Aflatoxin Contamination: Association with Foreign Material and Characteristic Fluorescence in Damaged Corn Kernels[1]

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ABSTRACT

Samples of corn from commercial markets, previously shown to contain aflatoxin, were examined to determine the distribution of toxin within the bulk of the corn. A greenish-gold fluorescence under ultraviolet light (365 nm.) was associated with the presence of aflatoxin. Damaged kernels with the characteristic fluorescence contained as high as 88,500 to 101,000 p.p.b. aflatoxin B_1, indicating that the toxin contamination could be concentrated within a few kernels in a corn sample. In two of thirteen contaminated corns examined, aflatoxin B_1 was found in high concentrations in the broken corn-foreign material, accounting for most of the toxin in the total sample of corn.

Levels and incidence of aflatoxin detected in corn (1-4) from commercial markets are low. However, because carcinogenic properties of aflatoxins have been

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demonstrated in some test animals, there is concern about their possible presence in corn being bought and sold for food and feed. Any method of rapid detection or easy removal of the toxin, if present, would be a valuable contribution. One study (5) showed that aflatoxin might be concentrated in "dockage"–broken kernels and foreign material–and could be easily removed by physical means. Since the corn investigated in that study had been stored at least 12 years, it was not typical of corn going through today's commercial markets. In our initial studies, we established a correlation between a characteristic greenish-gold glowing fluorescence in damaged corn and the presence of aflatoxin. Our findings on aflatoxin in broken corn-foreign material (BCFM)[2] and other fractions of corn moving in commercial channels are reported here.

MATERIALS AND METHODS

Separation of Corn Samples

The 34 samples, both aflatoxin-positive and -negative, came from commercial markets and were collected in previous surveys. As samples were received, each was divided into two 1-kg. portions, one of which was ground for the original assay of the total sample. Unground portions were shaken on a grain-grading 12/64 sieve (6). The material that passed through the sieve, BCFM, was collected and weighed. Particles that had a greenish-gold glowing fluorescence, as detected with a Blak-Ray B-100 high-intensity light or a UVL-22 hand lamp (each 365 nm.), and that were large enough to pick out with tweezers, were removed, weighed, and extracted. Particles and pieces of corn with other kinds of fluorescence were also separated for study. From all samples, large pieces of corn and kernels not passing through the 12/64 sieve, but having the same kind of greenish-gold fluorescence, were also taken out for extraction. The following fractions were hand-selected from portions of seven samples that did not pass through the sieve: large broken pieces, whole kernels with mold damage or cracks, and whole undamaged kernels (Fig. 1).

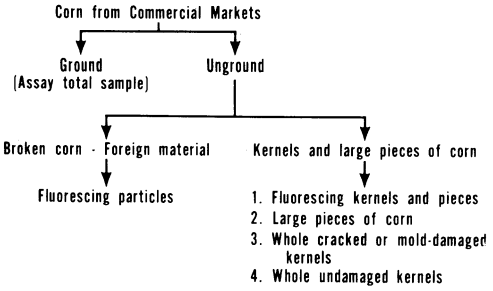

Fig. 1. Separation of corn into fractions.

[2]Kernels and pieces of corn and all matter other than corn which will pass readily through a 12/64 sieve.

Extractions

BCFM, large broken pieces, whole kernels with mold damage, cracked kernels, and whole undamaged kernels were extracted by the method recommended for peanuts and peanut products (7), but without previous grinding. Instead, a sample was simultaneously ground and extracted in a Waring Blendor. The unground sample (50 g.), water, diatomaceous earth, and chloroform were mixed in the Waring Blendor 5 min. before filtering. If there were more than 50 g. in a fraction, the entire sample was assayed in 50-g. portions to ensure significant results. The extracts were partially purified by column chromatography before thin-layer chromatography (TLC).

Particles from BCFM and damaged corn kernels were placed in small vials and steeped 48 hr. in chloroform (2 to 3 ml.) and a drop of water. The chloroform layer was removed, and solids were washed with small portions of chloroform until the chloroform wash did not fluoresce. The combined extract and washes were evaporated on a steam bath under nitrogen for TLC. The solids were extracted a second time in the same manner. A third extraction did not remove any more aflatoxin.

Thin-Layer Chromatography

Chromatoplates (20 X 20 cm.) were coated with a 0.5-mm. layer of Adsorbosil-1, dried about 30 min., heated 1 hr. at 105°C., and stored in a desiccating chamber.

Solutions of unknowns and of standards in benzene-acetonitrile (98:2, v./v.) were applied to plates alone and in admixture. The plates were developed with acetone-chloroform-water (12:88:1.5, v./v./v.) (8).

Confirmatory Tests

The presence of aflatoxin B_1 was confirmed by water and acetate adducts (7, p. 434).

RESULTS AND DISCUSSION

An unusual fluorescence under 365-nm. ultraviolet light was observed on damaged kernels and particles from corn samples containing aflatoxin. This fluorescence was greenish-gold and appeared to glow (Fig. 2). Extraction of particles and damaged kernels possessing this fluorescence revealed that they had high levels of aflatoxin (Table I). Some of the other damaged kernels fluoresced white, blue, or orange. A number of these were hand selected and extracted. Although a white or blue fluorescence was more typical of crystalline aflatoxin B_1 than the greenish-gold fluorescence, extracts of samples fluorescing white or blue contained no B_1. All the available unground samples of corn in which aflatoxin had been detected in previous surveys were inspected for particles and kernels that fluoresced greenish-gold. Seventeen of the eighteen fluorescing isolates contained high levels of aflatoxin B_1. The one that did not was such a small sample (0.002 g.) that B_1 would have had to be present at levels of 3,000 p.p.b. to be detected. Crystalline aflatoxin B_1 fluoresces pale blue under 365-nm. ultraviolet light. The greenish-gold fluorescence disappears when aflatoxin is removed from fluorescing BCFM particles and damaged corn kernels by chloroform extraction.

We did not detect this fluorescence in rice or wheat inoculated with *Aspergillus flavus* or *A. parasiticus* and incubated. However, the material responsible for the

greenish-gold fluorescence we observed in corn may be identical to that causing "bright greenish-yellow" fluorescence in cotton fibers (9,10). Evidence indicates that the fluorescing substance in cotton fibers is formed by a heat-labile enzyme in the living plant that oxidizes kojic acid produced concurrently with aflatoxin by *A. flavus*. Because the rice and wheat we inoculated and incubated had been autoclaved, the fluorescing substance described in cotton fibers could not have formed. We are now attempting to establish a relationship between the greenish-gold fluorescence we observed in corn and the bright greenish-yellow fluorescence found in cotton fibers and other living plant tissues.

Particles from BCFM could not always be identified as corn and could have been some other commodity. For example, a moldy peanut with 1,120 p.p.b. B_1 was found in the grade U.S. No. 3 sample that assayed 25 p.p.b. B_1 (4). Fluorescing particles contained 240 to 54,900 p.p.b. aflatoxin B_1 (Table I).

The greenish-gold fluorescing material in damaged kernels that did not go through the 12/64 sieve appeared to be located next to the germ, except for one

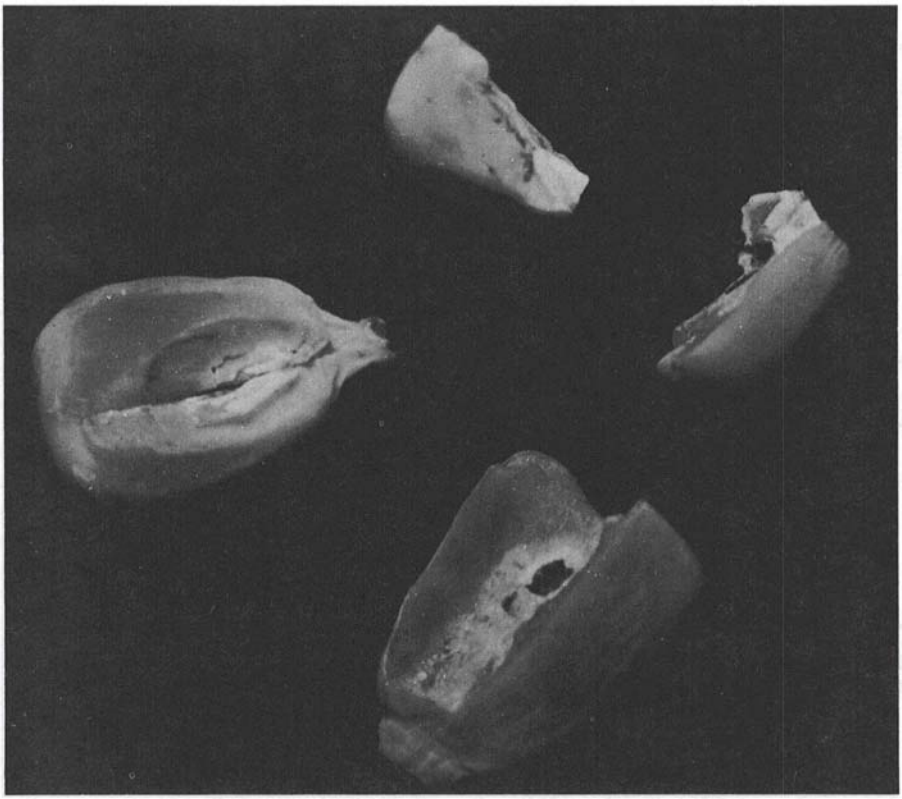

Fig. 2. Kernels and pieces of corn kernels with greenish-gold fluorescence under ultraviolet light (365 nm.).

TABLE I. AFLATOXIN B_1 PRESENT IN PARTICLES AND DAMAGED CORN KERNELS EXHIBITING FLUORESCENCE[a]

Grade U.S. No.	Aflatoxin B_1 in Total Sample p.p.b.	Particles from BCFM[b]		Damaged Corn Kernels[c]	
		Weight extracted g.	Aflatoxin B_1 p.p.b.	Weight extracted g.	Aflatoxin B_1 p.p.b.
1	0.7	None	...	0.104	88,500
2	47	0.278	5,640	0.139	5,320
3	25	0.07	54,900	0.030	2,080
		0.158	33,100		
3	18	None	...	0.246	1,060
3	15	0.063	956	0.534	8,090
4	16	0.306	6,650	0.648	284
4	12	0.002	ND[d]	None	...
4	<6	None	...	0.189	285
5	35	0.196	6,440	1.22	10,300
SG[e]	25	0.529	240	None	...
SG	8	0.171	2,900	0.123	101,000

[a]Greenish-gold glow under a 365-nm. ultraviolet light.

[b]Material that passed through a 12/64 grain-grading sieve.

[c]Damaged kernels that would not go through a 12/64 grain-grading sieve.

[d]ND = not detected. Considering the size of the sample extracted, there would have to be 3,000 p.p.b. to be detected.

[e]SG = sample grade.

kernel in which contamination had spread throughout. Kernel parts which fluoresced were structurally weakened and tended to crumble easily. Levels of 284 to 101,000 p.p.b. B_1 were detected in damaged kernels (Table I). The ground sample of grade U.S. No. 1 contained 0.7 p.p.b. B_1 (average of four values ranging from 0 to 1.5 p.p.b.). From the unground portion, a kernel with 88,500 p.p.b. B_1 was removed. If this portion had been the one ground originally and blended, the resulting meal would have analyzed 9 p.p.b. B_1. Similarly, if the kernel assaying 101,000 p.p.b. B_1 had fallen into the portion of corn to be ground, the assay would have shown at least twice as much B_1 to be present. The problems involved in obtaining a representative sample of corn, as discussed by Johnson et al. (11), are understandable.

Aflatoxin was not detected in any of the whole undamaged kernels separated by hand from material that did not pass through the 12/64 sieve. Results from two other types of corn selected from this material for assay are shown in Table II. Three of the seven samples of large broken pieces of corn contained aflatoxin B_1, one at a higher concentration (16 p.p.b.) than was detected in the original sample (<6 p.p.b.). Three samples of whole corn with mold damage and cracked kernels contained B_1 (1.5 to 8 p.p.b., all levels less than in the original sample).

The BCFM from available unground portions of aflatoxin-positive samples from previous studies was assayed for the presence of the mycotoxin (Table III). Seven samples had higher levels of B_1 in the BCFM than was determined in the total sample; two, about the same levels of toxin in both; and four, less in the BCFM than in the total sample. Assuming that the level of B_1 in the BCFM analyzed alone was the same as in the BCFM of the original total sample, we calculated the

TABLE II. AFLATOXIN CONTAMINATION IN DAMAGED CORN[a]

Grade U.S. No.	Aflatoxin B_1 in Total Sample p.p.b.	% in Sample	Hand-Picked Broken Pieces: Aflatoxin B_1 p.p.b.	Hand-Picked Broken Pieces: Aflatoxin B_1 % in total sample	Whole Kernels with Mold and Cracks: % in Sample	Whole Kernels with Mold and Cracks: Aflatoxin B_1 p.p.b.	Whole Kernels with Mold and Cracks: Aflatoxin B_1 % in total sample
3	25	4.5	12	2.2	18.8	8	5.8
3	18	5.9	4	1.2	18.3	2	1.5
3	12	3.8	ND[b]	0	13.1	ND	0
4	12	5.3	ND	0	25.6	ND	0
4	9	4.2	ND	0	21.0	ND	0
4	<6	4.3	16	>15	19.5	1.5	>5
SG[c]	25	8.8	ND	0	25.8	ND	0

[a]Whole corn and pieces that would not go through a 12/64 grain-grading sieve.

[b]ND = not detected.

[c]SG = sample grade.

TABLE III. AFLATOXIN B_1-CONTAMINATION IN BCFM[a] FROM COMMERCIAL CORN THAT CONTAINED AFLATOXIN

Grade U.S. No.	Aflatoxin B_1: Total sample p.p.b.	Aflatoxin B_1: BCFM p.p.b.	BCFM in Sample %	Aflatoxin B_1 % of Total in BCFM
1	0.7	94	1.6	100[b]
2	47	126	3.9	10
3	25	241	6.8	66
3	18	3	3.6	1
3	15	68	2.2	10
3	12	8	4.7	3
4	16	70	5.0	22
4	12	ND[c]	6.4	0
4	9	ND	5.6	0
4	<6	5	4.5	>4
5	35	122	5	17
SG[d]	25	5	31.5	20
SG	8	20	12.7	32

[a]Material that passed through a 12/64 grain-grading sieve.

[b]Assuming the same level of contamination in BCFM of original total sample.

[c]ND = not detected.

[d]SG = sample grade.

percentage of toxin in BCFM. In two samples, most of the aflatoxin B_1 was in the BCFM, but B_1 was not always concentrated in this part of a corn sample. As seen before, damaged kernels and large pieces of corn that do not pass through a 12/64 sieve can be highly contaminated with B_1.

The BCFM was separated from 20 samples of corn in which aflatoxin B_1 had not been detected previously, and each was analyzed for the presence of toxin (Table IV). Three samples of BCFM contained B_1 in levels of 3 to 20 p.p.b.; and if the BCFM were the only contaminated material in the whole sample of corn, the levels would be below the sensitivity limit (1 p.p.b.) of the assay. High levels of contamination were not detected in BCFM from aflatoxin-free corn; furthermore, most cleaning procedures remove BCFM.

TABLE IV. AFLATOXIN B_1 IN BCFM[a] FROM COMMERCIAL CORN IN WHICH B_1 HAD NOT BEEN DETECTED

Grade U.S. No.	BCFM	
	% in Corn sample from corn-graders' report	Aflatoxin B_1 p.p.b.
2	2.4	20
2	2.6	ND[b]
2	2.9	ND
2	2.5	ND
2	2.7	ND
3	3.9	3
3	3.5	ND
3	3.3	ND
3	3.6	ND
3	3.5	ND
3	3.4	ND
4	5.0	6
4	3.7	ND
4	4.1	ND
4	4.0	ND
4	4.6	ND
5	5.0	ND
5	6.6	ND
SG[c]	31.0	ND
SG	5.5	ND

[a]Material that passed through a 12/64 grain-grading sieve.

[b]ND = not detected.

[c]SG = sample grade.

CONCLUSIONS

The association of greenish-gold fluorescence with the presence of aflatoxin B_1 in pieces and kernels of corn may be one means of locating corn that is contaminated with toxin—particularly at levels of more than 10 p.p.b. When one kernel containing 90,000 to 100,000 p.p.b. in 1 kg. of corn represents an aflatoxin contamination of 10 p.p.b., we realize how difficult it is to obtain representative samples for assay. The greenish-gold fluorescence we observed in corn may be the same as the bright greenish-yellow described in cotton fibers by Marsh et al. (9,10). Our results show that aflatoxin could be concentrated in BCFM, in a few kernels, or in only pieces of kernels. Removal of BCFM or dockage by physical sorting according to size would not successfully eliminate aflatoxin from all corns. However, cleaning processes, including blowers as well as sieves, might remove the greater part of B_1, because contaminated kernels and pieces of kernels shatter easily.

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