# Aflatoxin Contamination of Corn Hybrids in Alabama[1]

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**ABSTRACT**

Aflatoxin contamination was determined for 215 corn hybrids; 38 were grown for 4–6 years and an additional 177 for 1–3 years. They were grown at 12 locations in Alabama from 1976 until 1981. In 1977, corn from the southern region of the state averaged 1,188 ppb of aflatoxin $B_1$ and that from the central and northern regions averaged 47 and 66 ppb, respectively. In 1980, each region averaged approximately 200 ppb. Contamination levels were mostly insignificant in 1978 with averages ranging from 5 to 12 ppb in all regions and in 1979 in the central and northern regions, whereas contamination averaged 110 ppb in the southern region in 1979. Contamination levels in 1976 and 1981 were low and uniform throughout the state, ranging from 5 to 39 ppb. It was concluded that there was no resistance to aflatoxin formation in any hybrid tested, and significant aflatoxin levels generally accompanied stress caused by high temperature, low rainfall, low-moisture-holding capacity of sandy soils, and insect infestation.

Aflatoxin in corn was considered primarily a stored grain problem during the 1960s. Anderson et al (1975) first demonstrated preharvest contamination of corn with aflatoxin in 1971 after a comprehensive survey involving essentially all of the corn-producing areas of the United States. Aflatoxin was found in the first field sampling when the corn was in the late milk state of development. However, there appeared to be no further increase in aflatoxin levels in corn left standing in the field up to three months after normal harvest time. The highest incidence of aflatoxin was found in corn from the warmer and more humid growing regions, i.e., the southeastern states. In 1972, aflatoxin was detected in 30% of 1,283 truckloads (12,000 metric tons) of white corn (1971 crop) delivered to an elevator from seven counties of southeastern Missouri (Shotwell et al 1975). Other investigations confirmed that preharvest contamination of corn by *Aspergillus flavus* and aflatoxin was widespread and prevalent in the Southeast (Hesseltine et al 1976, Lillehoj et al 1975a and b, Rambo et al 1974, Shotwell et al 1977).

Obviously, utilization of corn hybrids resistant to invasion by *A. flavus* and aflatoxin formation would be the most direct way to prevent or minimize aflatoxin contamination. In research with 50 hybrids and 15 sweet corn inbreds, Widstrom et al (1978) found no difference in 48 hybrids and sweet corn inbreds. One full-season and one short-season hybrid had significantly more natural infection than the other hybrids. Comparison of four hybrids and eight open-pollinated varieties in seven southeastern states and Hawaii showed that aflatoxin incidence and levels are correlated with location and crop year, but not with genotype, except for one highly susceptible variety (Stoloff and Lillehoj 1981, Zuber et al 1983). In South Carolina, a study with 26 corn hybrids revealed that kernels of short-season hybrids contain elevated levels of aflatoxin as compared to mid- and long-season hybrids (Lillehoj et al 1983). However, no hybrid showed significant resistance to aflatoxin formation.

Fortnum and Manwiller (1985) found no difference in aflatoxin levels in 15 commercial varieties regardless of whether the kernels were injured or not prior to inoculation; irrigation also had no effect. Inoculation of kernels of eight crosses of inbreds with *A. flavus* after pinboard injury resulted in less aflatoxin with two of the inbreds, suggesting to Thompson et al (1984) that resistance was under genetic control. In Georgia, sweet corn and dent corn single crosses were knife-inoculated, and the data led Widstrom et al (1984) to conclude that two of three inbreds within each set performed well enough as single crosses to be used as sources of resistance. They also noted that experimental inoculation methods did not always produce enough contamination to identify resistant genotypes. Thus, data obtained by injury plus *A. flavus* spore inoculation of kernel, ear, and silk were suspected of masking practical field resistance that might be present as well as failing to yield infection levels adequate to differentiate among genotypes (King and Wallin 1983).

This investigation was initiated in 1976 on a small scale, but after the aflatoxin epidemic of 1977 and development of the fluorometric-iodine rapid screen method of analysis (Davis et al 1981), we screened large numbers of hybrids for resistance to natural field contamination. This paper summarizes the results of aflatoxin analyses of samples of 215 commercial hybrids and breeding lines from 20 seed companies that were grown at 12 locations throughout Alabama during the six years from 1976 to 1981.

## MATERIALS AND METHODS

Corn hybrid yield trials of the Auburn University Department of Agronomy and Soils were conducted at 12 outlying units of the Alabama Agricultural Experiment Station. Locations in southern Alabama were Brewton Experimental Field, Brewton; Gulf Coast Substation, Fairhope; Monroeville Experimental Field, Monroeville; and Wiregrass Substation, Headland. Locations in central Alabama were Black Belt Substation, Marion Junction; Lower Coastal Plain Substation, Camden; Piedmont Substation, Camp Hill; Prattville Experimental Field, Prattville; and E. V. Smith Research Center, Shorter. Locations in northern Alabama were Sand Mountain Substation, Crossville; Tennessee Valley Substation, Belle Mina; and Upper Coastal Plain Substation, Winfield.

Corn hybrids were grown with recommended plant populations, fertilizer rates, and cultural practices (Carden 1976, 1977; Currier 1978, 1980, 1981, 1982). Corn ears were shucked by hand and dried in burlap sacks to approximately 14–16% grain moisture content. Grain was removed from cobs with motorized shellers, and the moisture content was determined with a model 700 Burroughs moisture tester. A 1-lb sample of shelled corn was then taken from each hybrid and placed in a paper sack, which was labeled, stapled, and sent to Auburn University. Samples were ground in a hammermill to pass a 1-mm screen and stored at 14–16% moisture (15.5% is the standard for No. 2 corn) and 35–40°C until subsampled for aflatoxin analyses.

Thirty-eight corn hybrids were grown for 4–6 years at one or more locations, sampled, and analyzed for aflatoxin: Coker 16, 22, 56, 77B; DeKalb XL-72B, 80, 394; Funks G-795W-1, 4507, 4507A, 4611, 4747W-1, 4776, 4810, 4864, 4949A, 5945; Golden Harvest H2500, 2775; McCurdy 67-14, 84AA; Northrup-King-McNair PX-79, 95, S-338, X-300, 508; Paymaster UC-8951; Pioneer 3030, 3145, 3147, 3368A, 3369A; Ring Around 1501, 1502, 2602W; and Trojan TXS-114, 115A, 119. The 177 corn hybrids that were grown for 1–3 years are identified by Davis et al (1985).

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Seed was supplied by seed companies for the agronomic trials, and the removal of old hybrids and the addition of new ones for testing were at least partly decided by the seed producers. In the first two years of this study, a random selection of hybrids for analysis from several locations was made by E. H. Carden. However, after the aflatoxin outbreak of 1977, each hybrid grown at the 12 locations was sampled and analyzed for several years. Among the 177 hybrids tested for 1–3 years, the number of hybrids × locations that were sampled and analyzed varied from 1 to 34, with over 82% of the hybrids being tested less than 10 times, whereas 31 hybrids were evaluated 10 to 34 times for aflatoxin.

The numbers of corn hybrid samples analyzed each year for aflatoxin were: 95 (1976), 86 (1977), 481 (1978), 668 (1979), 572 (1980), and 450 (1981). Samples of corn were analyzed for aflatoxin by the Pons aqueous acetone method for cottonseed (Pons and Goldblatt 1965), as modified and described in AOAC methods 26.052–26.060 in 1976 and 1977 (Association of Official Analytical Chemists 1980). From 1978 to 1981 analyses were made by the fluorometric-iodine rapid-screen method with one modification (Davis et al 1981).

## RESULTS

Data on the aflatoxin levels of 177 corn hybrids grown only 1–3 years at 12 locations were similar to those summarized in Table I. In years of low aflatoxin occurrence (1978, 1979), these hybrids showed little or no aflatoxin contamination. In 1977 and 1980, which were epidemic years for aflatoxin in corn in the Southeast, they showed high levels (100–350 ppb) as did the other reported hybrids (Table I). In 1981, most hybrids had low levels of aflatoxin contamination (10–50 ppb) at nearly every location.

Data from the aflatoxin analyses of 38 corn hybrids grown at 12 locations in at least four of the six years from 1976 to 1981 are summarized in Table I by region within Alabama and presented in detail by Davis et al (1985). Table I, which shows the level of aflatoxin contamination of 26 corn hybrids grown in four southern locations near Brewton, Fairhope, Monroeville, and Headland, reveals high mean aflatoxin levels in the epidemic years of 1977 and 1980 of 1,180 and 209 ppb, respectively, compared with aflatoxin levels that were much lower in 1976, 1978, 1979, and 1981. In 1976, when only 19 samples of 15 hybrids were analyzed, only one sample from Brewton was heavily contaminated (200 ppb), whereas samples of two other hybrids from Headland contained about 50 ppb of aflatoxin $B_1$. In 1978, Headland was the only southern location that had samples appreciably contaminated, with five hybrids averaging 198 ppb, while 18 others were uncontaminated. In 1979, samples from Monroeville were thought to have been contaminated postharvest because of the high aflatoxin level that averaged 319 ppb. Only three hybrids from Headland were heavily contaminated and 18 were uncontaminated. Also, the 23 hybrids at Brewton and the 19 hybrids at Fairhope were uncontaminated that year. In 1981, nearly every hybrid was contaminated, but at levels averaging only 14 ppb in 66 hybrids at three locations (a trial was not carried out at Headland in 1981).

**TABLE I**
**Aflatoxin Contamination of Corn Hybrids Grown 4–6 Years, 1976–1981**

| Location | No. of Varieties Infested[a] | | | | | |
|---|---|---|---|---|---|---|
| | 1976 | 1977 | 1978 | 1979 | 1980 | 1981 |
| Southern Alabama | | | | | | |
| Brewton | 11 | 10 | 39 | 37 | 40 | 39 |
| Fairhope | 0 | 0 | 43 | 65 | 76 | 77 |
| Monroeville | 10 | 10 | 40 | 37 | 39 | 39 |
| Headland | 8 | 1 | 73 | 71 | 37 | 1 |
| Aflatoxin (mean in ppb) | 5 | 1,180 | 12 | 110 | 209 | 14 |
| Central Alabama | | | | | | |
| Camden | 0 | 9 | 34 | 39 | 40 | 40 |
| Camp Hill | 10 | 0 | 39 | 39 | 40 | 0 |
| Shorter | 10 | 0 | 47 | 74 | 1 | 50 |
| Marion Junction | 9 | 0 | 35 | 31 | 35 | 38 |
| Prattville | 6 | 0 | 1 | 41 | 42 | 38 |
| Aflatoxin (mean in ppb) | 39[b] | 47 | 5 | 9 | 196 | 28 |
| Northern Alabama | | | | | | |
| Belle Mina | 10 | 20 | 43 | 74 | 43 | 38 |
| Crossville | 12 | 18 | 36 | 76 | 86 | 14 |
| Winfield | 9 | 19 | 24 | 51 | 43 | 39 |
| Aflatoxin (mean in ppb) | 21[c] | 66 | 8 | 2 | 178 | 24 |

[a] Number of varieties tested that year. Data by variety and year have been published by Davis et al (1985).
[b] Adjusted for one skewed sample from Shorter.
[c] Adjusted for three skewed samples from Crossville.

In 1977 and 1980, corn was under severe drought stress, and the level of insect infestation was high throughout the state, resulting in drastic yield reductions. Five-year average corn yields (1972–1976) for the northern, central, and southern locations were 128, 94, and 97 bu/acre, respectively, whereas in 1977 yields were 66, 0, and 50 bu/acre, respectively. In 1980, yields were reduced 50% at eight of 12 locations. Also in 1977 and 1980, almost every hybrid planted at all locations was contaminated with aflatoxin at levels exceeding 100 ppb. In 1977, four samples from Monroeville averaged 2,500 ppb, and six hybrids from Brewton averaged 300 ppb. Armyworm infestation was so severe at the other two locations that samples were deemed worthless. In 1980, 91 of 99 samples contained aflatoxin exceeding 100 ppb with a mean of 209 ppb. Drought (water stress) exacerbated the level of aflatoxin contamination as well as the incidence of contamination of hybrids tested in 1980. Finally, the incidence of aflatoxin was high in 1981, but aflatoxin levels were low, averaging only 14 ppb. Both aflatoxin level and incidence were low in 1976, 1978, and 1979, (omitting the Monroeville data of 1979).

Table I presents the aflatoxin contamination data of 21 corn hybrids grown in five central Alabama locations. Aflatoxin was comparatively high in 1977 and 1980 (47 and 196 ppb, respectively) compared with only 5 ppb in 1978, 9 ppb in 1979, and 28 ppb in 1981. The high mean of aflatoxin calculated for 1976 (170 ppb) is an anomaly and can be disregarded as this was skewed by the 2,000-ppb value of a single sample from Shorter, probably resulting from postharvest contamination; the mean level is then 39 ppb. Generally, in the five central Alabama locations, the incidence of aflatoxin contamination was high in three of the six years (1977, 1980, 1981), but the mean level of contamination was many times higher in 1980 than in 1981 (196 versus 28 ppb).

Table I also provides data on 22 corn hybrids grown in three northern locations near Belle Mina, Crossville, and Winfield. Here, a high incidence and high mean level of aflatoxin contamination occurred in 1976 (413 ppb), 1977 (66 ppb), and 1980 (178 ppb). Three highly contaminated samples (1,000–3,333 ppb) from Crossville are believed to have resulted from postharvest contamination, thereby distorting the data for 1976. Omitting these three samples from this one location, the mean aflatoxin level for 1976 was 21 ppb, similar to the levels at other locations in 1978 (8 ppb), 1979 (2 ppb), and 1981 (24 ppb). The incidence of aflatoxin contamination was also high in 1981 in the northern locations, but aflatoxin levels were low. Contamination was generally higher in hybrids grown at Crossville in 1980 than at the other northern locations.

## DISCUSSION

### Preharvest Contamination

In Alabama, preharvest contamination of corn with aflatoxin (1976–1981) generally ranged from 20 to 150 ppb, although most samples showed no visible fungal infection. However, under stress induced by drought, high temperatures, and insect infestations, preharvest toxin contamination occasionally reached much higher levels (2,000–5,000 ppb). Preharvest contamination occurs when *A. flavus* colonizes corn silks during the first two weeks of silking and just after pollination and invades the developing kernels 4–13 days later (Jones et al 1981, Payne 1983). Infection is favored by

temperatures of 30–34° C (Jones et al 1980). Water stress (drought) apparently increases the amount of inoculum (spore loads) rather than plant susceptibility (Jones et al 1981, Payne 1983, Thompson et al 1980). In many years, only a relatively small number of kernels is contaminated, but they may contain high levels of aflatoxin. Alternately, infection may occur following inoculation of kernels injured by insect vectors with subsequent production of moderate to high levels of aflatoxin in the damaged kernels.

**Postharvest Contamination**

Aflatoxin can be a serious postharvest problem in the South and anywhere in the world when commodities such as corn are harvested moist or are not promptly dried to safe storage moisture, which is the kernel moisture in equilibrium with 70% relative humidity. Corn, other grains, peanuts, and other crops require drying to safe storage moisture after harvest to prevent fungal deterioration. Safe storage moisture then must be maintained in facilities adequate to prevent moisture buildup to avoid fungus growth and subsequent toxin formation. Safe storage moisture for corn is approximately 13%, although No. 2 corn is marketed at 15.5%. Aflatoxin levels generally become high in postharvest-contaminated corn compared with preharvest-contaminated corn, frequently attaining levels of 2,000–5,000 ppb of aflatoxin $B_1$ in southeastern states.

In 1965, we verified the first instance of aflatoxin contamination of corn in Alabama (*unpublished data*), when a farmer near Selma lost most of his newly farrowed pigs after feeding corn that contained 2,000–8,000 ppb of aflatoxin from postharvest contamination.

**Genetic Control**

In our investigation, analysis of aflatoxin data for six years of over 200 corn hybrids grown throughout Alabama indicated that there was no resistance to aflatoxin formation in any hybrid tested (Davis et al 1985). In 1977 and 1980, which were epidemic years with high levels of aflatoxin being common through the Southeast, all hybrids tested were contaminated with moderate to high levels of aflatoxin. In these two years, preharvest contamination resulted in high levels that caused serious problems for both corn and animal agribusiness (Diener et al 1983, Nichols 1983). In those years, a considerable acreage of the corn crop was plowed under because of the combined effect of low yield and preharvest contamination. Corn from most locations showed little or no contamination in 1976, 1978, and 1979, except at southern locations of Headland in 1978 and Monroeville in 1979. Also, low levels of 10 to 50 ppb were common at all locations in all hybrids in 1981, which was a year of high incidence coupled with low levels of aflatoxin contamination.

The mean levels of aflatoxin contamination in corn hybrids by region were relatively low in all regions in 1976, 1978, 1979, and 1981 (Table I), except for the southern region in 1979. Aflatoxin contamination was high in all regions in 1977 and 1980, but it was highest in the southern region. The high incidence and high level of aflatoxin periodically occurring in preharvest corn in the southern region was generally the result of stress caused by a combination of high temperature, low rainfall, insect infestation, and low-moisture-holding capacity of the area soils.

After extensive research, Widstrom and Zuber (1983), convinced that aflatoxin production in corn is under genetic control, attributed the inability to repeat differences in aflatoxin levels in preharvest commercial maize hybrids in comparisons over locations and years as the major obstacle in developing a genetically resistant hybrid. Because the nature and mechanisms of resistance have not been elucidated, and because their results have not been consistently reproduced, it appears that little real progress has been made in developing hybrids with direct genetic resistance to aflatoxin contamination in corn. Nevertheless, development of genetic resistance to *A. flavus* invasion and aflatoxin formation in corn kernels remains the most practical way to seek control of the aflatoxin problem in corn. Resistance has been highly successful for the control of several fungal pathogens of corn. Unfortunately, *A. flavus* is not an aggressive plant pathogen. Instead it is a saprophyte or weak parasite, and thus, it is possible that control by plant breeding for resistance may not be attainable by traditional experimental procedures.

Although we believe that genetic resistance to aflatoxin contamination has not been demonstrated to exist in commercial maize hybrids, and that research in this area does not seem promising, the important search for resistance cannot be abandoned. Possible innovative and unique sources of resistance may be obtainable through the application of the tools of modern biotechnology. For example, recent research (Schmidt et al 1983) demonstrated the presence of extrachromosomal elements, including double-stranded RNA, in a nontoxigenic strain of *A. flavus*. When treated with cycloheximide or emetine (antifungal antibiotics), this strain became a toxin producer. Thus, genetic elements that prevent aflatoxin biosynthesis may be present in this strain of *A. flavus*. Attempts are now underway to transfer these genetic elements to toxigenic strains of *A. flavus* and *A. parasiticus* to determine if they will prevent aflatoxin formation by normally toxigenic strains of these fungi. If this research is successful, then by accomplishing transfer of the inhibitory genetic elements (double-stranded RNA, DNA plasmids, or whatever) to corn or other higher plants via protoplast fusion and other techniques, we might create a source of resistance to aflatoxin formation or *A. flavus* invasion for utilization by the plant breeder.

The possibility of developing and utilizing genetic resistance to aflatoxin contamination may still exist in exotic material, such as flint corn, maize endosperm mutants, teosinte (*Zea mexicana* K.), and diploid *Zea perennis* R. & M. Use of pinboard, knife, and needle injury plus inoculation techniques probably result in overkill and loss of potential practical field resistance. Silk inoculation for evaluating segregating generations in combination with site or controlled environment (phytotron) facilities might improve progress in selecting resistant varieties via experimental utilization of the natural mode of infection and induced water stress.

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