AFLATOXIN AND *ASPERGILLUS FLAVUS* OCCURRENCE IN 1975 CORN AT HARVEST FROM A LIMITED REGION OF IOWA[1]

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ABSTRACT

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Freshly harvested corn (18.4% moisture average) from the 1975 crop was examined for *Aspergillus flavus* Link ex Fr., bright greenish-yellow (BGY) fluorescence, and aflatoxin. The survey was in three counties of west central Iowa and in one centrally located county. In all, 214 samples of shelled corn were collected and dried to 13% moisture or less within 4–156 hr after harvest. About one-half of the samples exhibited BGY fluorescence in cracked fractions, and 17% contained detectable levels of toxin. However, only four samples had aflatoxin B_1 levels exceeding 20 ppb, with the highest concentration at 56 ppb. Visual examination of ears on standing stalks was carried out in 8 fields; 11 ears with greenish-yellow spores were found. Corn from these ears was contaminated with a wide array of toxin levels ranging from 1 ppb to 1560 ppb.

The presence of aflatoxin has aroused concern as surveys (1–3) and FDA actions (4,5) continue to identify its occurrence in corn. Although an early study (6) showed that *Aspergillus flavus* could infect kernels of developing corn, contamination of corn by *Aspergillus* spp. traditionally has been considered largely a problem of stored grain. Examination of preharvest corn in central and northern regions of the U.S. in 1971 and 1972 had shown only a limited incidence of *A. flavus* (7). Since then, the fungus has been found in corn before harvest, particularly in southern states (8–11).

In 1973, corn from a region of South Carolina was collected at harvest to determine field occurrence of *A. flavus* and aflatoxin (9). Thirty-two per cent of 297 samples contained aflatoxin at levels exceeding 20 ppb. Mycological tests demonstrated extensive incidence of *A. flavus* in the samples. These observations conclusively demonstrated that extensive *A. flavus* infection and aflatoxin production occur in corn before harvest.

Anderson *et al.* (12) also observed a higher incidence of aflatoxin in corn grown in warm, humid regions compared to that found in corn of temperate regions. Examination of factors contributing to the problem showed that stressed growing conditions such as dense populations of plants or reduced fertility appeared to increase the incidence of toxin contamination. Dickens *et al.* (13) observed a similar stress-susceptibility relationship in preharvest peanuts; their results suggest that drought conditions during development of the peanut increase *A. flavus* infection of the pod underground.

Several investigations have implicated insect damage as a critical factor in establishment of *A. flavus* infection of developing corn (9–11,14). Various insect species have been associated with observed infection by the fungus, but a specific

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insect-*A. flavus* relationship has not been established.

To extend the studies of field occurrence of aflatoxin in corn, we selected areas in Iowa for further investigation during the 1975 harvest. Specific regions were selected because reports had been made of the occurrence of bright greenish-yellow (BGY) fluorescence and aflatoxin in freshly harvested corn from those areas (15,16).

MATERIALS AND METHODS

Corn was acquired in a three-county region of west central Iowa and from a county in the central portion. During the last 2 weeks of October 1975, samples were collected either directly from picker-shellers or from trucks at country elevators. Weights of samples were: series B, C (1 kg); series D, E (2.5 kg); and series A, F (5 kg).

Each 2.5- and 5-kg sample was placed in a perforated drying tray and dried in a mechanical convection oven at 90° C. The 1-kg samples were dried in opened paper bags on racks in the same 90° C ovens. Moistures were determined for each sample before drying, and final moistures were tested periodically to ensure adequate drying for safe storage (13% moisture content or less). Moisture content was determined with a Motomco meter.

The entire dried sample was coarsely ground with a Hobart disk mill and examined under high-intensity ultraviolet (uv) light (365 nm) with a Blak ray lamp (Model B, 100-A) for BGY fluorescence (17). Subsequently, the samples were ground with a 12-in. Raymond hammer mill fitted with a screen containing 1/8-in. perforations, then blended 15–30 min in a Patterson-Kelley twin shell blender.

TABLE I
Distribution of Sampling Dates, Average Initial Moisture Levels, Sampling-to-Drying Times, Bright Greenish-Yellow (BGY) Fluorescence, and Aflatoxin Contamination

Sampling Series	Date	Moisture %	Sampling to Drying hr	Samples Total	BGY Positives	Aflatoxin Positives[a]
Western Iowa						
A	10/17–10/19	18.4	4	39	26 (67%)	6 (15%)
B	10/20–10/21	18.5	48	37	9 (24%)	3 (8%)
C	10/22–10/24	17.1	156	34	19 (56%)	6 (17%)
D	10/25–10/26	19.1	48	12	6 (50%)	2 (16%)
E	10/27–10/29	18.5	48	55	25 (45%)	16 (29%)
Central Iowa						
F	10/20–10/21	19.2	12	37	16 (43%)	4 (11%)
Total		...	...	214	101	37
Overall mean		18.4	...	...	(47%)	(17%)

[a]Aflatoxin B_1 levels ranged from 1 ppb to 56 ppb. Chi-square tests showed no significant variability in the percentage occurrence of aflatoxin between series.

In western Iowa, corn ears on upright stalks were examined in eight fields. Husks were pulled back on about 400 ears in each field, and individual ears were visually inspected. Of 81 fungal-contaminated ears, 11 exhibited characteristic greenish-yellow spores of *A. flavus*. Ears were dried at 65° C for 1 week and individually shelled. Corn from each ear was cracked, examined under uv light, finely ground, and assayed for aflatoxin.

Ground corn samples were assayed for aflatoxin by the Official First Action Method of the AOAC, sections 26.037–26.039 (18). Quantities of aflatoxin were determined on activated thin-layer chromatographic (tlc) plates coated with 0.5 mm Adsorbosil-1. Plates were developed with water:acetone:chloroform (1.5:12:88 v/v/v) in unequilibrated tanks, and fluorescent zones were measured densitometrically. Aflatoxin B_1 was confirmed in representative positive samples by the formation of the water adduct; see AOAC First Action Method, section 26.A17 (18).

Subsamples of series C and E test units were dried at 65° C for 4 days for microbiological evaluation. From each sample, 50 randomly selected kernels were surface-sterilized with 2% sodium hypochlorite for 2 min, rinsed twice with sterile water, placed on ME agar (malt extract, 30 g/L and agar, 15 g/L) in petri plates, incubated at 28° C, and examined under a stereoscopic microscope after 4 days.

RESULTS

A major objective in the examination of Iowa corn was to determine the extent of preharvest infection by *A. flavus* and the levels of aflatoxin contamination. To avoid post-harvest development of the fungus, the time between sample collection and drying was minimized and the samples were dried rapidly by operating dryers at 90° C. Three hours of drying reduced 18 to 20% moisture corn to 10 to 11%. The average initial moisture levels for each series and the mean time between collection and drying of test corn are presented in Table I. The overall initial moisture mean was 18.4% with no significant variation between series means. Sampling-to-drying time ranged from 4 to 156 hr. Of the 214 samples, 177 (83%) were collected in west central Iowa (series A–E) and 37 (17%) came from the center of the State (series F).

BGY-fluorescing pieces were observed in 47% of the coarsely ground samples. Many fluorescing particles appeared to be small fragments of immature kernels, integuments, or glumes that could not independently contaminate kilogram quantities of nonfluorescing corn with detectable levels of toxin. A similar type of fluorescence was observed on ear tips of Missouri corn examined in 1972 (8). In addition to fluorescence of the glume-like structures, the usual BGY emission at germ margin and endosperm was observed.

Aflatoxin was detected in 17% of the samples. The occurrence ranged from 8% (series B) to 29% (series E). However, there was no statistical evidence of significant variation in B_1 incidence between series. Since there was no association between aflatoxin incidence and longer collection-to-drying times, apparently all toxin contamination occurred in preharvest corn. Examination of the relation between BGY fluorescence and aflatoxin occurrence showed that 67% of the BGY-positive samples did not contain detectable levels of toxin. Of the aflatoxin-positive samples, 3/37 did not exhibit BGY fluorescence.

TABLE II
Distribution of Mean Moisture Levels in Corn Samples Based on BGY Fluorescence and Aflatoxin Occurrence

Sampling Series	Mean Moisture Content Levels (%)			
	BGY		Aflatoxin B_1	
	−	+	−	+
A	19.7	17.6	18.3	18.9
B	18.7	17.9	18.5	18.6
C	17.9	16.4	16.9	18.0
D	18.9	19.2	19.4	17.5
E	19.3	17.6	18.8	17.8
F	20.8	17.2	19.5	17.0
Overall mean	19.2*	17.4*	18.5	18.0

TABLE III
Number of Samples from Each Series in Sequential Aflatoxin Contamination Categories

Aflatoxin B_1 ppb	Series						Total
	A	B	C	D	E	F	
<5	2	1	2	0	1	1	7
6–10	3	2	1	2	6	1	15
11–20	0	0	1	0	8	2	11
21–40	1	0	2	0	0	0	3
41–56	0	0	0	0	1	0	1
Totals	6	3	6	2	16	4	37

TABLE IV
Incidence of *Aspergillus flavus* and Aflatoxin in Corn Samples from Sampling Series C and E

Sampling Series	Aflatoxin B_1	Number of Samples[a]		
		A. flavus-free	*A. flavus*-infected	Total
C	−	2	22	24
	+	0	6	6
	Total	2 (7%)	28 (93%)	30
E	−	27	11	38
	+	4	11	15
	Total	31 (58%)	22 (42%)	53

[a]From each sample, 50 kernels were surface-sterilized, plated on a nutrient medium, and incubated for 7 days at 28°C. Presence of one or more *A. flavus*-containing kernels identified the sample as infected.

Since previous studies (9) demonstrated an association between initial moisture content of freshly harvested corn and presence of aflatoxin, the relation between moisture, BGY fluorescence, and the toxin was examined (Table II). Distribution of mean moisture levels showed that BGY-fluorescent corn was significantly drier than BGY-negative samples. A similar trend was observed in moisture means based on aflatoxin occurrence, but differences were not statistically significant.

Of the aflatoxin-positive samples, 89% contained less than 20 ppb; the highest level of aflatoxin B_1 observed was 56 ppb (Table III). The distribution of toxin levels between series was somewhat uniform; most samples from all test areas were contaminated at levels below 20 ppb.

The incidence of *A. flavus*-infection was identified in series C and E samples (Table IV). Series C samples had been held for 156 hr before drying; in this group, all six aflatoxin-producing samples yielded one or more *A. flavus*-infected kernels. In addition, 22 toxin-negative samples had kernels infected with the fungus. Series E had the highest incidence of aflatoxin-positive samples. Microbiological tests detected *A. flavus* in 73% of the toxin-contaminated corn samples and in 29% of the aflatoxin-negatives. The overall incidence of samples infected by the fungus was significantly higher in series C (93%) than in series E (42%). The absence of detectable *A. flavus* in four of the series E toxin-positive samples may have been related to the low occurrence of contaminated kernels observed in the test; this incidence ranged from zero to 36%, with a mean of 8%. On the other hand, the occurrence of the fungus in kernels of series C samples ranged from zero to 100%, with a mean of 36%.

Visual examination of ears on standing stalks provided ears with greenish-yellow spores in 4/8 fields; all these ears contained detectable levels of toxin

TABLE V
Aflatoxin Occurrence in Corn from Ears Collected from Standing Stalks

	Number of Ears				
Field	Greenish-yellow spores	Other fungi	BGY Positive	Aflatoxin B_1 positive	Aflatoxin B_1[a] Mean (ppb)
1	3		3	3	27
1		10	2	3	4
2		10	3	0	
3	1		1	1	1,000
3		6	2	0	
4		6	3	0	
5		10	1	1	14
6		7	2	0	
7	3		3	3	880
7		10	0	1	5
8	4		4	4	260
8		11	1	1	2
Total	11	70	25	17	

[a]The mean toxin levels are based on all aflatoxin-positive ears. Aflatoxin B_1 concentrations in individual ears ranged from 1 ppb to 1560 ppb.

(Table V). The level of aflatoxin in contaminated ears ranged from 1 ppb to 1560 ppb. Of the fungal-infected ears not exhibiting greenish-yellow spores, 6/70 contained detectable levels of toxin. However, levels in these ears were significantly lower than in the ears with visible greenish-yellow spores; values ranged from 1 ppb to 9 ppb.

DISCUSSION

Our results support the conclusion that aflatoxin developed in the field because: a) series A corn was collected and entered into a 90° C dryer within 4 hr, b) the aflatoxin incidence of series A corn did not vary significantly from samples with longer collection-to-drying times, c) test corn was relatively mature with a mean moisture of 18.4%, d) four aflatoxin-positive samples had initial moisture levels below 15.5%, and e) ears collected from standing stalks contained the toxin.

Although the incidence of toxin-contaminated samples was 17%, less than 2% of the test corn contained aflatoxin B_1 at levels exceeding 20 ppb. In addition to limited incidence of toxin levels exceeding the FDA action guideline, the highest concentration of toxin detected was only 56 ppb. These observations indicate considerable *A. flavus* infection of corn in some Corn Belt fields but restricted toxin production.

An association was observed between occurrence of BGY fluorescence and initial moisture; drier corn had higher levels of fluorescence. Although a similar trend was observed in toxin incidence, the difference between moisture means was not significant. A relationship had been described between lower corn moisture and increased toxin occurrence in South Carolina samples (9). Although the current investigation suggests a similar pattern in Iowa corn, results are not definitive.

Microbiological tests showed that series C corn was heavily infected with *A. flavus* (93%). The increased incidence of the fungus can be attributed to the extended collection-to-drying time (156 hr) of corn in the series. In addition to appropriate conditions for *A. flavus* growth, a large number of spores must have been available to infect kernels almost totally in the predrying period. The higher incidence of *A. flavus*-infected kernels in this series did not increase BGY and aflatoxin occurrence. Apparently, the initial spread of *A. flavus* did not elaborate detectable levels of fluorescing metabolite(s) or toxin.

Corn taken directly from stalks showed that ears with greenish-yellow spores all contained aflatoxin. Levels of toxin from these ears were high; 4/11 contained aflatoxin above 900 ppb. Clearly, extensive toxin-contamination of particular ears occurred in the field. However, the wide range of aflatoxin levels (1 ppb to 1560 ppb) indicates a distinct heterogeneity of contamination. Furthermore, because no toxin-contaminated ears were obtained in 3/8 fields, contamination between fields must vary markedly. Absence of greenish-yellow spores in 6/17 toxin-contaminated ears points out that visual estimation is an inadequate measure of toxin-contamination in the field.

Factors that may contribute to *A. flavus* infection and aflatoxin contamination of preharvest corn include weather conditions during the growing season. During July and August 1975, central and western Iowa experienced drought conditions that might have predisposed the developing ears to attack by:

a) insects and *A. flavus,* b) other plant pathogens and *A. flavus,* or c) *A. flavus* directly. However, the etiological events associated with initial infection by the toxin-producing fungus remain speculative.

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