AFLATOXIN OCCURRENCE IN SOME WHITE CORN UNDER LOAN, 1971. II. EFFECTIVENESS OF RAPID TESTS IN SEGREGATING CONTAMINATED CORN[1]

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ABSTRACT

Rapid screening tests for the detection of aflatoxin were made on truckloads of white corn (1971 crop) under Commodity Credit Corporation as they arrived at an elevator in Missouri. Samples to be tested for the presence or absence of aflatoxin were selected on the basis of an inspection with ultraviolet light (365 nm) for bright greenish-yellow fluorescence associated with the presence of toxin. Results of the rapid field method were compared with those from quantitative determination and found to be effective, for the most part, in identifying aflatoxin-containing corn.

In August and September 1972, the Commodity Credit Corporation (CCC) asked farmers from seven counties in southeastern Missouri to deliver 1971 white corn under loan to an elevator leased in the area. There was some concern about possible aflatoxin contamination of the white corn in that area because of the Food and Drug Administration's (FDA) seizure of aflatoxin-tainted corn meal (1). Also a limited survey of white and yellow corn in the South from crop years 1969 and 1970 had indicated a 20% incidence of lots of corn containing more than 20 ppb aflatoxin (2). The Northern Regional Research Laboratory (NRRL) designed a program of rapid screening to separate aflatoxin-contaminated corn from toxin-free corn as farmers brought their truckloads to the elevator. Studies were also made on the incidence and levels of aflatoxin in the corn by quantitative analysis (3), factors related to occurrence of toxin, correlation of bright greenish-yellow (BGY) fluorescence in corn with the presence of aflatoxin, and the fungi occurring in corn.

MATERIALS AND METHODS

Sample Collection

As truckloads (200–400 bu capacity) of CCC corn were delivered to the elevator in southeastern Missouri, 10-lb probe samples were taken according to a standard pattern and divided in half in a Boerner divider (4). One 5-lb portion went to the Missouri State Inspection Service and the other was used in the rapid screening test at the elevator.

Fluorescent Tests. The 5-lb samples from probes were examined at the elevator under high-intensity ultraviolet light (365 nm) from a Blak ray lamp (Model B, 100-A) for the BGY fluorescence associated with the presence of aflatoxin in corn. The sample was spread out in a square box 3 ft × 3 ft for inspection, and the lamp was held about 10 in. from the sample. Corn samples

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that had BGY fluorescence were tested by the rapid screening method for aflatoxin (sensitivity 10 ppb); those that did not were coarse ground or cracked in a Straub disc mill (Model 4-E) before a second inspection in the same manner for fluorescence (Fig. 1). If BGY fluorescence was detected on uv inspection of cracked corn, these samples were also tested for aflatoxin by the rapid screening method. Corn samples that did not have BGY fluorescence even when ground were considered to be aflatoxin-free and the truckload was placed in a "clear" bin containing presumably aflatoxin-free corn (Fig. 1). Ultraviolet inspection for BGY fluorescence on either whole or cracked corn took about 5 min.

Rapid Screening Method. Corn samples that had BGY fluorescence either in the whole or cracked sample were analyzed at the elevator in Missouri by the minicolumn screening method developed by Pons *et al.* (5) and modified by Shannon *et al.* (6). An important decision had to be made on whether to grind and blend the entire 5-lb corn samples to obtain the 50-g sample required for the minicolumn test. All operations had to be carried out while farmers waited with their trucks, and grinding and blending would have been quite time-consuming (15–20 min per sample). Instead, it was decided to collect a 50-g sample by selecting and including as much of the BGY fluorescing material as possible from 5 lb of corn, together with the amount of nonfluorescing material needed to give 50 g. This selected sample would be expected to contain a higher concentration of aflatoxin than the total 5 lb since previous studies (7) indicated that BGY fluorescing material in a sample always contained aflatoxin and usually at rather high levels while many nonfluorescing undamaged kernels in the same sample were aflatoxin-free. Therefore, selection of the sample to be extracted and tested undoubtedly increased the sensitivity of the minicolumn test for detecting aflatoxin in the lot sample. For the sample actually extracted, expected test sensitivity is 10 ppb aflatoxin.

The 50-g corn samples were extracted in a blender with water:acetone (15:85 v/v). Extracts were filtered, treated with ammonium sulfate to precipitate impurities, and filtered. Toxin was partitioned from the partially purified extract

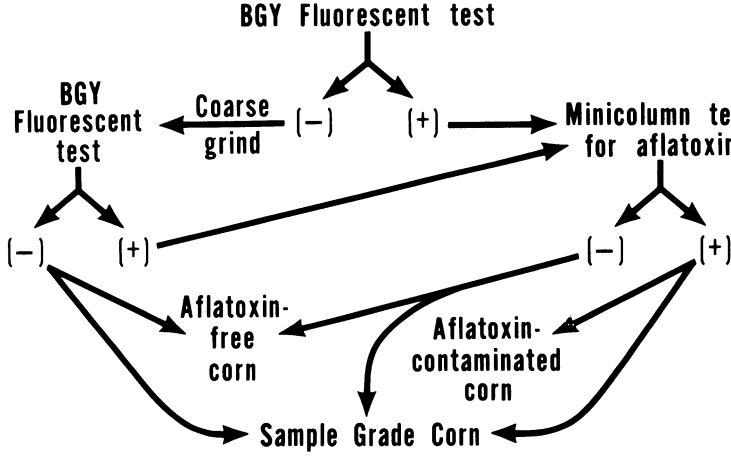

Fig. 1. Tests done at elevator in southeastern Missouri.

with benzene in a separatory funnel. The benzene solution was placed on a minicolumn for development as described (5,6). The preparation of the benzene solution took 13 min at the elevator, and development of columns took 5–7 min. Results were reported as positive or negative or, if columns seemed to indicate levels of aflatoxin greater than 10 ppb, results were rated based on intensity of blue fluorescence on columns as 2 or 3 with 1 being just positive.

Laboratory Analyses

After it was decided in which bin to store a load of corn, aflatoxin-free (clear), aflatoxin-contaminated, or Sample Grade (Fig. 1), a 50-lb sample was taken continuously from the stream of corn as each truck was unloaded and from this a 10-lb subsample was taken with a secondary sampler (3). When the 10-lb samples arrived at NRRL they were examined for BGY fluorescence, both as whole and as cracked kernels (3). All samples were then ground and blended to obtain 50-g samples for analysis. The samples were analyzed by rapid thin-layer chromatographic and quantitative methods recommended as Official First Action by the Association of Official Analytical Chemists (8). Limit of detection is 1 to 3 ppb aflatoxin for these methods. Identity of aflatoxin was confirmed by the formation of water adducts (9).

RESULTS AND DISCUSSION

There were 1283 truckloads (200–400 bu capacity) of white corn from 77 loans delivered to an elevator in southeastern Missouri within a period of 6–7 wk. From 30 to 40 truckloads of corn per day were delivered and tested for aflatoxin. As farmers waited, the BGY fluorescent test and then minicolumn test on BGY-positive loads were completed on probe samples. If no BGY fluorescing material was detected in either the whole or the cracked kernel sample, the load of corn was assumed to be aflatoxin-free and stored in the "clear" bin without doing a minicolumn test on it. Samples (420) that had BGY fluorescing material were further tested for the presence of aflatoxin by the minicolumn test using a 50-g sample selected to include the BGY fluorescing material from 5 lb of corn (5,6). If no aflatoxin was detected, the truckload (782 loads) of corn was placed in the aflatoxin-free storage bin; if positive (326 loads), in the aflatoxin-contaminated storage bin. All U.S. Sample Grade corn (175 loads) was stored separately because corn of this quality cannot be sold without a discount regardless of aflatoxin content (Fig. 1).

The success of the rapid screening test on 5-lb probe samples in southeastern Missouri in detecting aflatoxin contamination was judged by comparison with the results of the quantitative analysis at NRRL on 10-lb continuous samples. Aflatoxin levels as determined by the quantitative AOAC method in white corn samples judged at the elevator to be positive by rapid screening tests are shown in Table I. Some differences in results could be attributed to differing sampling procedures—probe and continuous.

As mentioned, samples (50 g) at the elevator were selected to contain as much BGY fluorescence as possible in the 5-lb corn sample. These specially selected samples were expected to increase the sensitivity of the minicolumn test, and they generally did. Usually the minicolumn test on a ground blended sample has a sensitivity of about 10 ppb aflatoxin.

Of samples from 367 truckloads of corn giving positive minicolumn tests at the elevator in southeastern Missouri, 100 (27%) loads had no detectable aflatoxin (less than 1 to 3 ppb) at NRRL where 10-lb ground and blended samples of the loads were studied. There were 64 (17%) loads of corn with positive minicolumn tests at the elevator that contained less than 10 ppb aflatoxin at NRRL and, therefore, were below the detection limit of the minicolumn test. If an assumption of no sampling error is made, these two groups comprising 164 of the samples which were aflatoxin-positive by the minicolumn test at the elevator probably would have been judged negative if the entire 5-lb samples had been

TABLE I
Distribution of Aflatoxin Levels[a] by Grades in Corn Samples Positive by Minicolumn Tests[b]

Grade, U.S. No.	Total	Total Aflatoxin, ppb					
		ND[c]	<10	10–19	20–29	30–100	>100
None	1	0	0	0	0	1	0
1	90	35	19	9	9	15	3
2	134	41	27	31	12	21	2
3	30	8	2	8	4	5	3
4	35	3	4	8	0	11	9
5	36	4	9	5	3	12	3
SG	41	9	3	2	3	16	8
Total	367	100	64	63	31	81	28

[a]As determined by method adopted Official First Action by AOAC for corn, sensitivity = 1-3 ppb aflatoxin.
[b]Done at elevator in southeastern Missouri.
[c]ND = not detected.

TABLE II
Distribution of Aflatoxin Levels[a] by Grade in Corn Samples Negative by Rapid Screening Tests[b]

Grade, U.S. No.	Total	Total Aflatoxin, ppb					
		ND[c]	<10	10–19	20–29	30–100	>100
1	281	245	26	7	2	1	0
2	343	313	13	11	4	2	0
3	77	65	12	0	0	0	0
4	37	29	3	1	1	3	0
5	44	34	3	3	3	1	0
SG	134	103	15	8	4	3	1
Total	916	789	72	30	14	10	1

[a]As determined by method adopted Official First Action by AOAC for corn, sensitivity = 1–3 ppb aflatoxin.
[b]Minicolumn and fluorescent tests done at elevator in southeastern Missouri.
[c]ND = not detected.

ground and blended to get 50-g representative samples for the rapid test. These samples, 44% of the positive samples at the elevator, would include the 100 loads in which aflatoxin was not detected and the 64 loads containing less than 10 ppb. Of the 367 samples giving a positive minicolumn test, 140 (38%) had more than 20 ppb aflatoxin, the present FDA action guideline (10). In truckloads of corn aflatoxin-positive by the minicolumn tests as carried out in southeastern Missouri, the poorest quality of corn (U.S. Grades 4, 5, and SG) tended to have the highest levels of aflatoxin.

Statistical evaluation of the means for aflatoxin levels in all loads from each of the loans delivered indicated that level of aflatoxin was related to grade but the correlation coefficient was low (0.29 for B-1; 0.27 for B-2). For example, contrary to the trend, three of the loads containing more than 100 ppb total aflatoxin were in U.S. No. 1 Grade.

In the 916 truckloads of corn that did not contain aflatoxin by the rapid screening test at the elevator in southeastern Missouri, 789 (86%) were also aflatoxin-negative at NRRL (Table II) by quantitative analysis (limit of detection, 1 to 3 ppb). There were 72 (9%) containing less than 10 ppb aflatoxin. Of the loads of corn aflatoxin-negative at the elevator two-thirds were in U.S. Grades 1 and 2, the best quality corn. Of the 916 loads of corn 25 (3%) had more than 20 ppb aflatoxin as determined at NRRL. In 18 of the 25 loads, BGY fluorescence was not detected in either the whole or cracked 5-lb sample at the elevator. These samples, therefore, were not analyzed by the minicolumn method. In the laboratory, BGY fluorescence was found in the continuous sample of all 25 truckloads. Of the 18 truckloads 14 had five or less BGY particles or kernels per 10 lb corn in the continuous sample. These 14 samples probably would have been missed at the elevator simply because of sampling error or the conditions under which rapid tests were done. The continuous sampling procedure should give a more representative sample than the probe. The other 4 loads with more than five BGY particles or kernels may have been missed for the

TABLE III
Distribution of Aflatoxin Levels[a] in Truckloads of 1971 White Corn Stored in Southeastern Missouri—by Storage Bin

Total Aflatoxin ppb	U.S. Sample Grade[b]	Aflatoxin-Free[c]	Aflatoxin-Contaminated[c]
None	112	686	91
<10	18	57	61
10–19	10	22	61
20–29	7	10	28
30–100	19	7	65
≥100	9	0	20
Total	175	782	326
Average aflatoxin content (ppb)	17	1	28

[a]As determined by AOAC method at NRRL.
[b]All Sample Grade corn was stored in one bin because of poor quality and regardless of aflatoxin content.
[c]As determined by rapid screening tests with BGY fluorescence and minicolumn.

same reasons. Five of the loads of corn containing more than 20 ppb aflatoxin at NRRL were reported to be aflatoxin negative by minicolumn tests carried out at the elevator after BGY material was detected.

The distribution of aflatoxin levels as determined on continuous samples from truckloads put in the three storage bins—aflatoxin-free (clear), aflatoxin-contaminated, and Sample Grade—are summarized in Table III. In the bin containing Sample Grade corn, 15% of the loads had more than 20 ppb aflatoxin. Assuming all trucks contained the same amount of corn, the average toxin level in the bin containing Sample Grade corn was 17 ppb. Marketability of this corn probably would be influenced more by its general poor quality than by the aflatoxin (17 ppb) contamination of the grain.

In the bin containing contaminated corn, the loads (326) averaged 28 ppb aflatoxin which is just above the present FDA guideline. Of the 326 truckloads of corn placed in the "contaminated bin," 28% were aflatoxin-free (less than 1–3 ppb aflatoxin) and 34% had levels greater than 20 ppb toxin. Of the 326 loads (excluding Sample Grade) giving positive minicolumn tests on probe samples at the elevator in southeastern Missouri, 151 (47%) had less than 10 ppb aflatoxin by quantitative determinations done at NRRL on continuous samples.

Most of the corn (71%) delivered in southeastern Missouri was identified as aflatoxin-free by the rapid screening test and placed in the appropriate storage bin. Analytical results indicated that 88% of the truckloads placed in the clear bin did not contain aflatoxin in levels (1–3 ppb toxin) detectable by the methods used. Only 2% of the loads in this bin contained more than 20 ppb aflatoxin. Once again assuming all loads of corn to contain the same quantity of grain, the overall average aflatoxin level was 1 ppb. Almost all the corn in this bin was U.S. Grades 1 and 2 and of high quality.

CONCLUSION

When rapid screening tests conducted at the elevator in southeastern Missouri and quantitative analyses done in the laboratory at NRRL are compared, differences in both sampling and assay methods must be considered. At the elevator, 5-lb probe samples of each truckload were examined; further tests were done only on samples in which BGY fluorescing material was detected in whole or cracked sample; 5-lb samples were not ground and blended to obtain a 50-g representative sample, but 50 g of corn was selected to contain as much BGY material as possible; and 50-g samples were assayed by the rapid minicolumn test that was qualitative and sensitive to 10 ppb aflatoxin. At NRRL, 10-lb continuous samples of each truckload were examined; each sample collected was analyzed; 10-lb samples were ground and blended to obtain a 50-g sample for analysis; and 50-g samples were quantitatively analyzed by the AOAC approved method that is sensitive to 1–3 ppb aflatoxin. The results indicate that the rapid screening tests carried out at the elevator in southeastern Missouri on white corn as delivered were effective, for the most part, in segregating contaminated corn from aflatoxin-free corn.

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