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Effect of Hybrids with Different Levels of Susceptibility to Second-Generation European Corn Borers on Aflatoxin Contamination in Corn

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Two corn hybrids, *Zea mays* L., grown from two planting dates in Georgia, Missouri, and Iowa were inoculated with two strains of *Aspergillus flavus* Link ex Fr (one a producer of aflatoxins B_1 and B_2 and the other a nonproducer) and a strain of *Aspergillus parasiticus* Spears (producer of aflatoxins B_1, B_2, G_1, and G_2). Half of the plots also were infested with European corn borer (ECB), *Ostrinia nubilalis* (Hübner), egg masses (in four applications of two masses each, spaced 1 day apart). Infestations were started 3 days after full silk. Corn hybrid B86 X SC213 was resistant to sheath-collar and stalk feeding by second-generation ECB, and Oh43 X W182E was susceptible. A higher incidence of *A. flavus* group isolates occurred in ECB larvae collected from the susceptible hybrid than from the resistant hybrid. Hybrid differences in levels of aflatoxin, however, exhibited significant intralocation variation, but the location by hybrid interaction was significant. The hybrid susceptibility to aflatoxin contamination was location dependent, and the specific environment at a corn-growing site is a critical component among the host of factors that are related to preharvest aflatoxin contamination.

Kernel damage in corn, *Zea mays* L., by larvae of second-generation European corn borers, *Ostrinia nubilalis* (Hübner) (ECB), has been implicated in the fungal infection by *Aspergillus flavus* Link ex Fr (Lillehoj and Hesseltine, 1977). Hybrid differences also have been noted for aflatoxin contamination in preharvest corn (Zuber and Lillehoj, 1979).

Resistance to leaf feeding by first-generation ECB has not been as difficult to locate as resistance to sheath-collar feeding by second-generation ECB (Guthrie et al., 1971). Only two agricultural experiment station inbred lines of corn (B52 and B86) have been identified with high degrees of resistance to second-generation borers, whereas inbred SC213 has a low level of resistance. More than 95% second-generation larval mortality occurs within 3 days after egg hatch on resistant inbred lines (Guthrie et al., 1971).

The primary purpose of the current study was to compare the ECB *A. flavus* group interaction in a hybrid resistant to second-generation ECB with a susceptible hybrid.

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MATERIALS AND METHODS

Two hybrids (B86 X SC213 and Oh43 X W182E) were grown at three locations: Ankeny, IA, Portageville, MO, and Tifton, GA. The field experiment at each location involved two replications of a split-plot design with main blocks containing planting dates (May 1 and June 1, Ankeny, IA; April 1 and May 1, Portageville, MO, and Tifton, GA). Hybrids were associated with plots within main plots. The subplot for each hybrid contained 12 treatments: (1) *A. flavus* inoculum (nonproducer of aflatoxin) placed in silks, (2) *A. flavus* (nonproducer) in silks plus ECB, (3) *A. flavus* (nonproducer) in leaf axils, (4) *A. flavus* (nonproducer) in leaf axils plus ECB, (5) *A. flavus* (producer of aflatoxin) in silks, (6) *A. flavus* (producer) in silks plus ECB, (7) *A. flavus* (producer) in leaf axils, (8) *A. flavus* (producer) in leaf axils plus ECB, (9) *Aspergillus parasiticus* (producer) in silks, (10) *A. parasiticus* (producer) in silks plus ECB, (11) *A. parasiticus* (producer) in leaf axils, and (12) *A. parasiticus* (producer) in leaf axils plus ECB. Fungal spores with varied aflatoxin producing potential included *A. flavus* (nonproducer) NRRL 1957, *A. flavus* (producer of aflatoxins B_1 and B_2) NRRL 3357, and *A. parasiticus* (producer of aflatoxins B_1, B_2, G_1, and

Table I. Effect of Hybrids and Planting Dates on Plant Damage Caused by the European Corn Borer

hybrid	planting date I[a] cavities, cm[c] GA	IA	rating,[c] IA	planting date II[b] cavities, cm[c] GA	IA	rating,[c] IA
B86 X SC213	7.9	2.7	2.4	11.2	2.2	3.0
Oh43 X W182E	35.4	14.3	6.0	55.0	35.9	7.5

[a] Planted April 1 at Tifton, GA, and May 1 at Ankeny, IA. [b] Planted May 1 at Tifton, GA, and June 1 at Ankeny, IA. [c] Differences between hybrids for cavity counts and ratings were significant at the 0.01 level of probability; means were based on a total of 120 plants per hybrid per planting date per state.

Table II. Incidence of *A. flavus* Group Isolates from European Corn Borer (ECB) Larvae Collected from Hybrids

location	hybrid	*Aspergillus* isolates, no.[a] +	−	χ^2 [b]
GA	B86 X SC213	24	216	0.19 n.s.
	Oh43 X W182E	28	212	
MO	B86 X SC213	11	229	5.74*
	Oh43 X W182E	26	214	
IA	B86 X SC213	9	223	0.73 n.s.
	Oh43 X W182E	14	218	
total	B86 X SC213	44	668	5.13*
	Oh43 X W182E	68	644	

[a] (+) = number of ECB larvae that contained *Aspergillus* isolates; (−) = number of ECB larvae that did not contain *Aspergillus* isolates. [b] χ^2 = chi-square value comparing hybrids; n.s. = not significant at the 5% level; (*) = significant at the 5% level.

G_2) NRRL 2999. Subplots were one row with eight hills/row and three plants/hill (100 cm between rows and between hills); one guard row of Pioneer Hybrid 3369A was planted between each plot. Plants were infested with ECB (eight egg masses/plant) in four applications of two masses each, spaced 1 day apart: 1, pinned to the husk near the silk; 2, pinned to the primary ear leaf; 3, pinned to the leaf below the primary ear; 4, pinned to the second leaf above the ear (all of the egg masses were pinned to the underside of leaves). Infestations were started 3 days after full silk. Plants were inoculated with *A. flavus* group spores with a 1.0-mL tuberculin syringe 3 days after silking. A 0.1 volume of spore suspension (1×10^8 spores/mL) was introduced into the upper seven leaf axils and into silk bundles. The spore inoculum was prepared at the Southern Regional Research Center (SRRC), New Orleans, LA (Lillehoj et al., 1980). At each location, five mature ECB larvae were collected from stalks and five from ears of each hand-infested subplot (15–20 days after egg hatch). Each insect was individually placed in a sterile screw-capped vial and sent to SRRC for mycological examination (Lillehoj et al., 1980).

Stalk damage was determined by dissecting the stalks and counting cavities (each 2.5 cm of damage = 1 cavity) as described by Guthrie et al. (1978). A visual rating of larval damage (1 = no damage to 9 = extensive damage to sheath-collar tissue) was made on a plot basis (Guthrie et al., 1978).

Cavity counts in Georgia were made 30 days after egg hatch because all larvae had pupated. Cavity counts and plant damage ratings in Iowa were made 60 days after egg hatch. Plant damage measurements were not made in Missouri.

At maturity, 10 ears were randomly collected from each treatment and placed in a 65 °C forced-draft dryer for 7 days. Dried ears were shelled, cracked, and ground in a Raymond hammer mill with a screen containing 3.2-mm perforations. Ground and blended kernel samples were assayed for aflatoxin by the Official First Action Method of the Association of Official Analytical Chemists (1975).

Analytical data were examined by means of analyses of variance using log $(X + 0.1)$, where X is the total aflatoxin level. A wide range of values, including samples with no aflatoxin detected, indicated that transformation of the data was necessary (Snedecor and Cochran, 1967). Significance statements are based on the analysis of variance *F*-ratio tests or for count frequencies use of χ-square tests.

Six of the twelve treatments within each of the two replications were artificially infested with ECB egg masses. The data in Table I were, therefore, analyzed as a split-plot design.

Table III. Aflatoxin Levels in Corn of Two Test Hybrids with Varied Exposure to European Corn Borer (ECB) and *Aspergillus* Inoculum

treatment	aflatoxin, ppb[a] Missouri PD 1 H-1	H-2	PD 2 H-1	H-2	Georgia PD 1 H-1	H-2	PD 2 H-1	H-2	means
silk (nonprod)[b]	T	5	0	2	19	2	175	38	20
silk (nonprod) + ECB[c]	T	13	0	0	2	24	231	20	25
axil (nonprod)[b]	0	T	0	0	T	9	122	21	13
axil (nonprod) + ECB[c]	T	T	0	149	5	4	240	33	36
silk (B_1, B_2 prod)[b]	T	2	6	0	2	T	36	3	4
silk (B_1, B_2 prod) + ECB[c]	T	T	2	70	23	4	265	18	32
axil (B_1, B_2 prod)[b]	3	12	12	T	3	3	344	40	35
axil (B_1, B_2 prod) + ECB[c]	2	58	0	T	T	0	250	2	26
silk (B_s, G_s prod)[b]	T	779[d]	T	15	97[d]	0	17	26	70
silk (B_s, G_s prod) + ECB[c]	T	118[d]	0	11	14	91[d]	216	21	39
axil (B_s, G_s prod)[b]	2	112	0	0	0	28[d]	T	169	26
axil (B_s, G_s prod) + ECB[c]	37	2	T	T	8	732	635	2	110
mean	4	91	2	21	14	73	211	33	

[a] PD = planting data; H-1 = B86 X SC213; H-2 = Oh43 X W182E; T = trace (less than 2 ppb); aflatoxin levels are sums of B_1, B_2, G_1, and G_2 (Iowa kernel samples had a limited incidence of aflatoxin (see the text). [b] Infestation by the natural moth population was at a very low level. [c] Infested with 8 egg masses (~200 eggs) per plant. [d] Samples with G_1 and G_2.

Table IV. Hybrid Comparison of Incidence of *A. flavus* Isolates from European Corn Borer Larvae Collected from Test Hybrids and Aflatoxin Levels in Mature Corn

location		*A.*[a] *flavus* group, no.	grain samples, no., for aflatoxin[b]		aflatoxin,[c] ppb
			−	+	
GA	B86 X SC213	24	9	39	113*
	Oh43 X W182E	28	13	35	54
MO	B86 X SC213	11	26	22	3*
	Oh43 X W182E	26	18	30	59
IA	B86 X SC213	9	39	9	1
	Oh43 X W182E	14	43	5	1
total	B86 X SC213	44	74	70	39
	Oh43 X W182E	68	74	70	38

[a] Isolates obtained from surface-sterilized ECB larvae. [b] (−) = number of grain samples without aflatoxin; (+) = number of grain samples with aflatoxin. [c] Aflatoxin levels are sums of B_1, B_2, G_1, and G_2. (*) = significant difference (5% level) between hybrids.

RESULTS AND DISCUSSION

The insect damage data show that B86 X SC213 was resistant to sheath-collar feeding and to stalk feeding by second-generation ECB, whereas Oh43 X W182E was highly susceptible (Table I). Stalk damage at both locations generally was greater in planting date II than in planting date I. Stalk damage was more extensive in both hybrids in Georgia than in Iowa. Sheath-collar feeding damage was somewhat greater in planting date II than in planting I (Iowa).

Comparison of the *A. flavus* group isolates obtained from ECB larvae collected from the two hybrids demonstrated significant variation over all locations and in the Missouri samples (Table II). A higher incidence of the fungi occurred in larvae collected in Missouri from the ECB-susceptible hybrid (Oh43 X W182E) than from the ECB-resistant hybrid (B86 X SC213). A similar pattern was observed in the Georgia and Iowa samples, but the differences were not significant. These observations linking different hybrids with fungal occurrence in larvae collected from them underscore the importance of the interaction among insects, their internal microbes, and ingested corn plant material. Although the current study does not elucidate the factors responsible for selection of *A. flavus* group propagules by plant materials in the insect digestive tract, the results identify a critical agro-ecosystem phenomenon; i.e., introduction of genetic variation in crop plants can induce a ripple effect in the entire associated biota.

An assessment of the aggregate aflatoxin levels in mature kernels from the three locations demonstrated a broad distribution of toxin in Georgia and Missouri samples (Table III), with limited incidence in Iowa kernels (seven samples in Iowa had a trace of aflatoxin, one sample had 1 ppb of aflatoxin, and three samples had 2 ppb of aflatoxin in the kernels). Except for five samples, all of the contaminated kernels contained only aflatoxins B_1 and B_2. The samples containing both B_s and G_s were obtained from plants that had been inoculated with *A. parasiticus*. Three samples with toxin levels exceeding 500 ppb also were from inoculated plants. A pattern of increased toxin levels was observed in most of the samples from plants that had been hand-infested with ECB. However, samples exhibited no consistent relationship between planting dates and toxin levels. No differences in toxin levels were observed between corn samples collected from plants that had been inoculated with spores of either toxin-producing or nontoxin-producing fungal isolates. Apparently, the presence of spores of a nontoxin-producing isolate did not block the introduction of toxin-producing propagules into the kernels of developing ears.

The association between hybrids and aflatoxin levels demonstrated no apparent linkage (Table IV). The resistant hybrid (B86 X SC213) had a higher level of aflatoxin in Georgia than did the susceptible hybrid (Oh43 X W182E), whereas in Missouri, the susceptible hybrid had a higher level of aflatoxin. The aflatoxin levels in both hybrids grown in Iowa were low.

Although a higher incidence of the fungi occurred in larvae collected from the ECB-susceptible hybrid than from the resistant hybrid, the distribution of aflatoxin grain samples from these hybrids did not reflect a similar association. The hybrid susceptibility to aflatoxin contamination was location dependent in this study and corroborates earlier observations (Lillehoj et al., 1978) that the specific environment at a corn-growing site is a critical component among the host of factors that are related to preharvest aflatoxin contamination.

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