South Carolina Corn Yield Trial Samples as Probes for the Natural Occurrence of Aflatoxin in Preharvest Kernels

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ABSTRACT

To explore the distribution of the aflatoxin-producing fungi and the accumulation of toxin in preharvest corn kernels, a study was conducted in the 1979 South Carolina yield trials. Thirty hybrids, 10 from each of three maturity groups (short, medium, and full season) were sampled at two locations (Blackville and Florence). A 10-lb sample of kernels was collected from each of six replications for each hybrid. The entire sample was examined for the bright greenish yellow (BGY) fluorescence that has been linked to development of the aflatoxin-producing fungi. Subsequently, the kernels were ground, blended, and assayed for aflatoxin. Over two thirds of the samples contained detectable levels of toxin, with distinct location differences. Kernels from the midseason hybrids at Florence had higher toxin levels and numbers of BGY-fluorescing particles than did other sample groups; 2% contained aflatoxin B_1 at levels above 640 ppb and had more than 320 BGY particles. Results from this location demonstrated a significant association between the number of BGY-fluorescing particles and the aflatoxin concentration in a sample.

In response to a growing awareness of the broad occurrence of aflatoxin in preharvest corn, the need has arisen for: 1) development of rapid detection techniques that can reliably estimate the extent of contamination, 2) identification of hybrid differences in susceptibility to infection by toxin-producing fungi, and 3) acquisition of representative samples for early determination of the regional scope of aflatoxin in the crop.

One of the major impediments to studies of the field occurrence of aflatoxin has been the absence of a rapid, definitive test. A simple, presumptive test has been developed for screening purposes that utilizes the presence of a bright greenish yellow (BGY) fluorescence induced by ultraviolet light in commodities that have become infected by some fungal species, including aflatoxin-producing strains (Kwolek and Shotwell 1979). Although the BGY fluorescence is broadly linked to the occurrence of aflatoxin in kernels, the test is imprecise, and chemical methods have been required for confirmation and quantitative determination of the toxin.

Recommendations for control of preharvest contamination by aflatoxin have generally consisted of techniques for the reduction of stress and pest damage (Zuber and Lillehoj 1979). Selection of planting date to escape periods of major plant stress, particularly during flowering, has been considered (Zuber and Lillehoj 1979), but unequivocal evidence linking maturity and toxin contamination over a number of years is not available. Under specific conditions, some commercial hybrids appear to exhibit relative resistance to toxin accumulation, but the results are compromised by observations of location-to-location and year-to-year differences (Lillehoj and Hesseltine 1977, Lillehoj et al 1980, Manwiller and Fortnum 1979, Widstrom et al 1978).

Preliminary studies of the distribution of aflatoxin in developing kernels showed that the toxin occurred sporadically (Lillehoj 1979). Heterogeneity of occurrence was observed between regions, among fields within a region, on ears within a field, and among kernels on individual ears (Lee et al 1980, Lillehoj 1979, Lillehoj and Hesseltine 1977). The extensive variation in toxin incidence and concentration requires a field experimental design that provides the best estimates of toxin presence; this objective might be attained by increasing sample size and number of replications. Representative conditions are particularly pertinent in interregional estimation of the natural occurrence of toxin. State corn yield trials are grown annually under uniform conditions and include hybrids and agronomic practices normally employed in a region. They provide a potential resource for acquiring representative samples for aflatoxin investigations.

The current study was carried out to provide information on: 1) variability in aflatoxin levels between test hybrids in plots of a state yield trial, 2) location differences in toxin distribution within a state, 3) identification of variation in susceptibility to aflatoxin contamination among hybrid maturity groups, and 4) association between BGY fluorescence kernels and aflatoxin concentrations.

MATERIALS AND METHODS

The study involved acquisition of 10-lb samples of shelled corn from each of six replications per hybrid from the 1979 state corn yield trials in South Carolina (Davis et al 1980, Whitaker and Dickens 1979). Ten hybrids were selected from each of three maturity groups, based on the time between planting and

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midsilking. The groups were: short season, 68–72 days; medium season (MS), 72–77 days; and full season, 77–83 days. The Florence and Blackville locations in the coastal plain of the state were selected for the study; although they are about 75 miles apart, soil types and weather conditions are similar at the two sites. Corn was planted during the third week of March at Blackville and the first two weeks of April at Florence. Samples were harvested during September at both locations. Corn samples were dried immediately after harvest to below 13% moisture and sent to the Southern Regional Research Center (New Orleans) for processing and aflatoxin assay. The number of BGY fluorescent particles in each 10-lb sample was determined in the instrument designed by Barabolak et al (1978). Subsequently, the samples were ground in a Raymond hammer mill with a screen containing 3.2-mm perforations; comminuted particles passed a No. 20 sieve. The ground sample was blended for 15–30 min in a Patterson Kelley twin-shell blender. Blended samples were assayed for aflatoxin by AOAC methods (1975). Quantities of aflatoxin were determined on activated thin-layer chromatographic plates covered with 0.5 mm of Adsorbosil-1; plates were developed with water:acetone: chloroform (1.5:12:88, v/v) in unequilibrated tanks, and fluorescent zones were measured densitometrically (AOAC 1975). Aflatoxin B_1 was confirmed in representative positive samples by the formation of the water adduct (AOAC 1975).

RESULTS AND DISCUSSION

Aflatoxin levels in 30 test hybrids from the state corn yield trials in Florence and Blackville demonstrated significant variation within each location (Table I). Analysis of variance of the results showed a significant interaction between variety and location; this observation underscores the importance of location effects on the susceptibility of a particular hybrid to preharvest aflatoxin contamination. The absence of a consistent pattern in hybrids of susceptibility to kernel infection by toxin-producing fungi reduces the utility of using only one hybrid as an interlocation probe for toxin accumulation.

Mean aflatoxin levels in kernels of hybrids grouped by maturity also demonstrated interlocation differences (Table II). Mean toxin levels in the three maturity groups were below 20 ppb in samples from Blackville; similar material from Florence exhibited levels exceeding 20 ppb. Although no differences in mean toxin accumulation were observed among the Blackville maturity groups, the variability in aflatoxin distribution at the location is highlighted by the MS hybrid samples, which had an 11-ppb mean with a range of 0–240 ppb. Maturity groups at Florence did exhibit significant variation; MS hybrid samples had the highest toxin levels. However, the absence of a pattern of maturity group differences common to the Florence and Blackville samples indicates that location effects contribute significantly to maturity group susceptibility to the toxin-producing fungi. The data suggest that the use of specific hybrid maturity groups as probes for aflatoxin contamination is confounded by unique location differences. Soil and weather conditions at the two locations are quite similar; however, the environmental factors responsible for the interlocation and hybrid maturity group differences in susceptibility to aflatoxin contamination may be subtle and reflect other ecological interactions. For example, corn was planted about two weeks earlier at Blackville than at Florence; differences in the subsequent maturity stages during crop development could contribute to variation in kernel infection by toxin-producing fungi.

The distribution of aflatoxin levels showed that even though the mean levels of toxin in kernels from Blackville were below 20 ppb, the toxin was detected in about two thirds of the samples (Table III). A 99% incidence of toxin-positive samples was found in the corn from Florence, with the MS maturity group exhibiting the

TABLE I
Aflatoxin B_1 Levels (mean ppb) in Corn Kernels of Thirty Hybrids Grown in South Carolina in 1979[a]

Brand	Hybrid	Location	
		Florence	Blackville
Pioneer	3145	222 a	2 b
Golden Harvest	2775A	158 ab	6 b
DeKalb	XL72B	156 abc	2 b
Funks	G4507	150 abc	13 b
DeKalb	394	140 abc	26 ab
McCurdy	779	140 abc	1 b
Pioneer	3369A	130 abc	13 b
FFR	929W	130 abc	48 a
Ring Around	2601	120 abc	6 b
Ring Around	1501	116 abc	0 b
Pioneer	3147	101 abc	8 b
DeKalb	XL80	92 abc	0 b
Northrup King	PX95	88 abc	2 b
Coker	22	88 abc	19 ab
Ring Around	1502	66 bc	10 b
McNair	X300	61 bc	2 b
Northrup King	PX79	61 bc	1 b
Paymaster	9792	52 bc	2 b
McCurdy	84AA	48 bc	4 b
Paymaster	12052	46 bc	2 b
McNair	488	43 bc	22 ab
McNair	508	41 bc	26 ab
Paymaster	8951	36 bc	5 b
McCurdy	6714	33 bc	1 b
Coker	77	32 bc	15 b
DeKalb	395A	32 bc	4 b
Pioneer	3030	20 bc	1 b
McCurdy	7625	19 bc	5 b
McCurdy	76101	17 bc	0 b
DeKalb	1295	16 c	22 ab

[a]Duncan's multiple range test was used; means followed by the same letter are not significantly different (5% level).

TABLE II
Mean and Range Levels of Aflatoxin in Preharvest Corn Kernels From Three Maturity Groups of Hybrids Grown in Blackville and Florence, SC, in 1979

Location	Hybrid Group[a]	Aflatoxin B_1 (ppb)	
		Mean	Range
Blackville	FS	10	0–128
	MS	11	0–240
	SS	7	0–72
Florence	FS	30[b]	0–146
	MS	121	3–867
	SS	93	3–571

[a]FS = full season, MS = medium season, SS = short season.
[b]The three values differ significantly (0.05 level).

TABLE III
Distribution of Aflatoxin B_1 Levels and Bright Greenish Yellow (BFY)-Fluorescing Kernels in Corn Samples from Three Maturity Groups of Hybrids[a] Grown in Blackville and Florence, SC, in 1979

Incidence[b]	Sample Distribution (%)											
	Blackville						Florence					
	Aflatoxin			BGY Kernels			Aflatoxin			BGY Kernels		
	SS	MS	FS	SS	MS	FS	SS	MS	FS	SS	MS	FS
ND[c]	48	39	20	8	4	10	...	...	2	...	...	2
⩽9	35	50	64	58	52	48	3	3	37	3	2	7
10–19	3	...	5	27	36	25	9	5	15	25	2	28
20–39	7	7	3	5	4	15	20	14	20	32	5	37
40–79	7	...	5	2	2	2	32	22	18	28	25	22
80–159	...	2	3	...	2	...	24	32	8	7	50	5
160–319	...	2	...	...	...	...	5	19	...	5	14	...
320–639	...	...	...	...	...	...	7	3	...	...	2	...
⩾640	...	...	...	...	...	...	...	2	...	...	...	...

[a]FS = full season, MS = medium season, SS = short season.
[b]Aflatoxin (ppb) or BGY kernels (number) per sample.
[c]ND = none detected.

highest mean toxin level (121 ppb) and having the most samples in the upper toxin level categories; 5% of the samples contained toxin exceeding 320 ppb. The general distribution of toxin resembles the pattern observed in a 1973 survey of South Carolina corn (Lillehoj et al 1975). Both 1973 and 1979 provided conditions that were considered good to excellent for corn production, whereas 1977 (McMillian et al 1978) was marginal for corn production in the southeastern United States because of elevated temperatures and drought conditions. Although the mean levels of toxin from the 1979 South Carolina yield trials in the current study were relatively low compared to samples obtained in the region in other years (Lillehoj et al 1980), the overall incidence of aflatoxin in the yield trials exceeded 80%. The results suggest that toxin-positive samples can be acquired with relative ease from the state yield trials in South Carolina during good crop production years.

More than 95% of the samples had at least one BGY-fluorescing kernel or kernel fragment (Table III). The pattern of BGY distribution resembled the aflatoxin occurrence; values from Blackville were lower than those from Florence. The highest incidence of fluorescing material was observed in the MS hybrids grown at Florence.

To examine the relationship between the number of BGY particles and aflatoxin concentrations, the linearity of the association was determined by regression analyses. The Blackville results were not significant, but the Florence data showed a consistent association. The best-fit equation from the Florence results was: aflatoxin B_1 (ppb) = 19.6 + 1.1 BGY (number of particles). The results suggest that BGY screening of 180 10-lb samples of 30 hybrids at a yield trial location could provide a meaningful estimate of the aflatoxin concentration if the mean number of fluorescing particles exceeds four and the minimum range is approximately two to six fluorescing units in each 10-lb sample.

An overview of the results obtained in the one-year study demonstrates areas of opportunity and limitations in the utilization of state corn yield trials as probes for the preharvest contamination of the crop by aflatoxin. Because the two test locations were only 75 miles apart, the differences in toxin incidence and levels confine the extrapolation of test results to corn fields that are proximate to the test sites. Because no specific hybrid or maturity group of hybrids provided a consistent pattern of enhanced sensitivity to aflatoxin accumulation, identification of an effective representative sampling procedure requires utilization of a number of hybrids from all three maturity groups. Because 1979 was a good corn production year and toxin levels were relatively low, the presence of detectable toxin in two thirds of the samples from the location with the lowest mean toxin levels demonstrates the ready access of toxin-positive samples if the appropriate number of hybrids, number of replications, and sample size are used to define a representative basis of aflatoxin contamination. The association between the number of BGY-fluorescing particles and aflatoxin concentrations was significant at the location with the highest incidence of the two variables; this suggests that the number of BGY-fluorescing units can be utilized as an indicator of the level of toxin in the sample if the means of six replications of 30 hybrids exceeds four fluorescing kernels, with an equivalent estimate of 80 ppb aflatoxin.

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LITERATURE CITED

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. 1975. Official Methods of Analyses, 12th ed. Sections 26.037–26.055 and 26.A17. The Association: Washington, DC.

BARABOLAK, R., COLBURN, C. R., JUST, D. E., KURTZ, F. A., and SCHLEICHERT, E. A. 1978. Apparatus for rapid inspection of corn for aflatoxin contamination. Cereal Chem. 55:1065.

DAVIS, N. D., DICKENS, J. W., FREIE, R. L, HAMILTON, P. B., SHOTWELL, O. L., and WYLLIE, T. D. 1980. Protocols for surveys, sampling, post-collection handling, and analysis of grain samples involved in mycotoxin problems. J. Assoc. Off. Anal. Chem. 63:95.

KWOLEK, W. F., and SHOTWELL, O. L. 1979. Aflatoxin in white corn under loan. V. Aflatoxin prediction from weight percent of bright greenish-yellow fluorescent particles. Cereal Chem. 56:342.

LEE, L. S., LILLEHOJ, E. B., and KWOLEK, W. F. 1980. Aflatoxin distribution in individual corn kernels from intact ears. Cereal Chem. 57:340.

LILLEHOJ, E. B. 1979. Natural occurrence of mycotoxins in feeds: Pitfalls in determination. Page 139 in: Interactions of Mycotoxins in Animal Production. Proceedings of a Symposium, July 1978, Michigan State University, E. Lansing. Nat. Academy of Sci.: Washington, DC.

LILLEHOJ, E. B., and HESSELTINE, C. W. 1977. Aflatoxin control during plant growth and development. Page 107 in: Rodericks, J. W., Hesseltine, C. W., and Mehlman, M. A., eds. Proceedings of the First International Conference on Mycotoxins in Human and Animal Health, October 1976, University of Maryland, College Park. Pathotox Publishers: Park Forest, IL.

LILLEHOJ, E. B., KWOLEK, W. F., SHANNON, G. M., SHOTWELL, O. L., and HESSELTINE, C. W. 1975. Aflatoxin occurrence in 1973 corn at harvest. I. A limited survey in the southeastern U.S. Cereal Chem. 52:603.

LILLEHOJ, E. B., KWOLEK, W. F., ZUBER, M. S., HORNER, E. S., WIDSTROM, N. W., GUTHRIE, W. D., TURNER, M., SAUER, D. B., FINDLEY, W. R., MANWILLER, A., and JOSEPHSON, L. M. 1980. Aflatoxin contamination caused by natural fungal infection of preharvest corn. Plant Soil 54:469.

MANWILLER, A., and FORTNUM, B. 1979. A comparison of aflatoxin levels in preharvest corn from the South Carolina Coastal Plain for 1977–78. Res. Series No. 101. SC Agric. Exp. Stn., Clemson University, Clemson.

McMILLIAN, W. W., WILSON, D. M., and WIDSTROM, N. W. 1978. Insect damage, *Aspergillus flavus* ear mold, and aflatoxin contamination in South Georgia corn fields in 1977. J. Environ. Qual. 7:564.

WHITAKER, T. B., and DICKENS, J. W. 1979. Variability associated with testing corn for aflatoxin. J. Am. Oil Chem. Soc. 56:789.

WIDSTROM, N. W., WISEMAN, B. R., MCMILLIAN, W. W., KWOLEK, W. F., LILLEHOJ, E. B., JELLUM, M. D., and MASSEY, J. H. 1978. Evaluation of commercial and experimental three-way corn hybrids for aflatoxin B_1 production potential. Agron. J. 70:986.

ZUBER, M. S., and LILLEHOJ, E. B. 1979. Status of the aflatoxin problem in corn. J. Environ. Qual. 8:1.

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