. maydis* indicate that an infestation by ECBs also contributes to appreciable damage by other SR pathogens. Hooker and White (9) and Kommedahl et al (14) reported that *Gibberella zeae* (Schw.) Petch and *Fusarium* spp. are common SR pathogens throughout the Corn Belt; these pathogens probably contributed to natural SR damage in our study. It would be of interest to know the pathogenic species present in the natural SR complex. From a practical viewpoint, however, we were more interested in determining the effect of ECB infestation on the incidence of SR than in its etiology.

Results of our study indicate that resistance to SR in 10 maize genotypes may break down if a high infestation of second-generation ECB is present. SR resistance did not break down, however, in genotypes that also were resistant to second-generation ECB. Therefore, breeders should select for resistance to both SR

TABLE 1. Significance of sheath-collar ratings, numbers of stalk cavities, and stalk rot damage for various combinations of *Diplodia maydis* inoculation and European corn borer (ECB) infestation

Source of variation	Degrees of freedom	Statistical significance[a]					
		European corn borer					
		Sheath-collar rating		No. of stalk cavities		Stalk rot damage	
		1979	1980	1979	1980	1979	1980
Genotype	9	***	***	***	***	***	***
D. maydis inoculation-ECB infestation	5	***	***	***	***	***	***
D. maydis inoculation	1	NS	NS	NS	NS	***	***
ECB infestation	2	***	***	***	***	***	***
D. maydis inoculation × ECB infestation	2	NS	NS	NS	NS	*	***
D. maydis inoculation-ECB infestation × genotype	45	***	***	***	***	***	***
D. maydis inoculation × genotype	9	NS	NS	NS	NS	*	***
ECB infestation × genotype	18	***	***	***	***	***	***
D. maydis inoculation × ECB infestation × genotype	18	NS	NS	NS	NS	NS	NS

[a]NS = not significant; * = significant, $P = 0.05$; and *** = significant, $P = 0.001$.

TABLE 2. Sheath-collar (Sh-C) ratings, numbers of stalk cavities (SC), and stalk rot (SR) damage for all combinations of SR, inoculation with *Diplodia maydis*, and European corn borer (ECB) infestation for 10 maize genotypes

Maize genotype	Type of damage	Free of ECB natural SR		Natural ECB natural SR		Free of ECB and inoculated with *D. maydis*		Artificially infested with ECB, natural SR		Natural ECB and inoculated with *D. maydis*		Artificially infested with ECB and inoculated with *D. maydis*	
		1979	1980	1979	1980	1979	1980	1979	1980	1979	1980	1979	1980
PI 171916	Sh-C[a]	1.0	1.0	1.5	1.7	1.0	1.0	3.7	6.7	1.1	1.2	4.0	7.2
	SC[b]	0.5	0.9	2.9	3.3	0.4	3.0	8.9	14.0	3.0	4.7	13.7	15.0
	SR[c]	1.7	1.2	2.4	0.9	2.1	3.3	3.8	3.1	2.0	2.8	4.4	5.2
A257	Sh-C	1.0	1.0	1.0	1.0	1.0	1.0	5.7	8.0	1.2	2.0	6.2	7.7
	SC	0.9	2.2	4.2	5.3	0.6	2.3	19.1	50.0	5.4	10.7	21.5	49.3
	SR	1.6	0.7	2.4	1.9	3.7	3.0	4.6	5.6	3.5	3.1	5.0	5.4
A295	Sh-C	1.0	1.0	1.2	1.7	1.0	1.0	7.0	8.0	1.2	1.2	7.5	8.2
	SC	0.6	1.3	4.2	9.3	0.7	1.8	29.8	43.5	3.5	5.3	31.8	51.8
	SR	3.3	0.5	4.6	1.9	3.3	2.4	6.8	5.7	4.5	2.7	7.2	6.6
CI187-2	Sh-C	1.0	1.0	1.7	1.5	1.0	1.0	7.5	7.5	1.0	1.2	7.5	8.0
	SC	0.6	0.9	6.9	6.0	0.2	2.5	30.9	40.6	6.9	7.5	34.6	44.6
	SR	3.4	0.8	4.8	1.9	3.7	2.7	7.7	6.6	4.1	3.9	8.5	6.9
PI 162927	SH-C	1.0	1.0	1.0	1.2	1.0	1.0	1.5	2.0	1.0	1.2	1.5	2.2
	SC	0.2	0.6	2.8	5.1	0.6	1.8	4.8	7.0	3.7	5.5	6.3	7.2
	SR	0.2	0.2	0.8	1.0	0.7	1.5	1.3	2.0	1.3	2.3	2.2	2.2
PI 186209	Sh-C	1.0	1.0	1.0	1.0	1.0	1.0	1.7	2.0	1.0	1.0	1.5	2.0
	SC	0.8	1.3	3.4	3.9	1.1	0.9	6.0	6.7	3.5	1.9	6.3	4.7
	SR	0.7	0.5	1.7	1.4	1.4	0.9	1.3	2.0	2.1	1.1	2.0	2.1
B52	Sh-C	1.0	1.0	1.0	1.0	1.0	1.0	1.5	2.0	1.0	1.0	1.5	1.7
	SC	0.3	0.7	1.1	0.6	0.2	0.5	5.0	2.9	1.3	0.7	7.3	4.5
	SR	1.8	0.2	2.2	0.1	1.6	1.2	1.5	0.7	1.0	1.2	2.9	1.9
B86	Sh-C	1.0	1.0	1.0	1.0	1.0	1.0	2.0	2.2	1.0	1.0	2.0	2.7
	SC	0.2	0.3	0.7	1.6	0.2	1.2	1.8	6.5	1.8	2.6	5.1	8.6
	SR	1.1	0.5	1.4	0.7	2.2	2.0	1.8	2.6	2.3	2.6	2.6	3.2
PI 172333	Sh-C	1.0	1.0	2.0	1.7	1.0	1.0	5.6	7.5	1.7	2.2	6.5	7.7
	SC	1.0	3.3	5.4	9.8	1.2	2.9	15.9	30.3	6.4	8.9	18.4	33.1
	SR	1.4	0.9	1.9	2.7	1.5	2.3	5.5	6.6	2.6	3.8	6.2	7.1
B14A	SH-C	1.0	1.0	4.4	1.0	1.0	1.0	6.5	7.5	3.3	1.0	7.8	7.7
	SC	0.4	1.5	5.9	5.8	0.9	2.4	11.4	28.5	3.3	3.0	14.3	31.9
	SR	0.8	0.6	1.8	2.1	1.3	2.2	3.8	5.9	2.1	2.9	5.0	6.2

	Sheath-collar rating		No. of stalk cavities		Stalk rot damage	
	1979	1980	1979	1980	1979	1980
Standard error of difference between any two means among genotypes	0.37	0.17	0.80	1.12	0.17	0.30
Any two means among SR damage-ECB infestation LSD, $P = 0.05$	0.14	0.11	0.57	0.89	0.15	0.14
Any two means among genotypes	0.75	0.35	1.65	2.30	0.34	0.61
Any two means among SR damage-ECB infestation	0.27	0.22	1.12	1.77	0.29	0.30

[a] Sh-C = sheath-collar feeding ratings (class 1 = no damage, 9 = extensive damage to sheath-collar tissue).
[b] SC = stalk cavities (centimeters of damage in the stalks).
[c] SR = number of internodes, or fraction thereof, that were rotted.

and second-generation ECB in the same plant populations. A recurrent selection breeding technique can be used to select for resistance to both generations of ECBs (6) and to *D. maydis* (11). All ECB-resistant genotypes included in our study were also resistant to SR; therefore, other genotypes resistant to second-generation ECB may be potential sources of SR resistance. The genotypes used in our study, however, were not selected at random, and, although a high correlation was obtained between ECB resistance and amount of SR damage, we are not suggesting that a breeder can automatically obtain resistance to SR by selecting for resistance to second-generation ECB. Evaluating a large number of genotypes might show little relationship of reaction to the insect and pathogen.

We believe that our study is the first in which the effects of insect resistance (where the insect is not a known vector) on resistance to plant pathogens have been investigated. Studies of this type also should be made with other crops, insects, and diseases to determine whether resistance to insects is associated with resistance to diseases.

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