Aflatoxin Contamination of Preharvest Corn: Role of *Aspergillus flavus* Inoculum and Insect Damage

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ABSTRACT

Twelve corn hybrids (*Zea mays* L.) were planted in Florida, Georgia, and Tennessee on two dates during 1978 to examine the interaction between hybrids, field environments, planting date, and *Aspergillus flavus* Link ex Fr. infection of developing kernels. At harvest, a broad occurrence of aflatoxin was observed in the samples, with no significant differences among hybrids. Silk channels of treated ears were inoculated with *A. flavus* spores; kernels from the inoculated ears exhibited significantly higher levels of toxin than did those of the controls. Insect damage of ears was assessed at harvest by visual examination; a trend of increased toxin levels was associated with greater damage. Presence of bright greenish yellow fluorescence in corn samples was closely linked to the occurrence of high levels of aflatoxin (> 300 ppb), but corn samples with no detectable toxin also routinely exhibited fluorescencing particles.

Detection of *Aspergillus flavus* Link ex Fr. and aflatoxin in corn (*Zea mays* L.) before harvest has prompted investigation of factors that contribute to the infection of developing kernels by the toxin-producing fungus (Lillehoj and Hesseltine 1977). Several studies identified an association between insect damage and toxin contamination (Fennell et al 1978, Lillehoj et al 1978, Widstrom et al 1975). However, large variability in the insect-fungal interaction had previously been observed. Some corn-growing regions have experienced extensive insect damage of maturing ears with no aflatoxin occurrence, and other areas with equivalent insect damage exhibited a relatively broad incidence of the toxin in kernels at harvest (Fennell et al 1978, Lillehoj and Hesseltine 1977). Several factors have been considered as possible causes of the interregional diversity; these include weather, hybrid differences, and insect variability.

The current study investigated the interregional heterogeneity of aflatoxin contamination of preharvest corn through examination of: 1) differences among hybrids in susceptibility to insect-fungal attack, 2) availability of fungal inoculum, and 3) variation in the association between insect damage and aflatoxin accumulation.

MATERIALS AND METHODS

This study involved hybrid corn replications grown in Georgia, Florida, and Tennessee. Samples were visually scored for insect damage, and within each damage category, the aflatoxin level of each sample was determined.

Twelve hybrids were grown in the study: B73 × Mo17 HT, Reciprocal Mo17 × B73, Pioneer 3369A, B73 × D71-4, Reciprocal D71-4 × B73, Pioneer 3968, DeKalb XL 395, McCurdy 67-14, Pioneer 3145, DeKalb XL 80, McNair 300, and three-way cross with MP496. The hybrids were grown at Gainesville, FL; Tifton, GA; and Knoxville, TN.

The field experiment involved a split plot design with two main blocks for planting dates, each with 12 randomized plots for hybrids. Each plot contained 2 subplots—for control and inoculated corn (10 plants/subplot). Corn was planted at two times: as early in April as possible and about June 1.

Treated ears were inoculated at 20 days after flowering by injecting 0.1 ml of spore suspension of *A. flavus* (NRRL 3357, spores: 1 × 10/ml) into the silk channel with a 1-ml tuberculin syringe. The inoculum was prepared by growing *A. flavus* on potato-dextrose agar in Roux flasks for two weeks at 28°C. Spores were washed from the agar surface with sterile distilled water.

At maturity, 10 ears were harvested from each subplot, sorted visually on the basis of insect damage (1 = light, 2 = moderate, 3 = heavy), and immediately placed in a 65°C forced-draft dryer for 5–7 days. Dried ears were shelled, and the kernels were cracked, examined under ultraviolet light for bright greenish yellow (BGY) fluorescence, and ground in a Raymond hammer mill with a screen containing 3.2-mm perforations. The ground sample was blended for 15–30 min in a Patterson-Kelley twin-shell blender.

BGY fluorescence in cracked kernels of each sample was rated from 0 to 2 on the basis of positive particles: 0 = none, 1 = 1–10, 2 = > 10. Ground and blended corn samples of each category of insect damage were assayed for aflatoxin by the Official First Action Methods of the AOAC (1975). Quantities of aflatoxin were determined on activated thin-layer chromatographic plates coated with 0.5 mm of Adsorbosil-1. Plates were developed with water/acetone/chloroform (1.5:12:88, v/v) in unequilibrated tanks, and flourescent zones were measured densitometrically (AOAC 1975). Aflatoxin B_1 was confirmed in representative positive samples by the formation of the water adduct (AOAC 1975).

RESULTS AND DISCUSSION

Aflatoxin levels varied between hybrids at a location, but uniform susceptibility of individual hybrids at all three locations was not observed (Table I). Detectable levels of aflatoxin were observed in corn from all hybrids except for the control in the late, Tennessee planting of entry 12.

The most apparent trend in the distribution of aflatoxin levels was the increase in toxin in ears that had been inoculated with *A. flavus* spores; toxin levels ranged from three times the control level in the Georgia samples to nine times in corn from Tennessee (Table I). Analysis of variance tests of the toxin levels demonstrated that the differences caused by *A. flavus* treatment were significant; variation in the interaction of location and treatment was also significant. Because the toxin levels in control samples were relatively uniform between locations, the interstate variation in response to inoculation suggests that environmental factors (temperature, rainfall, humidity, etc.) at a location affect the access of *A. flavus* spores to developing kernels and/or subsequent toxin production.

Further analysis of variance demonstrated no difference in toxin levels associated with planting dates. However, significant variation was observed in the interaction of planting date and location. The latter difference was primarily attributable to the

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TABLE I
Aflatoxin B_1 in Twelve Hybrids Planted at Two Dates in Florida, Georgia, and Tennessee

	Aflatoxin B_1 (ppb, geometric means)[a]											
	Florida				Georgia				Tennessee			
	Control		Treated[b]		Control		Treated[b]		Control		Treated[b]	
Hybrid	PD 1[c]	PD 2[c]	PD 1	PD 2	PD 1	PD 2	PD 1	PD 2	PD 1	PD 2	PD 1	PD 2
B73 × Mo17 HT	76	103	223	136	35	30	69	175	10	44	420	160
Reciprocal Mo17 × B73	113	91	795	485	17	36	75	129	77	47	387	171
Pioneer 3369A	36	13	110	290	35	20	130	183	69	4	489	220
B73 × D71-4	6	350	515	191	11	21	41	121	64	9	596	91
Reciprocal D71-4 × B73	11	29	118	248	76	41	201	57	39	1	330	123
Pioneer 3968	30	23	239	790	75	13	108	103	138	36	830	1081
DeKalb XL 395	9	1	41	2	84	6	491	98	194	3	213	65
McCurdy 67-14	9	49	4	113	156	8	512	28	22	52	307	91
Pioneer 3145	8	8	98	183	181	10	1242	184	46	16	681	119
DeKalb XL 80	15	69	140	193	296	15	494	39	37	39	82	127
McNair 300	5	148	28	336	55	7	274	42	72	8	1026	60
Three-way cross with MP496	1	10	61	131	742	10	542	13	46	0	847	59
Planting date means	13	32	101	159	77	16	222	76	53	11	431	130
Treatment means	20		127		35		130		24		238	

[a] Means of two replications. LSR = 66 (least significant ratio for comparing entries with equivalent treatment at each location).
[b] *Aspergillus flavus* spores introduced into the silk bundle of test ears.
[c] PD 1 = Corn planted as early in April as possible, PD 2 = corn planted about June 1.

TABLE II
Levels of Aflatoxin, Bright Greenish Yellow (BGY) Fluorescence, and Insect Damage in Corn Grown in Florida, Georgia, and Tennessee

			Corn			
			Control[c]		Treated[c,e]	
State	Planting Date[a]	Insect Damage Category[b]	BGY[d]	Aflatoxin B_1 (ppb)	BGY	Aflatoxin B_1 (ppb)
FL	1	1	0.6	17	1.1	125
		2	0.7	22	1.1	145
		3	1.1	84	1.7	752
	2	1	0.3	27	0.8	136
		3	0.8	104	1.0	257
GA	1	1	1.1	214	1.3	683
		2	1.1	140	1.2	286
		3	1.3	134	1.8	490
	2	1	1.2	21	1.6	72
		2	1.2	38	1.7	136
		3	1.2	461	1.3	160
TN	1	1	0.8	77	1.0	534
		2	0.5	55	1.0	425
		3	0.5	47	1.0	794
	2	1	0.2	9	1.0	140
		2	0.6	31	1.0	177
		3	0.8	39	1.0	2098

[a] 1 = corn planted as early in April as possible, 2 = corn planted about June 1.
[b] 1 = light, 2 = moderate, 3 = heavy.
[c] Means from 12 entries and two replications.
[d] Rating showing positive particles in sample: 0 = none, 1 = 1–10, 2 = >10.
[e] *Aspergillus flavus* spores introduced into the silk bundle of test ears.

TABLE III
Distribution of Aflatoxin B_1 (Afl) Levels and Bright Greenish Yellow(BGY) Fluorescence in Preharvest Corn Grown in Florida, Georgia, and Tennessee

	Preharvest Corn Grown in					
	Florida		Georgia		Tennessee	
Level of Aflatoxin B_1 (ppb)	Afl[a]	BGY[b]	Afl	BGY	Afl	BGY
> 700	4	100	11	100	19	100
500–700	3	91	3	100	5	100
300–500	3	100	6	100	11	100
100–300	21	97	21	98	24	93
1–100	46	81	56	88	33	74
0	23	66	3	60	8	93

[a] Percentage of all samples from a location.
[b] Percentage of samples within a toxin-level category exhibiting BGY fluorescence.

increased toxin levels in Florida corn and decreased toxin in samples from the other two locations from corn planted on the second date. The results demonstrated a distinct location-dependent pattern of increase or decrease in toxin levels of both control and *A. flavus*-inoculated samples between planting dates.

Although prior study has suggested that early planting dates might reduce susceptibility of developing corn to aflatoxin contamination (Zuber and Lillehoj 1979), the current results suggest that planting date variation as a causal factor in subsequent levels of aflatoxin is location-dependent. In spite of the location differences in planting date levels of aflatoxin, the *A. flavus*-inoculated ears routinely yielded increased levels of toxin. These results suggest that inoculum availability was a primary factor in aflatoxin contamination at the three locations but that differences in toxin accumulation between planting dates depend on local environmental conditions.

The association between insect damage, BGY fluorescence, and aflatoxin contamination was explored by visual identification of insect damage on test ears and the independent assay of corn from each of three damage categories, light, moderate, and heavy (Table II). An analysis of variance of the aflatoxin results showed significant differences among categories of insect damage. Although overall increased insect damage was associated with higher toxin levels, high toxin contamination was observed in a number of samples with light insect damage, for example, in Georgia corn from the initial planting. A general pattern of association between aflatoxin contamination and insect damage was found. However, the exceptions to the pattern suggest that undefined environmental factors can induce relatively high levels of toxin in corn with only limited insect damage.

No significant differences in aflatoxin levels were observed between the two planting dates, but the interaction of planting date and insect damage did show significant differences. The association between increased toxin levels and insect damage was greater in corn from the second planting date than in that from the first. The planting-date results show that plant maturity can exert an influence on the insect-toxin interaction. BGY fluorescence was

also higher with higher insect damage, with some distinct exceptions, for example, the Georgia second planting.

The distribution of aflatoxin levels in samples from the three locations indicated that more than 90% of the samples from Georgia and Tennessee and more than 75% from Florida contained detectable quantities of the toxin (Table III). Most of the corn was contaminated in the 1–300 ppb range, but 19% of the samples from Tennessee contained toxin in excess of 700 ppb. Because the characteristic BGY fluorescence associated with the presence of aflatoxin-producing fungi has been suggested as a determinant in a rapid technique for screening commodities for aflatoxin (Lillehoj 1979), the samples in the current study were examined for fluorescence. Results were computed as percentage occurrence within an aflatoxin-level category. Except for the 500–700 ppb category in Florida corn, all the samples with more than 300 ppb of toxin exhibited BGY flourescence. However, in the 1–300 ppb categories, the incidence of fluorescence ranged from 74 to 98%. Samples with no detectable aflatoxin demonstrated a common occurrence of BGY fluorescence. BGY fluorescence is a reasonably accurate qualitative indicator of aflatoxin presence when the corn is contaminated at high levels of toxin. However, the broad occurrence of the fluorescence in toxin-free samples indicates that a rapid screening process based on BGY would falsely identify significant quantities of corn.

The results of the current study demonstrated: 1) a general preharvest occurrence of aflatoxin in corn kernels of 12 hybrids grown in three states, with no indication that specific hybrids were uniformly susceptible to contamination at all locations, 2) a significant toxin increase in kernels from ears inoculated with *A. flavus* spores in silk bundles of test ears, 3) increased toxin levels of corn associated with increased insect damage, and 4) a close association between BGY fluorescence and kernels heavily contaminated with aflatoxin but also a routine occurrence of the fluorescence in toxin-free samples.

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