Effects of Irrigation and Kernel Injury on Aflatoxin B_1 Production in Selected Maize Hybrids

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ABSTRACT

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Effects of irrigation, simulated corn earworm (SCEW) damage, and naturally occurring insect damage on aflatoxin B_1 production in 15 selected maize hybrids were evaluated during 1979 and 1980. Aflatoxin production in preharvest maize among the hybrids attained levels ranging from 8,966 to 70,809 ppb in treatments receiving SCEW damage and *Aspergillus flavus* inoculation. Aflatoxin levels in hybrids inoculated with *A. flavus* without SCEW damage ranged from 32 to 1,863 ppb. Natural infection resulted in aflatoxin levels of 2–605 ppb. Irrigation suppressed aflatoxin production in kernels receiving both SCEW damage and *A. flavus* inoculation. Insect damage correlated ($P = 0.01$) with preharvest aflatoxin levels when averaged over hybrids and maturity groups for 1979 ($r = 0.50$) and 1980 ($r = 0.73$). Grain from inoculated and uninoculated maize hybrids varied significantly in levels of preharvest aflatoxin. There was little agreement between 1979 and 1980, however, suggesting strong environmental effects on aflatoxin production.

Surveys conducted in the southeastern United States demonstrated that *Aspergillus flavus* Link ex Fries and aflatoxin are present in maize before harvest (11,12,15). Plant stress, such as that caused by drought and high temperatures, during the silking to late dough stages of kernel maturity has been associated with the increased levels of aflatoxin found in preharvest corn (5,6,14,18,22). *A. flavus* colonization and aflatoxin production are most pronounced in kernels with damaged pericarps (16,22). Fennell et al (3,4) and Lillehoj et al (10) illustrated how insects damage kernels and thereby provide access for fungal invasion by *A. flavus*. Application of insecticides to developing corn ears for 6 wk after silking suppressed both southern corn earworm damage (*Heliothis zea* (Boddie)) and preharvest aflatoxin (9). Artificial wounding was as effective as insect injury in increasing aflatoxin levels (7). In a later study, however, Jones et al (6) demonstrated the ability of *A. flavus* to colonize silks and developing kernels where aflatoxin was produced, without a break in the pericarp.

Zuber et al (21) and LaPrade and Manwiller (8) showed that hybrids varied significantly in the amount of aflatoxin produced after natural or artificial inoculation with *A. flavus*. Zuber et al (21) suggested that aflatoxin B_1 formation in several single-cross hybrids was genetically controlled. Manwiller and Fortnum (13) showed significant differences in preharvest aflatoxin production among commercial hybrids. Similar studies by other researchers showed significant differences in toxin production between hybrids within a specific location but failed to show repeatability between locations, suggesting a strong environmental effect on *A. flavus* infection and aflatoxin production (19). This report shows the aflatoxin production in 15 maize hybrids (five in each of three maturity groups), the effect of irrigation and simulated corn earworm damage (SCEW) on aflatoxin production among selected hybrids after inoculation with a toxin-producing isolate of *A. flavus*, and the association of insect damage with preharvest aflatoxin formation.

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MATERIALS AND METHODS

Inoculum and field preparation. In 1979 and 1980 at the Pee Dee Research and Education Center in Florence, SC, 15 commercial hybrids (five from each of three maturity groups) were grown. Maturity group 1 included Pioneer 3369A, DeKalb XL72B, McCurdy 84aa, Coker 22, and DeKalb XL80. Maturity group 2 included Northrup King PX95, Paymaster 9792, Pioneer 3147, Golden Harvest 2775A, and McNair X300. Maturity group 3 included McNair 508, Coker 77, McCurdy 67-14, Pioneer 3030, and DeKalb 395A. A toxin-producing culture of *A. flavus* (N.R.R.L.-3357), obtained from the Northern Regional Research Laboratory, Peoria, IL, was used throughout the experiments.

Inoculum was prepared by washing a 10-day-old culture of *A. flavus* grown on Czapek-Dox agar with sterile distilled water containing 0.05% Tween 20 surfactant. Fresh aqueous suspensions of 10^6 conidia per milliliter were prepared daily and about 0.1 ml of inoculum was applied to wounded and unwounded kernels in a fine mist at 30 psi with a pressurized sprayer (Milwaukee Sprayer Manufacturing Company, Model A, Milwaukee, WI).

Single-row test plots were 6.1 m long and spaced 0.9 m apart. Climatological data were recorded at one location at the Pee Dee Research and Education Center. Test plots received 560 kg/ha of 5-10-30 NPK analysis fertilizer with an additional 448 kg/ha of anhydrous ammonium nitrogen applied as a sidedress. Butylate (9.4 kg a.i./ha), atrazine (2.24 kg a.i./ha), and carbofuran (2.24 kg a.i./ha) were applied for early-season control of annual grasses, broadleaf weeds, insects, and nematodes. The midsilk date (50% exposed silks) was recorded for each hybrid inoculated with *A. flavus*. Test plots of each maturity class were thinned to recommended planting populations about 3 wk after planting. All test plots were hand harvested during the last week of August or the first two weeks in September when the grain moisture level was 18–22%. Within 6 hr of harvest, ears were placed in a forced-hot-air drier at 60 C for 4 days or until the moisture content of the grain was 12% or less.

Natural contamination by aflatoxin. The 15 test hybrids were planted on 12 April 1979 under a center-pivot irrigation system in a randomized complete block design with six replicates. A duplicate set of hybrids was planted in an adjacent, unirrigated field. In 1980, the 15 test hybrids were planted in an unirrigated field in a randomized complete block design. Planting dates in 1980 were staggered to ensure uniform silking. Maturity groups 1, 2, and 3 were planted on 19, 18, and 2 April, respectively. The primary ear from each stalk was harvested and dried. Before mechanical shelling, harvested ears were rated for insect damage on a scale of 0–3, where 0 = no visible insect damage and 3 = severe insect damage. The principal insect pests damaging developing kernels were the corn earworm (*H. zea*) and the fall armyworm (*Spodoptera frugiperda* (J. E. Smith)). Grain was analyzed for aflatoxin after it was ground in a Wiley Mill (Model ED5) to pass through a 20-mesh screen.

Inoculation with *A. flavus*. *A. flavus* conidia were applied to uninjured kernels or SCEW-damaged kernels after hand removal of the husk. Corn earworm damage was simulated by lancing the pericarp with a series of pins set in a lead base (7). The husk was replaced and held in place with a rubber band. Each inoculated ear was then covered with a paper bag to prevent moisture loss and avian damage. The maize was inoculated 25 days after midsilk. Experimental plots consisted of five hybrids from each maturity group planted in a randomized split-block design, with overhead sprinkler irrigation representing whole blocks and hybrids representing subplots. Irrigated and unirrigated plots were separated by a 14-m buffer zone. Treatments were replicated nine times in 1979 and six times in 1980. Maturity groups 1, 2, and 3 were planted on a staggered schedule on 29, 22, and 16 April 1979 and on 6 April and 29 and 20 March 1980, respectively. Ten maize ears per plot were harvested, and the inoculated kernels or inoculated and SCEW-damaged kernels were removed from the cob. Kernels damaged by insects were excluded from the sample. Grain from inoculated plots was analyzed for aflatoxin after it was ground in a Wiley intermediate mill to pass through a 20-mesh screen.

Aflatoxin analyses. Grain from uninoculated and inoculated plots were ground, and a 50-g subsample was analyzed for aflatoxin. In plots receiving SCEW damage, a 5-g sample of ground maize was analyzed. In 1979, samples were analyzed by the technique described in the Official First Action of the Association of Official Analytical Chemists (1). Quantities of aflatoxin B_1 were determined on activated 0.5-mm Absorbasil-1 thin-layer chromatographic (TLC) plates (Applied Science Laboratories, Inc., State College, PA) that were developed in water:acetone:chloroform (1.5:12:88, v/v/v). Aflatoxin B_1 concentrations were determined visually under ultraviolet light (366 nm) by comparison with known standards. Dilute sulfuric acid (17) was used as an indicative test for aflatoxins. In 1980, to avoid using such hazardous solvents and to remove the error associated with visual estimation of fluorescence, the fluorometric-iodine (FL-I) method was used. The sensitivity, precision, and accuracy are similar to the AOAC method (2). The FL-I method does not distinguish between aflatoxin B_1 and G_1, and iodine does not increase the fluorescence of B_2 or G_2. Because of the infrequency of detecting G_1, G_2, or B_2 in previous samples and the lesser effect of iodine on G_1, sample means are listed as B_1. In representative samples analyzed by the FL-I method and indicating a positive aflatoxin reaction, a 50-ml aliquot of the aqueous extract was extracted with methylene chloride, evaporated to dryness, redissolved in solvent, and spotted on MK6F Silica Gel TLC plates (Whatman Inc., Clifton, NJ). The developing solution was chloroform-acetone (95:5, v/v). The presence of aflatoxin B_1 was confirmed by comparisons with known visual standards and the dilute sulfuric acid test under ultraviolet light.

RESULTS

Natural contamination by aflatoxin. Insect damage ratings varied significantly among hybrids (Table 1). Aflatoxin B_1 levels correlated with insect damage over maturity groups for 1979 ($r = 0.50$) and 1980 ($r = 0.73$) and were highly significant ($P = 0.01$). Correlations between insect damage ratings and aflatoxin means within each maturity group were not significant, and correlations between maturity groups reflected wide variations in insect damage and aflatoxin means. Preharvest aflatoxin levels in unirrigated test plots under natural contamination by *A. flavus* were generally higher in maturity group 1 during 1979 and 1980

Table 1. Mean insect damage rating in 15 hybrid maize entries grown at Florence, SC, in 1979 and 1980

	Insect damage[y]		
	1979		1980
Entry	No irrigation	Irrigation	No irrigation
Maturity group 1			
Pioneer 3369A	1.9 a[z]	1.8 ab	2.0 a
DeKalb XL72B	2.0 a	1.9 a	1.9 a
McCurdy 84aa	1.9 a	1.8 ab	2.0 a
Coker 22	2.0 a	2.0 a	1.9 a
DeKalb XL80	1.9 a	1.6 b	1.5 b
Maturity group 2			
Northrup King PX95	2.3 a	1.9 a	1.7 b
Paymaster 9792	2.0 bc	1.7 a	1.5 b
Pioneer 3147	2.2 ab	1.8 a	2.2 a
Golden Harvest 2775A	1.8 c	1.7 a	1.7 b
McNair X300	1.9 c	1.7 a	1.5 b
Maturity group 3			
McNair 508	1.8 a	1.7 a	1.7 a
Coker 77	1.8 a	1.6 a	1.4 b
McCurdy 67-14	1.9 a	1.6 a	1.5 b
Pioneer 3030	1.9 a	1.7 a	1.6 ab
DeKalb 395A	1.9 a	1.7 a	1.7 a

[y] Rated on a scale of 0–3, where 0 = no visible damage and 3 = heavy or severe damage.
[z] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Mean aflatoxin B_1 levels in 15 hybrid maize entries grown at Florence, SC, in 1979 and 1980

	Aflatoxin B_1 (μg/kg)[x]		
	1979[y]		1980
Entry	No irrigation	Irrigation	No irrigation
Maturity group 1			
Pioneer 3369A	45 a[z]	2 a	605 a
DeKalb XL72B	125 a	7 a	224 ab
McCurdy 84aa	83 a	12 a	232 ab
Coker 22	80 a	16 a	174 b
DeKalb XL80	60 a	16 a	288 ab
Maturity group 2			
Northrup King PX95	36 a	15 a	240 a
Paymaster 9792	59 a	3 a	31 b
Pioneer 3147	57 a	2 a	222 a
Golden Harvest 2775A	80 a	3 a	122 ab
McNair X300	40 a	17 a	102 ab
Maturity group 3			
McNair 508	5 b	10 a	112 a
Coker 77	35 ab	11 a	3 a
McCurdy 67-14	37 ab	3 a	32 a
Pioneer 3030	16 ab	9 a	34 a
DeKalb 395A	47 a	10 a	42 a

[x] Aflatoxin content determined by the fluorometric iodine method during 1980.
[y] Maize hybrids were planted under a center-pivot irrigation system during 1979, with an adjacent field serving as an unirrigated control.
[z] Means followed by the same letter are not different ($P = 0.05$) according to Duncan's multiple range test.

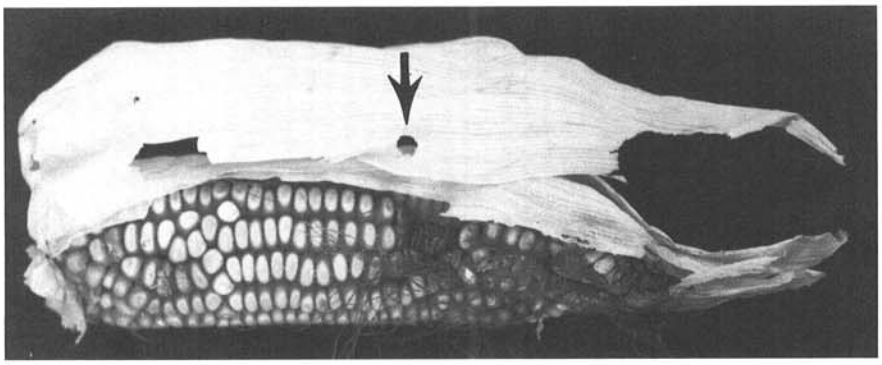

Fig. 1. Earworm exit hole (arrow) and accompanying sporulation of *Aspergillus flavus* on insect-injured kernels.

Table 3. Aflatoxin formation in uninjured corn kernels or kernels receiving simulated corn earworm damage (SCEW) after inoculation with *Aspergillus flavus* 25 days after midsilk

	Aflatoxin B_1 (μg/kg)[y]			
	1979		1980	
Entry	SCEW	Not injured	SCEW	Not injured
Maturity group 1				
Pioneer 3369A	19,613 a[z]	74 b	20,702 ab	372 a
DeKalb XL72B	17,677 a	69 b	15,531 ab	216 a
McCurdy 84aa	17,513 a	407 a	15,203 ab	394 a
Coker 22	8,966 a	316 a	32,984 a	643 a
DeKalb XL80	16,817 a	179 b	11,239 b	498 a
Maturity group 2				
Northrup King PX95	14,581 b	39 a	21,306 a	1,641 a
Paymaster 9792	26,812 a	51 a	14,780 a	125 b
Pioneer 3147	24,267 ab	96 a	21,485 a	1,115 a
Golden Harvest 2775A	15,747 b	72 a	13,261 a	564 ab
McNair X300	22,042 ab	41 a	14,746 a	1,186 a
Maturity group 3				
McNair 508	10,372 c	79 a	70,809 a	413 ab
Coker 77	16,906 ab	48 a	15,042 bc	192 b
McCurdy 67-14	12,740 bc	32 a	18,996 b	1,863 a
Pioneer 3030	16,310 ab	89 a	10,392 c	1,050 ab
DeKalb 395A	22,000 a	126 a	9,868 c	150 b

[y] Concentrations are given as the geometric mean (antilogarithm of the logarithmic mean) of the aflatoxin concentration (ppb); aflatoxin content determined by the fluorometric iodine method during 1980.

[z] Means followed by the same letter are not different ($P = 0.05$) according to Duncan's multiple range test.

Table 4. Effect of irrigation on aflatoxin B_1 levels for *Aspergillus flavus*-inoculated hybrid maize, averaged over entries, in 1979 and 1980

	Aflatoxin B_1 (μg/kg)[b] levels according to maturity group[c]		
Treatment[a]	1	2	3
1979			
Irrigation	117	52	76
No irrigation	230	61	59
Irrigation + SCEW	17,752	20,234	12,306*
No irrigation + SCEW	13,659	19,995	18,683
1980			
Irrigation	621	555	322
No irrigation	256	852	690
Irrigation + SCEW	11,760**	13,242**	17,085
No irrigation + SCEW	27,092	21,217	19,687

[a] Concentrations of aflatoxin in inoculated kernels or inoculated kernels with simulated corn earworm damage (SCEW) are significantly different from their respective unirrigated controls; * = $P = 0.1$ and ** = $P = 0.05$.

[b] Concentrations are given as the geometric mean (antilogarithm of the logarithmic mean) of the aflatoxin concentration (ppb); aflatoxin content determined by the fluorometric iodine method during 1980.

[c] Mean of five hybrids within each maturity group, with nine and six replicates per hybrid for 1979 and 1980, respectively.

than in maturity groups 2 or 3. During 1979, the 15 test hybrids were grown under a center-pivot irrigation system, and aflatoxin production in hybrids was low (Table 2). An adjacent field, serving as an unirrigated control, had higher levels of aflatoxin, and insect damage was observed. Sporulation of *A. flavus* was frequently observed on insect damaged kernels (Fig. 1). During 1980, aflatoxin production in the unirrigated test plots was much higher than in the similar test conducted during 1979 (Table 2). Maximum day temperatures during silking were higher and less rainfall was observed in 1980 than in the previous year. There was little uniformity in the levels of aflatoxin produced among hybrids during the 2 yr of the study.

Inoculation with *A. flavus*. SCEW damage enhanced aflatoxin B_1 production in preharvest maize after inoculation with *A. flavus*. Levels of aflatoxin for the 15 hybrids ranged from about 9,000 to 70,000 ppb. Hybrids varied in the levels of preharvest aflatoxin formed after SCEW damage and *A. flavus* inoculation (Table 3). However, there was considerable year-to-year variation in hybrid response. Aflatoxin was produced in hybrids inoculated with *A. flavus* without SCEW damage but at a much lower level than in those with SCEW damage. Aflatoxin levels ranged from a low of about 30 ppb in 1979 to a high of about 1,900 ppb in 1980. Aflatoxin levels were higher during the drought of 1980. Hybrids inoculated with *A. flavus* varied significantly in the level of preharvest aflatoxin. However, there was little agreement in aflatoxin levels among hybrids from year to year.

Irrigation reduced aflatoxin production in SCEW-damaged, *A. flavus*-inoculated kernels in maturity group 3 (1979) and those in maturity groups 1 and 2 (1980) (Table 4). Adequate rainfall minimized the effect of irrigation in 1979. No irrigation effect was observed on kernels inoculated with *A. flavus* without SCEW damage.

DISCUSSION

In a recent review, several authors were cited as having screened commercial hybrids for aflatoxin production (19). Differences in fungal growth or aflatoxin accumulation among commercial hybrids was reported and the authors suggested a genetic basis for resistance to *A. flavus* and aflatoxin production. It was also noted, however, that the adequate rating of hybrids for susceptibility to toxin formation was prevented by inadequate methods of assessing genotypic response. Test results have not been consistent among hybrids from location to location or year to year. In our tests, aflatoxin levels in various hybrids in 1979 differed from those in 1980, probably because of weather. We used three methods of *A. flavus* contamination: natural, inoculation of kernels with spore suspensions, and

inoculation of kernels with SCEW damage. No method appeared to reduce year-to-year variation. Staggering the planting dates minimized the effect of environmental variation during the critical growth stages of corn because of the more uniform silking date of maturity groups. Within a maturity group, however, silking occurred over a period of 7–10 days. Aflatoxin formation in maize is sensitive to high temperatures and moisture stress, both of which may vary during silking and may be responsible for some of the variation (5,6,14). Aflatoxin accumulation occurs during a short interval of kernel development, and the environment at that time may significantly affect aflatoxin production (8).

We concluded that the enhancement of aflatoxin production by SCEW damage uniformly observed over hybrids and growing seasons results from breaching the protective barrier, the pericarp. The significantly suppressed aflatoxin formation in inoculated kernels in irrigated plots receiving SCEW damage agrees with several studies on the effect of moisture stress on aflatoxin development (5,14). Only wounded kernels showed the irrigation effect; this suggests that insect damage may be more instrumental in increasing preharvest aflatoxin during drought years than during years with adequate rainfall or irrigation. Our tests showed similar insect damage ratings for 1979 and 1980; however, aflatoxin levels were much higher in the drought year 1980 than in 1979. Reports of insect enhancement of aflatoxin formation in maize have been inconclusive, with positive correlations occurring in some studies but not others (3,4,12,16,20). The influence of insect damage on aflatoxin formation may be dependent on the physiological state of the kernel when the wound occurs.

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