AFLATOXIN OCCURRENCE IN SOME WHITE CORN UNDER LOAN, 1971. III. ASSOCIATION WITH BRIGHT GREENISH-YELLOW FLUORESCENCE IN CORN[1]

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ABSTRACT

Studies were made on the relation between the occurrence in white corn samples from 1283 truckloads of Commodity Credit Corporation corn delivered at an elevator in southeastern Missouri of bright greenish-yellow (BGY) fluorescence under ultraviolet light (365 nm) and the presence and levels of aflatoxin. Although numbers of BGY fluorescing particles were related to levels of aflatoxin, the BGY fluorescent test could not be used to determine levels of toxin. Of the 10-lb whole-kernel corn samples having at least one BGY fluorescing corn particle or kernel, 55% contained measurable aflatoxin; 28% contained more than 20 ppb toxin, the present Food and Drug Administration guideline. The analytical method was sensitive to 1–3 ppb aflatoxin. Of the corn samples that had more than 20 fluorescing kernels and particles, 95% were contaminated with aflatoxin. Sixty-five per cent of them had more than 20 ppb toxin. Cracking corn before ultraviolet light (365 nm) inspection revealed more samples with BGY fluorescence, and 19% of these contained more than 1–3 ppb aflatoxin.

A bright greenish-yellow (BGY) fluorescence under ultraviolet light (365 nm) has been associated with the presence of either *Aspergillus flavus* or aflatoxin, or both, in cottonseeds and corn (1,2,3). The fluorescence is thought to result from the action of plant tissue enzymes on kojic acid formed by *A. flavus* concurrently with aflatoxin (1). A number of individual kernels showing BGY fluorescence, either externally or after crushing, and kernels showing no fluorescence from several contaminated lots of corn were analyzed for aflatoxin (4). Kernels with BGY fluorescence so far examined have contained aflatoxin; kernels that did not have BGY fluorescence did not. However, in a mixture containing only a few individual kernels or fragments with BGY fluorescence, fluorescing kernels may contribute so little aflatoxin that when the entire lot is ground and blended, it would not contain detectable or appreciable levels of toxin. Therefore, the presence of BGY-fluorescent material is only a presumptive indication of toxin.

The objective of the work reported here was to determine the limitations of the BGY fluorescent test as a rapid presumptive test for aflatoxin in corn. Our opportunity to study the correlation of BGY fluorescence with aflatoxin contamination came when the Commodity Credit Corporation (CCC) accepted delivery of white corn from the crop year 1971 under loan in southeastern Missouri. Because one lot of white corn originating in this area in 1971 had contained aflatoxin (5), it was suspected other corn might be contaminated.

Incidence and levels of aflatoxin have been determined for the 1283 truckloads of corn from 77 CCC loans (6). The effectiveness of the rapid screening tests on

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truckloads containing BGY fluorescence, completed in Missouri, in segregating aflatoxin-contaminated corn has also been reported (7). The correlation of results from quantitative analyses and a rapid screening test known as the minicolumn method (8) for aflatoxin with various inspections for BGY fluorescence under ultraviolet light (365 nm) is reported here.

MATERIALS AND METHODS

Sample Collection

As truckloads of CCC white corn were delivered to the elevator in southeastern Missouri, 10-lb samples were collected with grain probes or triers, as recommended by Grain Inspection Division, Agricultural Marketing Service, USDA. From 12 to 20 probes were required to collect 10 lb of corn. After each sample was split in half in a Boerner divider, 5 lb was used for rapid tests at the elevator and 5 lb for grading and inspection for BGY fluorescence by the Missouri State Inspection Service.

As all trucks were unloaded, 50-lb continuous samples were taken with a primary sampler. A secondary sampler split a 10-lb sample from the 50 lb (6). Each 10-lb portion of these continuous samples was used at NRRL for inspection for BGY fluorescence under ultraviolet light (365 nm) and for quantitative determination for aflatoxin.

Fluorescent Tests

At the elevator in southeastern Missouri, one of the 5-lb probe samples of white corn was inspected for BGY fluorescence under high-intensity ultraviolet light (365 nm) with a Blak Ray lamp (Model B, 100-A). The sample was spread out in a 3 by 3 ft square box for inspection, and the lamp was held about 10 in. from the sample. If no BGY fluorescence was observed, the 5-lb sample was cracked in a Straub disc mill (Model 4-E) before a second inspection for BGY fluorescence. A rapid minicolumn assay (8) was then conducted on those samples containing BGY fluorescence.

Examination for BGY fluorescence by the Missouri State Inspection Service was done in the same manner as it was at the elevator but included an exact weight on the whole sample and the weight of all kernels and particles with fluorescence from the unground sample. Results of the test were reported as grams of BGY fluorescing material per 5 lb of corn.

The 10-lb continuous samples sent to NRRL were also examined for BGY fluorescence when poured out into a flat pan under ultraviolet light (365 nm) with a Blak Ray lamp. The lamp was 10 in. from the sample. It took 10 min to count up to 20 BGY positive kernels and particles in 10 lb of corn. If no BGY-fluorescing particles were observed, the samples were ground, blended, and assayed directly. However, after 76 samples were treated, we decided to crack or coarse grind for a second inspection. Therefore, corn that did not show BGY fluorescence in the whole-kernel sample was ground in a Straub disc mill adjusted to crack the kernels. Inspection for BGY fluorescence with a Blak Ray lamp was made on the cracked corn as it came from the mill. Whole corn was fed into the mill at such a rate that the stream of cracked corn could be thoroughly inspected.

Aflatoxin Analysis and Confirmation

At the elevator in Missouri, corn samples that had BGY fluorescence under ultraviolet light (365 nm), either in the whole kernel or cracked sample, were assayed by the rapid screening procedure for aflatoxin known as the minicolumn test (8) having a detection limit of 10 ppb. Rather than grind and blend the 5-lb sample, the minicolumn test was run on 50-g samples of whole corn selected to include as many BGY fluorescing kernels and particles as possible. The 50-g samples were not representative but were chosen to increase the probability of detecting aflatoxin if it were present at a measurable level.

At NRRL, all samples, both BGY positive and BGY negative, were ground and blended for aflatoxin analysis by the rapid thin-layer chromatography (tlc) and quantitative methods approved in Official First Action by the AOAC (9). These methods are sensitive to 1–3 ppb. The confirmatory test was the formation of the water adduct on a tlc plate with trifluoroacetic acid (10).

RESULTS AND DISCUSSION

There are three types of BGY fluorescence associated with aflatoxin in corn: a) obvious fluorescence in more-or-less damaged kernels, b) BGY fluorescence visible under the seed coat that can be identified by an experienced technician as a dull gold color in the germ area, and c) subsurface BGY fluorescence that can only be detected after crushing corn kernels (4). Experience is required to distinguish between the BGY fluorescence and the many other types and colors of fluorescence that occur in corn.

The first comparisons were between results of the BGY fluorescent testing on the whole kernel samples at three locations: elevator in southeastern Missouri (Diehlstadt), Missouri State Inspection Service (Sikeston), and NRRL (Table I).

TABLE I
BGY Fluorescent[a] Test Results on Whole-Kernel Corn Samples, Three Locations

Location and Result				
Southeastern Missouri[b]	Missouri State Inspection[b]	Northern Regional Research Laboratory[c]	Total	Per Cent
–[d]	–	–	626	49
–	+	–	6	0.5
–	–	+	263	21
–	+	+	6	0.5
+	–	–	16	1
+	+	–	63	5
+	–	+	24	2
+	+	+	271	21
			1,275[e]	100

[a]Bright greenish-yellow fluorescence under ultraviolet light (365 nm).
[b]At least one fluorescing particle in a 5-lb sample.
[c]At least one fluorescing particle in a 10-lb sample.
[d]– = Negative; + = positive.
[e]Eight of the original 1283 samples did not have complete information and were eliminated in these evaluations.

There was agreement at the three locations on 70% of the corn samples. On 49% of the samples, all three inspections for BGY fluorescence were negative; on 21%, all three inspections were positive. BGY fluorescent testing at NRRL identified more positive samples (at least one fluorescing kernel or particle per 10 lb) than did tests at other locations. NRRL found BGY fluorescence and neither of the other locations found any in 21% of the samples. NRRL inspected the 10-lb sample under conditions that allowed more time for examination. At the Missouri State Inspection Service, only 5 lb of corn was inspected and at the elevator, inspection of 5 lb had to be completed as the farmer waited. Differences between probe and continuous sampling procedures may explain why the results of 26% of the fluorescent tests at NRRL did not agree with those at the other two locations. The other two locations differed in their results from each other in only 3.5% of the samples.

When whole-kernel samples of corn were inspected under ultraviolet light (365 nm) for BGY fluorescence at the elevator in southeastern Missouri, 29% (378 samples, of which four were excluded from Table I) had fluorescence. Of the 378 BGY-positive whole-kernel samples, 87% gave positive minicolumn tests. This high percentage of positive minicolumn tests would be expected because 50-g samples were obtained by selecting as many BGY fluorescing kernels and particles as possible out of 5 lb of corn. After coarse grinding or cracking, 47 out of 905 corn samples gave positive BGY fluorescent tests and 74% of these gave positive minicolumn tests. The remaining 858 samples that were BGY-negative when cracked were not assayed by the minicolumn test but were assumed free of aflatoxin to facilitate delivery at the elevator.

Probably the most meaningful correlations between BGY fluorescence and the presence of aflatoxin were made at NRRL where the 10-lb continuous samples of corn were inspected for BGY fluorescence under ultraviolet light (365 nm) and all samples were analyzed quantitatively for aflatoxin. Quantitative determinations of the aflatoxin in 1283 truckloads of corn delivered to the Missouri elevator revealed an overall incidence of 30% (6). The large number of aflatoxin-positive samples available permitted valid statistical evaluations of the relation between BGY fluorescence and presence of aflatoxin.

Of 1283 whole-kernel corn samples, 569 contained at least one BGY fluorescing particle or kernel in 10 lb. Of these positive BGY samples, 55% contained aflatoxin by quantitative analyses at NRRL (9) and 28% contained more than 20 ppb toxin (Table II). The presence of BGY fluorescing particles or kernels should not be used as the sole criterion for rejecting corn. There was a positive relation between numbers of fluorescing kernels and particles observed and levels of aflatoxin determined (9) (Table III). A positive relation does not necessarily mean one could depend on counting fluorescing particles to determine aflatoxin levels. The grams of BGY fluorescent material per approximately 5 lb of corn were also related to levels of aflatoxin. Correlation coefficient for aflatoxin B-1 was 0.54; for B-2, 0.51. This correlation is not high enough to encourage use of weight of BGY fluorescent material as an indication of aflatoxin content.

Relationships between numbers of BGY particles and kernels in whole corn and levels of aflatoxin are shown in Table III. Of the samples without BGY fluorescing particles or kernels when unground, 98% contained less than 20 ppb aflatoxin. If one to three fluorescing particles or kernels were observed, 84% of

the sample contained less than 20 ppb aflatoxin. However, for corn samples that contained more than 20 fluorescing particles and kernels, 65% contained more than 20 ppb aflatoxin, the Food and Drug Administration guideline (11). Table IV summarizes the levels of aflatoxin determined in these corn samples at NRRL (9). Twenty-nine of the 1283 southeastern Missouri corn samples contained more than 100 ppb aflatoxin; 28 of these had more than 20 BGY fluorescing particles and kernels. Corn samples having many BGY fluorescing particles and kernels have a high probability of containing more than 20 ppb aflatoxin, the present guideline level.

Of the 638 corn samples that were cracked or coarse ground for a second inspection under ultraviolet light (365 nm) at NRRL, 393 had BGY fluorescence

TABLE II
Whole Corn Samples Containing BGY Fluorescence[a]

Grade U.S. No.	Total	Aflatoxin, ppb[b]					
		ND[c]	<10	10–19	20–29	30–100	>100
Ungraded	1					1	
1	141	70	30	12	10	16	3
2	185	90	27	33	12	21	2
3	44	18	8	6	4	5	3
4	49	12	5	9	1	13	9
5	54	20	7	6	5	13	3
SG[d]	95	45	9	8	7	17	9
Total	569	255	86	74	39	86	29

[a]Bright greenish-yellow fluorescence under ultraviolet light (365 nm). At least one fluorescing particle in a 10-lb sample.
[b]As determined at NRRL by AOAC Official First Action Method.
[c]ND = Not detected.
[d]SG = U.S. Sample Grade.

TABLE III
Relation of Aflatoxin Level to Number of BGY Fluorescing Particles[a] and Kernels in Unground Samples

Aflatoxin Level ppb[b]	Number of BGY Fluorescing Particles and Kernels			
	0	1–3	4–20	>20
0	88[c]	60	41	6
<10	7	14	20	14
10–19	3	10	17	15
20–29	1	6	11	7
30–100	1	9	11	34
>100	0	0.3	0	24

[a]Bright greenish-yellow fluorescence under ultraviolet light (365 nm).
[b]As determined at NRRL by AOAC Official First Action Method.
[c]Percentage of total sample.

TABLE IV
Analytical Results on all White Corn Samples with more than 20 BGY Fluorescing[a] Particles

Grade U.S. No.	Total	Aflatoxin, ppb[b]					
		ND	<10	10–19	20–29	30–100	>100
Ungraded	1					1	
1	14	0	5	0	1	5	3
2	30	2	7	6	4	9	2
3	16	0	2	4	2	5	3
4	25	1	1	7	0	7	9
5	14	3	2	0	1	5	3
SG	17	0	0	1	0	8	8
Total	117	6	17	18	8	40	28

[a]Bright greenish-yellow fluorescence under ultraviolet light (365 nm) in unground sample.
[b]As determined at NRRL by AOAC Official First Action Method.

TABLE V
Coarse Ground Corn that Contained BGY Fluorescence[a]

Grade U.S. No.	Total	Aflatoxin, ppb[b]					
		ND	<10	10–19	20–29	30–100	>100
1	106	89	12	4	1	0	0
2	146	119	12	9	4	2	0
3	44	37	5	2	0	0	0
4	16	13	2	0	0	1	0
5	24	16	5	2	1	0	0
SG	57	44	9	2	0	2	0
Total	393	318	45	19	6	5	0

[a]Bright greenish-yellow fluorescence under ultraviolet light (365 nm). At least one fluorescing particle in a 10-lb sample.
[b]As determined at NRRL by AOAC Official First Action Method.

TABLE VI
Coarse Ground Corn with no BGY Fluorescence[a]

Grade U.S. No.	Total	Aflatoxin B-1, ppb[b]	
		ND	<10
1	79	77	2 (3, 4 ppb)
2	125	124	1 (5 ppb)
3	14	14	
4	5	5	
5	2	2	
SG	20	20	
Total	245	242	3

[a]Bright greenish-yellow fluorescence under ultraviolet light (365 nm).
[b]As determined at NRRL by AOAC Official First Action Method.

after cracking (Table V). Aflatoxin was detected in 19% of these samples, but only 15% of the aflatoxin-positive samples contained more than 20 ppb aflatoxin (9). There were 165 corn samples, out of the 1283 collected in southeastern Missouri, containing more than 20 ppb aflatoxin, and only 7% of these were corn samples that had to be cracked before BGY fluorescence became visible. Undoubtedly more BGY fluorescence was detected in cracked samples at NRRL than at the elevator in southeastern Missouri because of the differences in carrying out the test and the increased size of sample. At NRRL, the stream of cracked corn was inspected under ultraviolet light (365 nm) as it came from the mill whereas in Missouri, the whole sample of corn was inspected after cracking.

Of most concern to people using the fluorescent test to locate contaminated corn is whether corn samples without BGY fluorescence may contain aflatoxin and at what levels. The 245 samples that did not have BGY fluorescence after cracking included only three aflatoxin-positive samples, and the levels were 3, 4, and 5 ppb toxin (9) (Table VI). If there were no BGY fluorescing particles even when a corn sample was cracked, aflatoxin was not present in appreciable levels.

CONCLUSIONS

In conclusion, inspection of corn for BGY fluorescence pinpointed about twice as many samples for analysis as were actually found to contain measurable amounts (over 1–3 ppb) of aflatoxin. Use of the fluorescent test to minimize the analytical work load obviously would have more value at low levels of incidence than at the high level encountered in southeastern Missouri. Informal reports from corn mills using both the fluorescent test and quantitative analysis indicate that at low levels of incidence the relation between fluorescence and measurable aflatoxin is about the same as observed in this study. Therefore, for example, the 2% incidence of aflatoxin we observed in our survey of market corn (12–15) would give rise to about 4% of samples which would require analysis because of BGY fluorescence. The advantage over analysis of all samples is obvious in that case.

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