Relation of Insect Damage, Vector, and Hybrid Reaction to Aflatoxin B_1 Recovery from Field Corn

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ABSTRACT

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Mature ears from nine short-season commercial corn hybrids were harvested and separated into three different insect-damage severity levels due mostly to the rice weevil, *Sitophilus zeamais*. Thirty-nine percent of these naturally infested ears had detectable levels of aflatoxin B_1. The lightly, moderately, and severely damaged ears had an average of 0, 3.0, and 16.2 μg/kg of aflatoxin B_1, respectively. Artificially-infested rice weevils apparently were unable to infect ears of corn in the field when they were caged on the ears in fiberglass mesh bags. Only two of 28 ears each exposed to 25 weevils taken from a corn storage bin contained detectable levels of aflatoxin B_1. Two noninfested control ears were positive at a concentration of 2.0 and 15.0 μg/kg of aflatoxin B_1, respectively. In these tests, the rice weevil appeared to be a nonvector or very inefficient vector of *Aspergillus flavus*, since no detectable aflatoxin B_1 was found in corn on which the infested weevils had fed. Significantly less aflatoxin B_1 was recovered from corn of the South Carolina long-season cultivars and from their opaque-2 counterparts artificially inoculated with *A. flavus* than from nine short-season hybrids similarly inoculated.

Additional key words: mycotoxin, *Zea mays*.

Aflatoxin B_1 contamination in corn prior to harvest was demonstrated by Lillehoj et al. (5) in 1973. Since this survey was conducted, considerable work has been done to determine factors affecting the infection of corn by toxin-producing fungi. Physically damaged corn is reportedly more likely to have toxin accumulated than nondamaged corn (2, 3, 4). Toxin accumulation in field corn in South Carolina occurs more often during the late-milk to early-dough stage of development (3). Different corn genotypes are reported to differ in toxin production (3). Lillehoj et al. (5) suggested that the rice weevil, *Sitophilus zeamais,* may be associated with increased toxin production possibly because it is a vector of *Aspergillus flavus* in field corn in the southeastern USA.

To test for differences in aflatoxin production ability by corn hybrids, nine commercial short-season open-shuck hybrids, 27 long-season tight-shuck hybrids, and seven opaque-2 counterparts of the long-season hybrids were artificially inoculated with an aflatoxin-producing isolate of *A. flavus*. Also studied, was the role of corn-feeding insects with emphasis on the rice weevil in the preharvest infection of field corn by frequency and amount of aflatoxin B_1 recovered by chemical assay.

MATERIALS AND METHODS

Effect of nonartificially-infested rice weevil invasion.—Nine early maturing corn hybrids were grown for 154 days in a field where insects (particularly the rice weevil) caused considerable damage. Four ears were harvested at random from each of two plots of each corn hybrid. Insects damage ratings were assigned to each ear of corn, and ears with similar damage ratings were paired and hand-shelled. The corn from each pair of ears was kept separate and dried within 6 hr after harvest to $\leq 10\%$ moisture in a forced-air oven at 90 C. The corn was ground and blended, and a 50-g sample from each pair of ears was analyzed for aflatoxin B_1 content by the technique described in the Official First Action of the Association of Official Analytical Chemists (1). Quantities of aflatoxin present in the extracts were determined on thin-layer plates coated with 0.5 mm Adsorbosil-1. Plates were developed with water:acetone:chloroform (1.5:12:88, v/v) and fluorescent zones were measured densitometrically. The identity of aflatoxin B_1 was confirmed among representative positive samples by the formation of the water adduct with trifluoroacetic acid (6).

Effect of rice weevil artificial infestation.—The production of aflatoxin B_1 was determined after weevil infestation in the field of the following seven corn hybrids: three early maturing (Golden Harvest H2666, Pioneer 3369A, and McNair X194); three late maturing (SC 44 × 413, SC 441 × 413, and SC 31 × 76); and one opaque-2 counterpart of one of the late maturing hybrids (SC 31.02 × 76.02). Weevils were obtained from a corn storage bin and 25 weevils were trapped on each ear. Prior to use, the weevils were treated as follows: (i) infested with *A. flavus* conidia by placing them on a 3-wk-old culture of *A. flavus*

on a Czapek-Dox agar slant; (ii) confined on *A. flavus*-infected corn seeds 48 hr prior to being trapped on corn ears; or (iii) trapped on ears within 2 hr after capture from a storage bin, but without artificial infestation by *A. flavus*. Each test comprised two ears from each hybrid treated according to (i) and (ii) above and four ears according to (iii), while an additional four ears of corn were protected from insects by enclosure in fiberglass mesh bags with openings approximately 1 mm in diameter. These same type bags also were used for trapping weevils on ears. The fiberglass mesh bags were secured over each ear 3-5 days after first silk with black electrical tape at the point of attachment of the ear to the stalk. The weevils were trapped on the ears when the corn shucks had started to turn brown (8-9 wk after first silk). A slit was cut in the bags and the weevils were poured through the opening from a 2.27-cm diameter test tube. Then the slits were closed with tape. The bags were monitored closely for torn places throughout the study and when found were closed with tape.

The corn was harvested 3, 4, and 8 wk after exposure to the insects. Half of the exposed ears of the early maturing hybrids, and the late maturing hybrids were harvested 3 and 4 wk, respectively, after exposure to weevils; the remaining ears were harvested 8 wk after exposure.

The corn was dried immediately after harvest in a forced-air oven at 90 C for up to 6 hr as necessary to reduce the moisture level to 10% or less. Each ear of dried corn was hand-shucked and inspected for insect damage. Each ear was hand-shelled, ground in a Thomas-Wiley mill, and a 50-g sample from each ear was blended prior to analysis for aflatoxin B_1 by the method previously described.

Artificial inoculation.—A single isolate (3357) of *A. flavus* obtained from the National Regional Research Laboratory in Peoria, Illinois, was used as inoculum. The inoculum dose (0.1 ml of a freshly prepared aqueous suspension with 2.0% Tween-20 surfactant containing 10^8 conidia per ml) was injected forcefully using a 1.0-ml pipetting syringe and a 0.56-mm diameter needle into each of three seeds at three sites of each ear (in the mid-region and at two points about equidistant between the midpoint and opposite ends of the ear). Twenty ears of each of the short-season hybrids (Table 2), and eight ears of each of the long-season hybrids and their opaque-2 counterparts were inoculated (Table 3). The opaque-2 counterparts of the long-season hybrids are isogenic lines. The opaque-2 gene present in the opaque-2 counterparts enhances the quantity of the amino acids, lysine, and tryptophan. Only one ear that developed first on each plant was inoculated. Ears were inoculated 6 wk after silks first appeared (during the late-milk to early-dough stage). Both inoculated and noninoculated control ears were hand-harvested 3 wk later. The corn was dried within 0.5 hr after harvest as described previously. Dried corn was hand-shelled and stored at about 21 C and at $\leq$ 10% relative humidity for no more than 3 wk prior to aflatoxin quantitation of 50-g replicated samples as described previously.

RESULTS

The insect damage ratings on four single ear samples and the corresponding toxin levels (μg/kg) recovered from each ear are shown in Table 1. No aflatoxin was recovered from any ear that was rated light for insect damage. Twelve ears rated as moderate for insect damage had an average aflatoxin B_1 content of 3 μg/kg; however,

TABLE 2. Comparison of aflatoxin production in nine short-season hybrid corn cultivars artificially inoculated with a suspension of *Aspergillus flavus* conidia

Hybrid	Aflatoxin B_1 (μg/kg)[z]
Speight D31	88.5 a
Pioneer 3369A	100.0 ab
FFR 808C	122.0 ab
Coker 4018	140.5 ab
McNair X194	154.2 b
Golden Harvest H2666	157.5 bc
Funk G4525	161.8 bc
McCurdy 88	214.8 cd
DeKalb XL78	245.0 d

[z]Mean amount of aflatoxin B_1 in 50-g samples from 10 ears of corn in each of two replications harvested 8 wk after silks first appeared. Inoculations were performed 3 wk prior to harvest by injection of 0.1 ml of a conidia suspension of *A. flavus* into three positions on each ear. Toxin levels followed by the same letter were not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 1. Comparison of aflatoxin B_1 production in insect-damaged (primarily by rice weevils, *Sitophilus zeamais*) ears of nine short-season corn hybrids

	Insect damage[a] and toxin production (μg/kg)[b]							
	Reps							
Hybrid	1		2		3		4	
Funk G4525	(M)	0	(H)	50	(L)	0	(H)	2
Golden Harvest H2666	(H)	50	(M)	0	(H)	2	(L)	0
FFR 808C	(M)	30	(H)	18	(L)	0	(H)	0
Pioneer 3369A	(H)	45	(H)	45	(H)	0	(H)	6
Speight D31	(L)	0	(H)	18	(L)	0	(H)	200
McNair X194	(H)	75	(M)	6	(H)	0	(L)	0
DeKalb XL78	(M)	0	(H)	0	(L)	0	(H)	6
Coker 4018	(L)	0	(M)	0	(L)	0	(L)	0
McCurdy 88	(L)	0	(L)	0	(L)	0	(L)	0

[a]Insect damage ratings: H was heavily damaged, M was moderately damaged, and L was lightly damaged.
[b]Average amount of aflatoxin B_1 recovered from two equivalent insect-damaged corn ears.

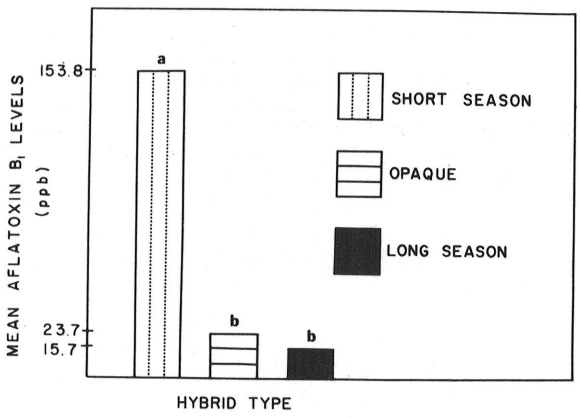

Fig. 1. Comparison of mean amounts of aflatoxin B_1 recovered from nine short-season commercial hybrids, 27 long-season South Carolina hybrids, and seven of their opaque-2 counterparts. The mean aflatoxin B_1 quantities shown were obtained from Tables 2 and 3. Mean toxin levels with the same letter shown in the figure were not significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 3. Comparison of aflatoxin production among long-season corn hybrids and their opaque-2 counterparts artificially inoculated with a suspension of *Aspergillus flavus* conidia

Hybrid	Aflatoxin B_1 (μg/kg)[y] Long-season hybrids[z]		Opaque-2 hybrid counterparts[z]	
SC 31 × 44	0	a	0	a
SC 417 × 401	0	a		
SC 402 × 401	0	a		
SC 12 × 44	0	a	97.8	e
SC 415 × 441	0	a		
SC 31 × 54	3	ab	31.7	d
SC 44 × 76	3	ab	0	a
SC 402 × 12	5	ab		
SC 416 × 401	8	ab		
SC 415 × 401	8	ab		
SC 441 × 413	9	ab		
SC 343 × 401	11	bc		
SC 402 × 413	11	bc		
SC 12 × 31	15	c	9.6	b
SC 12 × 401	15	c		
SC LVT × 401	16	c		
SC 476 × 413	17	c		
SC 402 × 55	23	d		
SC 12 × 54	23	d	7.4	b
SC 481 × 413	24	d		
SC 76 × 413	28	d		
SC 31 × 413	29	d		
SC 44 × 413	30	d		
SC LE × 401	30	d		
SC 484 × 413	31	d		
SC 443 × 413	39	e		
SC 44 × 54	46	f	19.2	c

[y]Mean amount of aflatoxin B_1 in 50-g samples from four ears in each of two replications harvested 9 wk after silks first appeared. Inoculations were performed 3 wk prior to harvest by injection of 0.1 ml of a conidia suspension of *A. flavus* into three positions on each ear.

[z]Toxin levels followed by the same letter within a column were not significantly different ($P = 0.05$) according to Duncan's multiple range test.

eight of the 12 ears contained no aflatoxin. Thirty-two ears rated as severe for insect damage, had an average aflatoxin B_1 content of 16.2 μg/kg, but eight of the 32 ears contained no aflatoxin.

Only two ears on which adult rice weevils were caged that were collected from a corn storage bin contained aflatoxin B_1 and two of 28 noninfested ears, from which all insects were excluded, contained very low levels of aflatoxin. Furthermore, all of the 28 ears on which the artificially-infested weevils were caged were free of aflatoxin B_1. The levels of aflatoxin B_1 found were all very low; the highest among all single ear samples was 15 μg/kg. These data suggest that the rice weevil either is not a vector of *A. flavus* or a very inefficient one.

When hybrid corn cultivars and lines were inoculated artificially with a conidia suspension of *A. flavus* during the early-dough stage, the short-season hybrids produced significantly more aflatoxin B_1 than the long-season hybrids or the opaque-2 counterparts of the long-season hybrids (Tables 2 and 3). The mean level of aflatoxin B_1 recovered per hybrid was significantly less for the long-season hybrids and opaque-2 counterparts than for the short-season hybrids (Fig. 1).

DISCUSSION

Insect-damaged corn can be highly contaminated with aflatoxin (as much as 100 μg/kg per ear). However, corn heavily damaged by rice weevils may have little or no aflatoxin contamination. It has been shown that mechanical wounds are necessary for *A. flavus* infection of corn prior to harvest and for production of significant levels of aflatoxin (3). Since the rice weevil was shown to be a poor vector of *A. flavus,* we assume that under our test conditions fungal propagules were transported by wind and possibly rain to the insect-induced wound sites where infection took place. If this is the predominant mode of infection, it would be expected that harvesting corn as early as possible should reduce the toxin formed in the field, since the time of exposure to insect damage caused primarily by the rice weevil would be shortened. Air-borne conidia populations monitored throughout the growing season in areas where toxin production is known to occur would help support or refute this theory.

Previous work has shown that potential for toxin production varies with different corn hybrids (3). It appears that the long-season hybrids and their opaque-2 counterparts (which were developed and are grown in South Carolina) are less prone to produce aflatoxin than the short-season hybrids, and thus, might be useful as breeding material for production of hybrids with less tendency to produce aflatoxin. All of our long-season hybrids have a tight-shuck cover which may prevent or reduce infection by *A. flavus*. Also, natural infestations of rice weevils are less common on the long-season hybrids presumably because of their tight shucks. However, since artificial inoculations, in which inoculum was injected through the shucks, induced less aflatoxin production in long-season hybrids, it appears that the difference is not due to tightness of shucks. It seems more likely that seeds of the long-season hybrids are more resistant to infection, growth of *A. flavus,* or to production of aflatoxin than are those of the short-season hybrids.

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