Aflatoxin: Distribution in Contaminated Corn[1]

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ABSTRACT

Individual kernels with the bright greenish-yellow fluorescence associated with the presence of aflatoxin and kernels without the fluorescence from contaminated white and yellow corn samples were assayed individually. Some kernels having a greenish-yellow fluorescence under the seed coat had to be split open before the typical fluorescence became visible. All fluorescing kernels contained aflatoxin; none of the nonfluorescing ones did. The following fractions from 10 lots of aflatoxin-contaminated corn were analyzed: a) fluorescing intact kernels and pieces; b) kernels with fluorescence visible under the seed coat; c) damaged, cracked, or discolored kernels, or in any combinations; and d) outwardly sound kernels. Aflatoxin was in all the listed fractions, but amounts and distribution of the toxin in each depended on the lot of corn. Aflatoxin was found in the outwardly sound kernel portion because some kernels with fluorescence under the seed coat do not have an abnormal appearance even under ultraviolet light (365 nm.). Fluorescing particles were observed in ground meals of outwardly sound kernels.

Finding aflatoxin in southern corn from the crop years 1969 and 1970 (1) led to more interest in rapid detection methods and procedures to remove the toxin if present. A bright greenish-yellow (BGY) fluorescence under ultraviolet light (365 nm.) associated with the presence of aflatoxin (2) has been used by industry for the rapid examination of corn. The corn fluorescence is related to that in cotton fibers that Marsh et al. (3) first reported. The BGY fluorescence is not caused by aflatoxin; rather, it probably results from a product of the action of plant enzymes on kojic acid. Kojic acid is made by *Aspergillus flavus*, the same mold that produces aflatoxin.

This fluorescence makes possible a rapid means of identifying lots of corn that may contain aflatoxin. It also enabled us to study further the distribution of the toxin in contaminated corn. We had examined some lots of yellow corn with low levels of aflatoxin (1 to 47 p.p.b. B_1), but within the past year white and yellow corn containing higher levels (61 to 325 p.p.b. B_1) became available. Our findings are reported here on the distribution of aflatoxin among individual kernels, as well as in fractions from these more highly contaminated corns. One purpose of our study was to establish more definitely the relationship between the occurrence of the BGY fluorescence and the presence of aflatoxin. Another purpose was to provide knowledge on aflatoxin content in individual kernels in contaminated lots as a basis for selecting or evaluating sampling procedures. Still another was to relate toxin distribution in contaminated lots to the likelihood of success in detoxification by physical separation.

MATERIALS AND METHODS

Fractionation of Corn Samples

Ten 1- to 2-kg. samples of white and yellow corns known to be contaminated

[1]Presented at the 57th Annual Meeting, Miami Beach, Oct.-Nov. 1972. Contribution from the Northern Regional Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Ill. 61604. The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms similar products not mentioned.

were collected and graded. Samples available to us were in every grade except U.S. No. 2. Each sample of unground corn was shaken on a grain-grading 12/64 round-hole sieve (4) to remove broken corn-foreign material (BCFM)[2] which was weighed and analyzed.

From each of two samples, 100 kernels, picked at random, were fractionated into four categories listed in Fig. 1 and analyzed individually. All fluorescing pieces and kernels and all kernels with fluorescence visible under the seed coat were selected from the remainder of these two samples for individual kernel assay. The remaining portions of these samples were divided into two fractions–discolored, cracked, and damaged kernels and outwardly sound kernels. The two fractions were ground separately and analyzed.

Kernels from the other eight samples were not analyzed individually but separated by hand selection into: a) fluorescing kernels; b) kernels with fluorescence visible under the seed coat; c) damaged, cracked, or discolored kernels; and d) outwardly sound kernels (Fig. 1). From an ungraded, contaminated sample 32 kernels with fluorescence visible under the seed coat and 28 apparently sound kernels were removed for analysis of individual kernels.

Analytical Methods

BCFM, fluorescing kernels, and kernels with fluorescence visible under the seed coat were ground and simultaneously extracted in a Waring Blendor for analysis by the method recommended for corn (5,6). An improved thin-layer chromatographic developer (7) and densitometric quantitation were used. Each corn sample was mixed with water, diatomaceous earth, and chloroform in the Waring Blendor for 5 min. before filtering. Because the two fractions containing outwardly sound kernels and damaged, cracked, or discolored kernels were large, they were ground in a Raymond hammer mill to pass a No. 14 sieve and blended in a twin-shell blender. Of each blended, ground fraction 50 g. was extracted. Extracts were partially purified by column chromatography before thin-layer chromatography.

Kernels to be assayed individually were crushed with pliers, weighed, placed in small vials, and steeped 48 hr. in chloroform (2 to 3 ml.) and a drop of water (2). A second extraction removed all the toxin that could be removed in this manner.

Confirmatory Tests

The presence of aflatoxin B_1 was confirmed by formation of water and acetate adducts (5, p. 436).

RESULTS AND DISCUSSION

The BGY fluorescence under ultraviolet light (365 nm.) was one of the properties used to visually select kernels and fractions for assay. BGY fluorescence can occur in three general forms: a) fully visible on the exterior of kernels, either whole or broken, and in foreign material; b) subsurface BGY which is detected as a dull gold color under the seed coat, usually in the germ area, and becomes fully visible when the kernel is broken open; and c) fully hidden internal fluorescence

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

which is detected only by breaking the kernel. The recognition of subsurface fluorescence (form b) often is difficult because of other fluorescences on some corn kernels and the low intensity of the glow. Experience and visual acuity of the observer become critical in this case. Cracking or coarse grinding of samples before ultraviolet inspection is highly recommended for critical examination even though most samples we have encountered with aflatoxin levels above about 20 p.p.b. have contained at least one or two kernels or fragments with visible BGY in a 1-lb. portion.

Individual Kernels

Single-kernel analyses on two of the corn samples demonstrate the occurrence of the aflatoxin at very high but variable levels in a small percentage of the kernels and the resulting effect that sample size can have on the analysis of the entire sample. Of the 100 kernels taken at random from the U.S. No. 1 corn sample containing 88 p.p.b. aflatoxin B_1, only three kernels had BGY fluorescence either before or after cracking, and they contained B_1 (Table I). Aflatoxin was not detected in the remaining 97 kernels, which were not BGY fluorescent, although the analytical method is capable of detecting about 10 p.p.b. B_1 in a single kernel. The three contaminated kernels would account for only 12 p.p.b. of B_1 in the 100-kernel sample or about 15% of the level in the 2-kg. sample. When all kernels having either external or visible subsurface fluorescence were selected from the whole 2-kg. sample (approximately 6,000 kernels) of the same U.S. No. 1 corn and

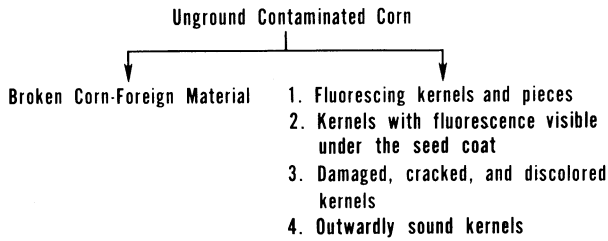

Fig. 1. Separation of corn into fractions.

TABLE I. AFLATOXIN B_1 IN 100 KERNELS SELECTED AT RANDOM FROM CONTAMINATED CORN

Description of Kernels	U.S. No. 1 88 p.p.b. aflatoxin B_1		U.S. Sample Grade 145 p.p.b. aflatoxin B_1	
	Number of kernels	Aflatoxin B_1 p.p.b.	Number of kernels	Aflatoxin B_1 p.p.b.
Fluorescent[a]	2	12, 14	3	408,294, 3,500
Fluorescence[a] visible under seed coat	1	1,155	1	125
Discolored and damaged	13	ND[b]	26	ND
Outwardly sound	84	ND	70	ND

[a]Bright greenish-yellow (BGY) fluorescence under 365 nm. ultraviolet light.
[b]None detected.

TABLE II. AFLATOXIN LEVELS IN INDIVIDUAL KERNELS AND LARGE PIECES OF CORN (2-kg. SAMPLES)

Levels Aflatoxin B_1 p.p.b.	Number of Fluorescing[a] Kernels and Pieces		Number of Kernels with Fluorescence[a] Visible Under Seed Coat		
	U.S. No. 1 (88 p.p.b. B_1)	U.S. Sample Grade (145 p.p.b. B_1)	U.S. No. 1 (88 p.p.b. B_1)	U.S. Sample Grade (145 p.p.b. B_1)	Ungraded (200 p.p.b.)
>0-100	2	16	8	12	7
101-1,000	1	11	9	10	6
1,001-5,000	2	11	5	1	6
5,001-10,000	1	7		4	3
>10,000	2	5	4	6	5
	Individual Levels in Kernels Containing >10,000 p.p.b.				
	24,800	13,300	18,700	13,500	13,800
	51,500	15,000	122,000	32,500	15,500
		22,400	187,000	35,300	17,700
		40,200	207,000	32,800	20,300
		84,100		53,100	37,900
				79,800	

[a]BGY fluorescence under 365 nm. ultraviolet light.

TABLE III. CONCENTRATION OF AFLATOXIN B_1 (p.p.b.) IN FRACTIONS OF CORN SAMPLES

U.S. No.	Total Corn Sample[a]	BCFM[b]	Fluorescing[c] Kernels and Pieces	Fluorescence[c] Visible Under Seed Coat	Damaged, Cracked and Discolored Kernels	Outwardly Sound Kernels
1	88	1,090	10,800	16,800	1,530	9
3	144	43	876	3,260	259	44
3	150	228	3,190	7,000	157	38
4	116	261	2,510	3,000	61	53
4	132	317	2,810	3,140	162	31
4	276	451	2,250	2,910	252	68
5	61	23	43,400	36,600	14	46
Sample Grade	61	198	2,890	65	9	3
Sample Grade	145	44	5,110	7,730	250	60
Sample Grade	321	689	3,220	4,220	50	250
Average activity		206	2,250	3,020	126	56

[a]Total corn samples weighed 1,000 to 2,000 g.
[b]BCFM = broken corn-foreign material.
[c]BGY fluorescence under 365 nm. ultraviolet light.

TABLE IV. DISTRIBUTION OF AFLATOXIN B_1 IN CORN FRACTIONS

U.S. No.	Type of Corn[a]	Weight of Sample Examined kg.	Total Sample Aflatoxin B_1 p.p.b.	Aflatoxin B_1 in: BCFM Aflatoxin B_1 p.p.b.[b]	BCFM Total %	Fluorescing[c] Kernels and Pieces Aflatoxin B_1 p.p.b.	Fluorescing[c] Kernels and Pieces Total %	Fluorescence[c] Visible Under Seed Coat Aflatoxin B_1 p.p.b.	Fluorescence[c] Visible Under Seed Coat Total %	Discolored and Damaged Kernels Aflatoxin B_1 p.p.b.	Discolored and Damaged Kernels Total %	Outwardly Sound Kernels Aflatoxin B_1 p.p.b.	Outwardly Sound Kernels Total %	Origin
1	W	2.0	88	<1	<1	10	11	59	67	10	11	9	10	Arizona
3	Y	1.0	144	2	1	7	5	51	35	51	35	33	23	South Carolina
3	W	2.0	150	8	5	32	21	38	25	47	31	25	17	Missouri
4	W	2.0	116	7	6	30	26	27	23	16	14	36	31	Texas
4	W	2.0	132	14	11	27	20	33	25	37	28	21	16	Texas
4	W	2.0	276	8	3	59	21	95	34	70	25	44	16	Missouri
5	Y	1.0	61	2	3	7	11	15	24	2	3	35	57	Virginia
Sample Grade	Y	2.0	61	48	79	3	5	5	8	3	5	2	3	South Carolina
Sample Grade	W	2.1	145	4	3	32	22	33	23	29	20	47	32	Alabama
Sample Grade	W	2.0	321	10	3	37	12	123	38	21	6	130	40	Missouri
Average					12		16		31		19		25	

[a] W = White; Y = yellow.
[b] All aflatoxin values are calculated as p.p.b. of the total sample weight.
[c] BGY fluorescence under ultraviolet light (365 nm.)

analyzed, seven of them accounted for 65 p.p.b. of B_1 in the entire sample, or about 75% of the total.

Somewhat similar results were obtained with the U.S. Sample Grade (SG) corn containing 145 p.p.b. aflatoxin B_1. Four of the 100 kernels selected at random exhibited BGY fluorescence and they contained aflatoxin at levels that would account for 40 p.p.b. B_1 in the 100 kernels (Table I), or about 28% of level in the entire 2-kg. sample. The 96 kernels that did not fluoresce, even when broken open, did not contain detectable amounts of aflatoxin. For a large sample of this SG corn, a complete examination revealed that about one-fourth of the kernels were contaminated with aflatoxin.

These results on 100-kernel samples indicate that sample size is extremely important for aflatoxin analysis in corn. The data are not sufficient to provide a basis for recommendations concerning optimum sample size. For such purposes distribution data on large samples, each having several thousand kernels, would have to be obtained by analysis of every kernel in the sample.

The 32 kernels, selected from an ungraded sample containing 200 p.p.b. aflatoxin B_1 because they looked as though there was BGY fluorescence under the seed coat, did contain B_1. The levels were from 7 to 37,900 p.p.b. (Table II).

Kernels with BGY fluorescence fully visible or visible under the seed coat from two graded samples were assayed individually and the results are also shown in Table II. Levels of aflatoxin ranged from 3 to 84,000 p.p.b. in kernels and pieces of corn with fully visible fluorescence. It was impossible to judge from the size or intensity of the fluorescent area what level of toxin might be present. One of the kernels with fluorescence visible under the seed coat had 207,000 p.p.b. B_1. The endosperm of kernels with highest levels of aflatoxin had become powdery and fluorescent.

Distribution in Fractions

Concentrations of aflatoxin in the different fractions of corn varied from one lot to another (Table III). Even though the BCFM in U.S. No. 1 corn contained 1,090 p.p.b. B_1, this amount was not significant because there was so little BCFM (0.001%) in that lot of corn. Fractions containing fluorescing kernels and pieces had from 876 to 43,400 p.p.b. aflatoxin B_1 with an average level of 2,250 p.p.b. The average and range in concentration of aflatoxin in kernels and pieces fluorescing greenish yellow have been a concern of industry when using the fluorescence to determine whether a lot of corn might contain levels of mycotoxin in excess of FDA guidelines. The highest levels of aflatoxin B_1 were found in fractions containing kernels with fluorescence visible under the seed coat. All of the fractions of outwardly sound kernels that could not be distinguished by any visible characteristics, such as damage, color, or fluorescence, contained aflatoxin B_1 – one as much as 250 p.p.b. When these fractions were ground, particles with the BGY fluorescence could be seen in the meal. These particles would have come from kernels having hidden internal fluorescence.

The variations in distribution of aflatoxin indicate that physical separation would not be suitable for decontamination of corn. Analysis of the fractions separated from different lots of corn indicated that any one of the fractions could contain most of the aflatoxin in the lot (Table IV). The BCFM of only one fractionated lot, the SG corn containing 61 p.p.b. B_1, had most of the aflatoxin. A cleaning operation would remove 79% of the toxin from this lot of corn and would

lower levels to 10 to 15 p.p.b. B_1. Fractions of fluorescing kernels and large pieces of corn accounted for 5 to 26% of the total B_1 in the 10 lots of corn; electronic sorting to remove glowing particles would not remove appreciable amounts of aflatoxin from these lots. Whole kernels with the BGY fluorescence detectable under the seed coat would not be removable by physical cleaning or electronic sorting. In every lot of corn except one, there were significant amounts of aflatoxin B_1 in the kernels with fluorescence visible under seed coat. In one, for example, 67% of the activity was in these kernels. Discolored and damaged kernels from the 10 lots contained 3 to 35% of the total aflatoxin. Hand sorting to separate BCFM, fluorescing kernels and pieces, kernels with visible fluorescence under the seed coat, and damaged and discolored kernels from sound kernels removed enough aflatoxin in two lots to lower levels of aflatoxin B_1 below 10 p.p.b.; the apparently sound kernels in the other eight lots had more than 20 p.p.b. B_1.

CONCLUSION

All individual kernels having a characteristic BGY fluorescence either externally or internally have contained aflatoxin as shown by analyses in a previous study (2) and in this study. The level of aflatoxin in a lot of corn cannot be predicted from the number of kernels and fragments with BGY fluorescence, although small numbers may be associated with low levels. Much of the fluorescent pigment associated with aflatoxin may be under the seed coat. In some cases such fluorescence is detectable as a glow under the seed coat; in others it is detectable only by breaking open the kernel. Cracking a sample before visual inspection under ultraviolet light to identify possibly contaminated corn is advisable. Because aflatoxin may occur at high levels in a few corn kernels in a lot, comprehensive sampling procedures are necessary to achieve a valid analysis. The toxin may be present in all fractions of corn – BCFM, fluorescing kernels, kernels with fluorescence visible under the seed coat, damaged and discolored kernels, and even apparently sound kernels – but in varying ratios depending on the lot of corn. In general, most aflatoxin-contaminated kernels would not be removed by physical separation, including electronic sorting devices.

Acknowledgments

We thank O. L. Brekke for collecting some of the lots of corn. Anna M. Jepson assisted in sorting corn into various fractions.

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[Received September 24, 1973. Accepted December 7, 1973]