Use of Bright Greenish Yellow Fluorescence as a Presumptive Test for Aflatoxin in Corn[1]

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ABSTRACT

Corn samples from the 1978 crop were examined under ultraviolet light (365 nm) for the bright greenish yellow (BGY) fluorescence associated with *Aspergillus flavus* or *A. parasiticus* and possibly the aflatoxin produced by these fungi. Two methods were used to test 248 samples for BGY fluorescence. First, whole-kernel samples were examined in a black light viewer, and BGY particles were counted. Then, the corn samples were coarse-ground in a disk mill; the BGY particles were counted as the stream from the mill was examined under ultraviolet light (365 nm). The counts obtained by the two methods were similar. Particle counts were identical in 61% of the samples and differed by one in 20%, two in 8%, three in 6%, and four in only 5%. Tinopal BHS, a compound that is stable to air, can be used as a color reference standard in the BGY test. In samples of 1978 corn having one BGY particle per kilogram, 92% had less than 20 ng of total aflatoxin per gram, the Food and Drug Administration's action guideline. In 1978 samples having four or more particles per kilogram, only 25% had less than 20 ng/g. In an examination of 1973 South Carolina corn, 98% of the samples in which no BGY fluorescence was detected had aflatoxin levels less than 20 ng/g; the two samples that were BGY-negative had 21 and 27 ng/g.

The bright greenish yellow (BGY) presumptive test is widely used by government agencies in monitoring corn for aflatoxin to identify lots that should be tested further. The test is based on the association of a BGY fluorescence in corn under ultraviolet light (365 nm) with invasion by *Aspergillus flavus* or *A. parasiticus*, the molds that produce aflatoxin (Shotwell 1977). If BGY fluorescence is used as the only criterion in deciding the presence or absence of the toxin, many false positive tests but very few false negative tests exist[3] (Shotwell et al 1975). Because the BGY test is so easily done, attempts have been made to establish aflatoxin levels by weight of BGY material in whole-kernel samples or by number of BGY particles present. Use of BGY weight for a quantitative estimation

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[2]The mention of firm names or trade products does not imply endorsement or recommendation by the USDA over other firms or similar products not mentioned.

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TABLE I
Comparisons of Aflatoxin Levels and BGY[a] Particles and Kernels per Kilogram in Unground 1973 South Carolina Corn Samples[b]

Total Aflatoxin Level (ng/kg)	Number of BGY Particles and Kernels per Kilogram									
	None		< 1		1		2		> 2	
	N[c]	%	N	%	N	%	N	%	N	%
ND[d]	72	88	33	65	37	47	3	10	0	0
< 20	8	10	13	25	24	30	8	25	2	4
20-49	2[e]	2	4	8	16	20	11	34	15	28
50-99			1[f]	2	2	3	8	25	15	28
100-500							2	6	18	33
> 500									4	7
Total	82		51		79		32		54	

[a]Bright greenish-yellow fluorescence.
[b]Of the 82 samples that were BGY-negative when unground, 21 were BGY-positive when coarsely ground.
[c]Number of samples.
[d]Not detected.
[e]These samples had 21 and 27 ng/g total aflatoxin.
[f]This sample had 51 ng/g total aflatoxin.

of aflatoxin level was unsatisfactory because prediction equations in different corn lots showed wide variation (Kwolek and Shotwell 1979). Although a relationship exists between numbers of BGY particles or kernels and aflatoxin levels, the correlation is not high enough to encourage use of the numbers as an indication of aflatoxin content (Shotwell et al 1975). Barabolak et al (1978) also support the conclusion that BGY count is not a quantitative measure of aflatoxin levels in corn samples.

The recommendation has been made that the BGY test be conducted on coarsely ground (cracked) corn because studies on aflatoxin distribution in contaminated corn revealed that BGY fluorescence might be hidden under the seedcoat (Shotwell 1977). However, a black light viewer designed by Barabolak et al (1978) for the BGY test on whole-kernel corn appeared to give satisfactory results. The viewer allows a monolayer of grain to move on a vibrating tray while being inspected.

We now report further information on relationships between number of BGY particles (pieces and cracked kernels) per kilogram and aflatoxin levels. An optical brightener was found for a color reference standard. We also compared results obtained by using the black light viewer for the BGY test on whole-kernel corn with results obtained by inspecting the stream of the same sample coarsely ground through a disk mill.

MATERIALS AND METHODS

Collection of Corn Samples

1973 South Carolina Corn. Corn was collected either in the field or at elevators in northeastern South Carolina (Lillehoj et al 1975). Corn samples (4.5 kg) were taken by probes of truckloads or directly from the picker sheller during transfer to a truck. The sample size was the one recommended for aflatoxin analysis of corn.

1978 Corn. Samples were collected from 15-ft rows of corn in fields that fieldmen from the Statistical Reporting Service, U.S. Department of Agriculture, harvested for projected yield surveys in eight states. The average weight of shelled corn samples was 2.1 kg. Although the sample size was about half the size recommended for aflatoxin analysis, it is the one frequently used by the industry because it gives reliable results economically.

TABLE II
Aflatoxin in Ground[a] 1973 South Carolina Corn That Was BGY[b]-Negative as Whole Kernels

	BGY After Coarse Grinding	
Number of Samples	Positive	Negative
Total	21	61
Aflatoxin not detected	17	55
Aflatoxin detected	4[c]	6[d]

[a] In a disk mill and inspected under ultraviolet light.
[b] Bright greenish yellow fluorescence.
[c] Total aflatoxin levels (ng/g): 10, 11, 16, 27.
[d] Total aflatoxin levels (ng/g): 4, 7, 8, 9, 10, 21.

Color Reference Standard

Three optical brighteners (Tinopal BHS, Tinopal CBS, and Tinopal GS; Ciba-Geigy) were compared with aflatoxin-contaminated corn kernels and particles with BGY fluorescence under a Blak-Ray high-intensity B-100 light or under a UVL-22 hand lamp and in a Chromato Cabinet (all with 365-nm light).

Inspection for BGY

1973 South Carolina Corn. Whole-kernel samples were inspected in trays under a Blak-Ray high-intensity light, and the number of BGY particles and kernels in each sample was counted. Those whole-kernel samples in which no BGY fluorescence was observed were coarsely ground (cracked) in a Straub disk mill at such a rate that the stream of cracked corn could be thoroughly inspected for BGY fluorescence under a Blak-Ray light (Shotwell et al 1975). BGY particles were counted. Particles from each shattered kernel would be counted as one as they came from the mill.

1978 Corn. Whole-kernel samples were inspected in a black light viewer designed to allow inspection of 4.5 kg of whole corn in less than 10 min (Barabolak et al 1978). Corn was discharged in a monolayer onto the vibrating feeder tray moving under ultraviolet light (365 nm). The feeder tray and light were enclosed in a cabinet with a viewing port. BGY kernels and particles were counted.

The same samples were then coarsely ground in a Straub disk mill and inspected as described above.

Analysis and Confirmation

All corn samples were analyzed for aflatoxin by the CB Method approved by the Association of Official Analytical Chemists (AOAC 1975) and the American Association of Cereal Chemists (AACC 1977). The identity of aflatoxin B_1 detected was confirmed by formation of the water adduct (AACC 1977, AOAC 1975).

RESULTS AND DISCUSSION

The color of the fluorescence of Tinopal BHS under ultraviolet light (365 nm) was the same as that of BGY fluorescence observed in corn containing *A. flavus* and *A. parasiticus* and possibly the metabolite, aflatoxin, produced by these fungi. The color of the fluorescence of Tinopal CBS and of Tinopal GS was too yellow. Tinopal BHS is an excellent standard for the BGY test because it not only has the correct fluorescence but is also stable on exposure to air and thus can be supplied in screw-cap vials. The BGY color reference standard previously used, Tinopal BV, hydrated and changed to a pale blue fluorescence upon exposure to moisture in the air. To retain the color of the dehydrated compound, Tinopal BV had to be heated 48 hr at 140° C and sealed immediately in glass vials while still hot.

Comparisons of numbers of BGY particles and kernels with aflatoxin levels in 1973 South Carolina corn samples (298) made within three months of collection are summarized in Table I. The main purpose of the 1973 study was to establish whether aflatoxin was formed in the field (Lillehoj et al 1975). All samples were dried

TABLE III
Comparisons of Aflatoxin Levels and BGY[a] Particles in Coarsely Ground 1978 Corn

	BGY Particles per Kilogram									
	1		2		3		4		> 4	
Total Aflatoxin Level (ng/g)	N[b]	%	N	%	N	%	N	%	N	%
ND[c]	41	63	17	57	9	41	4	36	9	8
< 20	19	29	5	17	7	32	3	27	16	15
20–49	1	2	6	20	2	9			19	18
50–99	4	6	1	3	3	14	2	18	12	11
100–500			1	3	1	4	1	9	35	33
> 500							1	9	16	15
Total	65		30		22		11		107	

[a] Bright greenish yellow fluorescence.
[b] Number of samples.
[c] Not detected.

TABLE IV
Comparisons of Two Methods of Measuring BGY[a] in 1978 Corn:[b] Inspection of Whole Kernels with Black Light and Inspection of Coarse Ground Corn

Source of Corn	Number of Samples: Total	With Same Number of BGY Particles by Both Methods	In Which Difference in BGY Particles Between the Two Methods Equals: 1	2	3	4
Georgia	58	41	7	5	1	4
Indiana	1	1				
Kansas	12	6	6			
Kentucky	13	7	3	2	1	
Missouri	10	5	3	2		
North Carolina	63	38	13	5	4	3
Texas	59	34	10	5	6	4
Virginia	32	19	7	2	3	1
Total	248	151	49	21	15	12
Percent	100	61	20	8	6	5

[a] Bright greenish yellow fluorescence.
[b] Average weight of samples was 2.1 kg.

TABLE V
Comparison of Particle Numbers Obtained by Two Methods of Measuring BGY[a] in 1978 Corn

Number of Particles	Number of Samples, Determined by: Inspection of Unground Corn with Black Light Apparatus	Inspection of Coarse Ground Corn From Disk Mill
0	59	69
< 1	45	21
1	27	32
2	21	27
3	16	12
4	23	25
> 4	57	62
Total	248	248

[a] Bright greenish yellow fluorescence.

immediately after harvesting. BGY particles in excess of two per kilogram were not counted individually. Of the 165 samples with one or more BGY particles or kernels per kilogram, 45% had total aflatoxin levels less than 20 ng/g and 55% had total aflatoxin levels equal to or exceeding 20 ng/g. Seven of the 133 samples with less than one BGY particle or kernel per kilogram had levels equal to or exceeding 20 ng/g total aflatoxin, with the highest being 51. Total aflatoxin levels were highest in samples containing the most BGY particles per kilogram; but even in this category, a few corn lots had less than 20 ng/g. The 82 South Carolina samples that were BGY-negative when examined under ultraviolet light as whole kernels also were examined for BGY particles when coarsely ground in a disk mill. BGY particles were observed in 21 of these samples (Table II). One of the 21 samples had an aflatoxin level greater than 20 ng/g and would be in violation of the FDA guideline.

As part of a study of 1978 corn, the BGY examination was designed to determine aflatoxin levels in corn lots containing one, two, three, four, or more than four BGY particles per kilogram (Table III). Of the 751 samples in the study, 516 had less than one BGY particle per kilogram. Of the 235 samples containing one or more BGY particles per kilogram, 55% had total aflatoxin levels less that 20 ng/g and 45% had levels equal to or exceeding 20 ng/g. In samples with one BGY particle per kilogram, 8% had levels of more than 20 ng/g and 6% had levels of 50–99 ng/g. In corn lots containing more than four BGY particles per kilogram, only 23% had total aflatoxin levels of less than 20 ng/g and 48% had more than 100 ng/g.

Two methods of observing for BGY fluorescence in corn, the use of the black light viewer on whole-kernel corn and the inspection of coarsely ground corn falling from a disk mill under ultraviolet light, were applied to the first 248 corn samples collected in the 1978 survey (Table IV). The number of samples was more than enough to obtain significant results. Results by both tests were similar. The same number of BGY particles were counted in 61% of the samples. In only 5% of the corn samples was a difference of four particles found between the two methods, a difference of about two BGY particles per kilogram. Table V compares the number of particles determined by each of the methods. Although a few more particles were counted in the coarsely ground corn, the difference was not significant.

In laboratories where efficient exhaust hoods are available, examination of streams of coarsely ground corn under ultraviolet light is convenient. The process is even necessary before the fine grinding needed to prepare subsamples suitable for aflatoxin analysis; counting the BGY particles as the coarsely ground corn comes from the mill takes no more time than does grinding the corn. However, in an elevator in the field, use of the disk mill is difficult as well as hazardous to unskilled workers. Corn dust generated by grinding must be thoroughly exhausted because, if it contains aflatoxin, it presents a potential hazard to workers inhaling it. The grinding process also creates dust under circumstances where dust is highly undesirable. Also, workers must wear safety glasses to protect their eyes from ultraviolet light while inspecting a stream of cracked corn. The black light viewer has eye protection built into it.

CONCLUSIONS

Tinopal BHS is a satisfactory color reference standard for the BGY test; it has been used with success in the field. The BGY test can be carried out equally well by using the black light viewer on whole-kernel corn or by inspecting a stream of coarsely ground corn from a mill under ultraviolet light (365 nm). From the results of the present studies on 1973 South Carolina corn and 1978 Statistical Reporting Service samples of corn and of a previous study on 1971 white corn under loan in southeastern Missouri (Shotwell et al 1975), a count of one BGY particle per kilogram obtained on a given corn lot appears to be an indication that the sample should be tested for aflatoxin by chemical means. The higher the BGY count in a corn sample, the more likely it is to contain aflatoxin in levels equal to or exceeding 20 ng/g.

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