Distribution and Measurement of Aflatoxin in 1983 Iowa Corn[1]

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Cereal Chem. 66(3):165-168

ABSTRACT

Six corn samples from each of Iowa's 99 counties were collected in December, 1983, to determine the degree of aflatoxin contamination in 1983 Iowa corn. While the statewide average was 18.8 parts per billion (ppb), 23% of the counties had no detectable aflatoxin and 11% were over 100 ppb. Aflatoxin concentrations were highest in the southeast and south-central regions, coinciding with the extreme drought conditions suffered there during August. The coefficient of variation among samples within counties averaging over 20 ppb was 105%. The data support other reports that high temperatures and drought stress in the three to four weeks following silking promotes aflatoxin contamination. The accuracy of a whole-kernel black-light test as a presumptive test for aflatoxin concentration in excess of 20 ppb was examined for two sample sizes (10.9 and 1.8 kg). The black light identified all 10.9-kg samples that contained aflatoxin contamination above 20 ppb. For the 1.8-kg samples, 11% of samples with aflatoxin levels above 20 ppb were missed. Of the large samples with bright greenish-yellow fluorescent particles, 55% contained less than 20 ppb compared with 33% of the small samples.

Aflatoxin is a product of the fungus, *Aspergillus flavus*, which can grow in corn, peanuts, and cottonseed. Aflatoxin is less common in Midwest corn (Lillehoj et al 1976) than corn from the southeastern United States. Aflatoxin production in corn is stimulated by temperatures, drought, and crop stress (Lillehoj et al 1976, Tuite et al 1984, Millian et al 1985). The severe drought and high temperatures during the summer of 1983 in Indiana appeared related to aflatoxin contamination (Tuite et al 1984). Parts of Iowa experienced similar weather patterns in 1983 and thus were subject to conditions favorable for *A. flavus* growth.

The distribution of aflatoxin among lots within a region is important, as is the ability to quickly predict aflatoxin contamination of a given lot. Bright greenish-yellow fluorescent (BGYF) particles, as detected in a black-light test, can be used as a presumptive test for the presence or absence of aflatoxin (Shotwell 1983). The black-light test uses long-wave ultraviolet light (365 nm) to detect a fluorescence associated with actively growing *A. flavus*. Therefore, the black-light test is not a direct indication of aflatoxin contamination. The fluorescence was first observed in corn samples collected during 1969–1970 from the South (Shotwell et al 1972). Studies conducted on the effectiveness of black-light testing for aflatoxin are summarized by Shotwell (1983). In a study of 1973 South Carolina corn, 98% of the samples with no BGYF particles had less than 20 parts per billion (ppb), the Food and Drug Administration's (FDA) action guideline (Shotwell and Hesseltine 1981). The variability of the relationship between BGYF and aflatoxin may depend on the specific fungal strain on the BGYF kernels (Calvert et al 1983). The black-light test was too imprecise to quantify aflatoxin (Kwolek and Shotwell 1979, Dickens and Whitaker 1981), but because of the cost and time required for chemical quantitative analysis, the black-light test is often used for screening corn lots for possible aflatoxin contamination. The recommended black-light procedure is to crack the kernels before viewing because fluorescence occurs in the endosperm under the seed coat (Shotwell 1983). However, inspection of whole kernels is simpler, faster, and the procedure most often used in grain elevators. Preharvest reports of high aflatoxin concentrations in Iowa corn led the FDA to require state officials to develop an action plan for preventing contaminated lots from entering the marketplace.

A study to determine the distribution and level of aflatoxin in the 1983 Iowa corn was conducted to aid in the development and targeting of this plan. The diverse weather patterns across Iowa offered an opportunity to assess forces that promote aflatoxin contamination. The study objectives were to estimate the average and variability of aflatoxin content in 1983 crop Iowa corn, to associate weather patterns with aflatoxin content of corn, and to estimate the accuracy of a whole-kernel black light test in identifying aflatoxin content greater than 20 ppb.

MATERIALS AND METHODS

Materials

The Iowa Cooperative Extension Service county and area directors collected six samples of corn from the 1983 corn crop

[1]Journal paper no. J-12339 of the Iowa Agriculture and Home Economics Experiment station, Ames 50011. Research supported by the Iowa Corn Promotion Board and the Iowa Agricultural Home Economics Experiment Station. Project 2339.

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from each of Iowa's 99 counties. Samples were collected between December 1 and 20, 1983. These six samples (sector samples) were collected from farm bins geographically distributed across the county. Each sector sample represented one-sixth of the county area and was either drawn from a combination of two producers' on-farm storage or from one producer's on-farm storage if more than two hybrids were represented. Producers were not selected on the basis of prior knowledge of crop conditions or storage abilities. The corn was to be dry and in storable condition, although moisture data subsequently showed some lots to be of much higher moisture than the producer thought they were.

Samples were collected either by probing the bins in five locations or by sampling an unloading stream. Seventy-three percent of the samples were probe samples, 23% were stream samples, and 4% were either not reported or taken by other means. Samples were transported to Iowa State University and stored at 3° C until analyzed. The average moisture content of the samples was 15.5% (wet basis), measured by a Dickey-john GACII moisture meter, with a standard deviation of 2.0 percentage points, and a range of 23.2–8.8%.

Methods

Each sector sample was divided in a Boerner divider. Half was stored as a sector-file sample. The weight of the other half (sector-work sample) was taken. The sector-work samples and file samples averaged 1.8 kg each. The whole-kernel work sample was then inspected for BGYF particles in a Seedburo grain viewer. Whole kernels passed under an ultraviolet light (365 nm). A similar apparatus is described by Barabalok et al (1978). BGYF particles were removed, counted, weighed, and returned to the work sample.

Each of the six sector-work samples from a county were combined to form a county composite weighing about 10.9 kg. The county composites were black-lighted, then coarsely ground through a Viking hammer mill with a 0.1-cm screen. The ground county composite was divided in a Boerner divider to give a county-analysis sample and a county-file sample. The average weight of the county-analysis samples was 5.4 kg. Each county-analysis sample was sent to the Iowa Department of Agriculture (IDA), where it was subsampled to 50 g, finely ground, and analyzed for aflatoxin by thin-layer chromatography (TLC) (AOAC 1984).

If a county composite tested above 15 ppb aflatoxin, then the individual sector-file samples for that county were also analyzed. Each sector's file sample was weighed and inspected for BGYF particles. The sector-file sample was then coarsely ground in the Viking hammer mill. The ground sample was reduced to approximately 200 g in a Boerner divider and analyzed for aflatoxin at IDA or the ISU Veterinary Diagnostic Laboratory. The Veterinary Diagnostic Laboratory also used the AOAC procedure.

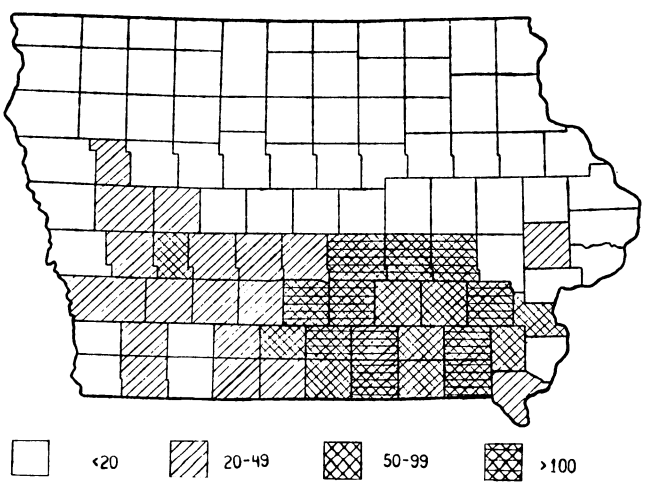

Fig. 1. Aflatoxin level (in parts ber billion) in 1983 Iowa corn as estimated from thin-layer chromatography results in county-composite samples.

A 10-sample paired t test showed no significant difference ($P = 0.05$) between the laboratories. Duplicate analyses had a within-laboratory coefficient of variation (CV) of 2.7%.

RESULTS AND DISCUSSION

Distribution and Level of Aflatoxin

The TLC results of the county composite samples are shown in Figure 1. The areas with highest aflatoxin concentration were in the southern portion of the state where the drought stress was greatest. Eleven counties (11%) had an average aflatoxin content above 100 ppb, 11 counties (11%) were between 100 and 50 ppb, and 16 counties (16%) were between 49 ppb and 20 ppb. Of the remaining 62 counties, 15 (15%) were between 19 and 10 ppb, 24 (25%) were between 9 and 1 ppb, and 22 (23%) had no detectable aflatoxin. The average statewide level of aflatoxin, weighted by 1983 county corn production, was 18.8 ppb.

By comparing the distribution of aflatoxin with crop development and weather patterns, an insight can be developed for the forces behind this particular aflatoxin outbreak. Chronologically, the first event to increase the possibility of *A. flavus* infestation was the wet and cool spring. This delayed emergence and moved the flowering stage into hotter months.

The next two important factors were the extreme drought and high temperatures from the end of July through August. Drought stress, when coupled with high temperatures, can result in significant number of kernels infected through silk colonization by *A. flavus* (Jones et al 1981). The silking process started during the middle of July and was essentially completed statewide by August 7. Table I indicates the temperature and cumulative precipitation departures from the 30-year normal that existed in the crop-reporting districts (groupings of 10 counties) of Iowa for July and August of 1983 (IDA 1983a). August temperatures resulted in the second hottest August on record and, in some areas of southern Iowa, rainfall was less than 10% of normal (IDA 1983b). In addition to affecting colonization of *A. flavus*, drought stress may alter the nutritional status of developing kernels, thus increasing aflatoxin synthesis (Jones et al 1981). However, variability in the weather-aflatoxin relationship is apparent in Table I.

Statistics were not used to correlate weather and aflatoxin because of uncontrolled variables and incomplete local weather information. Weather data were only available by crop reporting district. The high variability of aflatoxin within a district lead to a large error term in the analysis of variance. The low range in temperature departure between districts made it difficult to predict aflatoxin concentration from temperature departures. Since each district went through the silking process at a different time, the weather influence could not be isolated from silking-date influence.

Late-season rainfalls in October could have increased aflatoxin content. For the late-season rainfalls to play a role in aflatoxin

TABLE I
Temperature and Cumulative Precipitation Departures During July and August 1983 in Nine Iowa Crop Reporting Districts

District	Average Aflatoxin Concentration (ppb)[a]	Departure from 30-Year Normal: Temperature (°C) July	Temperature (°C) Aug.	Precipitation (cm) since April 1 July	Precipitation (cm) since April 1 Aug.
Northwest	5	2.2	4.4	9.1	4.1
North central	2	2.2	4.4	−0.3	−5.1
Northeast	2	1.7	3.9	3.0	−2.0
West central	21	1.7	4.4	0.3	−4.8
Central	28	2.2	4.4	4.3	0.8
East central	25	2.2	3.9	−8.1	−15.0
Southwest	30	2.2	5.0	−8.4	−16.3
South central	107	2.8	5.6	−0.2	−18.2
Southeast	89	2.8	5.0	−11.9	−18.3

[a] Parts per billion, average of county composites.

TABLE II
Aflatoxin Content in 1983 Iowa Corn Related to the Number of Bright Greenish-Yellow Fluorescent (BGYF) Particles per Kilogram for 10.9- and 1.8-kg Samples[a]

Total Aflatoxin Level (ppb)[b]	No. of BGYF Particles per Kilogram											
	None		0.1–0.9		1.0–1.9		2.0–2.9		3.0–4.9		4.0+	
	n[c]	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
10.9-kg samples												
0	11	69	11	27	...	...	...	...	...	...	...	...
1–19	5	31	26	65	4	29	3	20	2	33	...	...
20–49	...	...	3	7	7	50	4	27	1	17	...	...
50–99	...	...	...	...	3	21	4	27	1	17	3	37
100–499	...	...	...	...	...	...	4	27	2	33	4	50
500+	...	...	...	...	...	...	...	...	...	...	1	13
Total	16	100	40	100	14	100	15	100	6	100	8	100
1.8-kg samples												
0	20	47	6	17	6	14	4	10	2	5	...	...
1–19	18	42	18	51	12	29	15	38	3	8	4	7
20–49	4	9	9	26	16	38	13	33	17	47	13	23
50–99	1	2	1	3	4	10	4	10	12	32	15	26
100–499	...	...	1	3	2	5	2	5	2	5	24	42
500+	...	...	...	...	2	5	1	3	1	3	1	2
Total	43	100	35	100	42	100	39	100	37	100	57	100

[a] Whole-kernel black-light screening.
[b] Parts per billion.
[c] Number of samples.

TABLE III
Presumptive Reliability of Whole-Kernel Black-Light Test by Sample Size

Sample Size	% of Samples with <20 ppb of Aflatoxin	
	No BGYF[a] Particles	BGYF[a] Particles
10.9 kg	100	55
1.8 kg	89	33

[a] Bright greenish-yellow fluorescence.

development, the kernels must be infected with *A. flavus* (Jones et al 1981) as they clearly were in 1983. Several other factors that can influence aflatoxin concentration, but which were not measured, are *A. flavus* spore loads, insect-enhanced secondary spread, pericarp damage, and relative humidity (Jones et al 1981).

Variability of Aflatoxin within a County

The variability of aflatoxin within a county was measured by the CV for the 43 counties that had the six sector-file samples analyzed. The average within-county CV of these 43 counties was 105%, with a range of 36 to 193%. CV was not dependent upon the aflatoxin level. Because the CV is the standard deviation divided by the mean, the actual variability of aflatoxin within a county increased proportionately with aflatoxin content.

Because of the large CV, the confidence interval of aflatoxin in a county region included zero even for the highest county average (504 ppb). Therefore, condemning or halting corn shipments from a county based on some average result would penalize the corn that is not contaminated. This was a key point in the formulation of an action plan, that each lot regardless of origin must be treated individually. The wide difference between sector samples within a county could be caused by localized forces, such as *A. flavus* spore loads, soil conditions, hybrid, insects, agronomic practices, and local weather patterns.

Bright Greenish-Yellow Fluorescence

There were 352 samples that showed a direct comparison between aflatoxin content and the number of BGYF particles per kilogram. The 99 county composite samples (about 10.9 kg each) made up the first comparison sample set. The second BGYF comparison set of 253 samples came from the sector-file samples (average size of 1.8 kg) for the counties testing over 15 ppb.

There was decreased reliability (ability to identify samples in excess of 20 ppb) for the small sample size. Table II presents the data for 10.9-kg county-composite samples and the 1.8-kg sector-file samples. No large samples without BGYF particles contained more than 20 ppb aflatoxin, while 11% of the small samples with no BGYF particles had more than 20 ppb aflatoxin. Of the large samples with BGYF particles, 55% contained less than 20 ppb aflatoxin. In contrast, 33% of the small samples with BGYF particles had less than 20 ppb. A comparison of the large and small samples is shown in Table III.

The large sample size had a higher reliability by not missing contaminated samples but overpredicted the number of samples that contained aflatoxin. If one or more BGYF particles were found in a small sample, the more certain it was that aflatoxin would be found, but the small sample had a higher risk of missing contaminated corn. Therefore, when using the black-light test, one must consider the consequences of using BGYF to predict aflatoxin contamination. The 1.8-kg size is the approximate sample weight used to determine the U.S. grade by the Federal Grain Inspection Service (FGIS). County elevators typically collect about 0.5 kg per lot for grading purposes. If the black-light test is used as a presumptive test to determine further analysis requirements, then any sample, regardless of size, with BGYF particles should be tested further.

We attempted to quantify aflatoxin from the black-light test results. There was great variability in the relationship between the black-light test (quantified by either number or weight of BGYF particles per kilogram) and aflatoxin concentration. The smallest root mean square error that could be obtained (dropping the low and high aflatoxin samples and taking number, weight, and the interaction of number and weight of BGYF particles) was 51 ppb. An approximate CV for this type of a test would be 100%. Clearly, this level of accuracy is not acceptable in testing for aflatoxin concentration.

In this project, the black-light test was not as reliable as in other studies. In 1973 South Carolina corn (Shotwell 1983), only 2% of the 4.5-kg samples without BGYF particles contained above 20 ppb. By linear interpolation from our data, a 9.1-kg sample would be required to achieve the same 2% rate. This difference may be attributed to different geographic origins of corn, use of whole-kernel corn vs. cracked corn, absence of a color reference for glowing kernels, and possibility of different fungal strains. Although it is unclear which of these factors are most significant in the black-light test effectiveness, it seems that sample size has a large effect.

CONCLUSIONS

From TLC and black-light analysis of six corn samples per county, the following conclusions are drawn:

1. There was significant incidence of aflatoxin (27% of counties) in excess of 20 ppb in 1983 Iowa corn. However, the coefficient of variation for aflatoxin content within a county was 105%. Thus, it is unreliable to assume without analysis that all lots even in susceptible areas are contaminated.
2. Aflatoxin contamination was highest in southern Iowa where delayed emergence, extreme drought, and extreme temperatures were contributing factors.
3. For 10.9-kg samples, the black-light test did not miss contaminated samples, but 55% of 10.9-kg samples with one or more BGYF particles contained less than 20 ppb aflatoxin.
4. Small samples (1.8 kg) increased the probability of missing contaminated samples to 11%, but only 33% of the samples with one or more BGYF particles contained less than 20 ppb aflatoxin.

ACKNOWLEDGMENTS

The authors thank the Iowa Cooperative Extension Service county and area directors for their help in sample collection, the Iowa Department of Agriculture and the Iowa State University Veterinary Diagnostic Laboratory for aflatoxin assays, Cindy West for manuscript preparation, and the following Grain Quality Laboratory employees for sample analysis: Scott Schnicker, Lynn Paynter, Mitch Hushak, and Lisa Hushak.

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[Received September 23, 1988. Accepted December 15, 1988.]