Response of Corn Hybrids to Aflatoxin Formation by *Aspergillus flavus*

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Response of Corn Hybrids to Aflatoxin Formation by *Aspergillus flavus*[1]

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INTRODUCTION

CONTAMINATION OF CORN (*Zea mays* L.) by *Aspergillus flavus* (yellow mold) was first reported in 1920 by Taubenhaus (*36*), who also recognized that the fungus was a saprophyte that required kernel damage in the milky state for invasion. From 1930 to 1959, similar observations were made by investigators in Florida (*15*), Illinois (*21*), Kansas (*26*), and Indiana (*39*) prior to the discovery of the toxic effects of aflatoxin in peanuts in 1961 (*22*) and its chemical characterization in 1963 (*2*). Moldy corn toxicosis, probably caused by aflatoxin, occurred in the fall of 1952 with 24 outbreaks being recorded in Georgia; 23 of these affected swine and one affected cattle (*34*). Burnside et al. (*4*) isolated *A. flavus* and *Penicillium rubrum* from the toxic corn and found that these two fungi, when cultured on sterilized corn and fed to swine, reproduced symptoms of moldy corn toxicosis.

Aflatoxin in corn was considered primarily a stored grain problem during the 1960's. Anderson et al. (*1*) first demonstrated preharvest contamination of corn with aflatoxin in 1971 following a comprehensive survey and sampling program 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 stage of development. However, there appeared to be no further increase in aflatoxin levels in corn left standing in the field up to 3 months after normal harvest time. The highest incidence of aflatoxin was found in

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corn from the warmer and more humid growing regions, i.e. the Southeastern States. In 1972, aflatoxin was detected in 30 percent of 1,283 truckloads (12,000 tons) of white corn (1971 crop) delivered to an elevator from seven counties in southeastern Missouri (33). Other investigations soon confirmed the contention that preharvest contamination of corn by *A. flavus* and aflatoxin was widespread and prevalent in the Southeast (*17,23,24,30,32*).

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; consequently, considerable research has been conducted to identify such resistance in corn hybrids. In one experiment (*41*), 10 full-season and 10 short- to mid-season hybrids from three locations in the Georgia Coastal Plains were evaluated for aflatoxin levels resulting from natural infection by *A. flavus* during 1974-76. In each group, one hybrid had significantly more aflatoxin than the other 9 hybrids, but no differences were detected among 30 dent and 15 sweet corn inbreds. In a 1979-80 study (*35,43*), four widely grown hybrids and eight open-pollinated varieties were grown in seven Southeastern States and Hawaii to determine susceptibility to preharvest aflatoxin contamination. It was found that aflatoxin incidence and levels were correlated with location and crop year, but not with genotype, except for one highly susceptible variety. In another study (*25*), 26 corn hybrids from field trials in South Carolina were evaluated for interrelationships among aflatoxin level, moisture content at harvest, and bright greenish-yellow (BGY) fluorescence resulting from natural field infection by *A. flavus*. Results showed that kernels of short-season hybrids contained elevated levels of aflatoxin, moisture, and BGY fluorescence as compared to mid-season and full-season hybrids. No hybrid showed significant resistance to aflatoxin.

The effects of kernel injury, irrigation, inoculation of uninjured kernels with *A. flavus* spores, and natural insect damage on aflatoxin formation in 15 commercial hybrids (5 each of short-, mid-, and full-season) were evaluated during 1979 and 1980 in South Carolina (*16*). No hybrid showed consistently lower aflatoxin levels in comparison to the other 14 hybrids, regardless of whether kernels were injured prior to inoculation. Also, there was no difference in the level of contamination of the three maturity groups whether irrigated or not. In a study by Thompson et al. (38), developing kernels of eight maize single crosses (two A-inbreds X four B-inbreds) were inoculated with *A. flavus* after kernel injury via pinboard in four environments and assayed for aflatoxin. Two B-inbreds averaged 50 percent less aflatoxin than the other two B-inbreds (17-23 p.p.m. vs. 30-42 p.p.m.). The authors, therefore, suggested from this study that susceptibility or resistance to aflatoxin accumulation in developing kernels was under genetic control. Widstrom et al. (*40*) grew an eight-line diallel of sweet corn single crosses and a nine-line diallel of dent single crosses in replicated experiments for 3 years (1978-81) to identify resistant genotypes for incorporation into a breeding population for recurrent selection. Ears were knife-inoculated 20 days after full silk with an *A. flavus* spore suspension and grain was analyzed at maturity for aflatoxin contamination. The data led these authors to conclude that two or 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 seldom produced enough contamination to allow them 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 (*20*).

The research reported in this bulletin was initiated in 1976 on a small scale, but after the aflatoxin epidemic of 1977 and the development of the fluorometric-iodine rapid screen method of analysis (*12*), large numbers of hybrids were screened for possible resistance to natural field contamination. This report presents 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 6 years from 1976 to 1981.

MATERIALS AND METHODS

Corn hybrid yield trials were conducted at 12 outlying units of the Alabama Agricultural Experiment Station. Locations in southern Alabama were: Brewton Experiment Field, Brewton; Gulf Coast Substation, Fairhope; Monroeville Experiment 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 Experiment 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 (*5-10*). Corn ears were shucked

TABLE 1. CORN HYBRIDS GROWN FOR 1-3 YEARS AT ONE OR MORE LOCATIONS IN ALABAMA, 1976-81

Hybrid ident. and years x location tested	
Asgrow	
RX-90	5
RX-112	2
RX-114	7
RX-140	1
RX-140A	9
RX-777	2
RX-909	5
Aztec	
SX-640	1
SX-644	1
Big D (Taylor)	
2249	2
4204	2
4791	5
4862	12
6986	2
7220	2
Cargill (Security)	
495	4
949	4
951	1
967	1
SS-111	1
SS-112	8
Coker (Greenwood)	
18	17
18A	2
19	13
19A	19
21	2
44	2
45	1
54	13
4034	1
DeKalb	
XL-71	2
XL-72AA	8
XL-72BB	2
XL-78	15
XL-80A	3
XL-80B	4
XL-82	6
XL-95	2
XL-390	2
XL-390A	3
XL-390B	2
XL-395	9
XL-395A	7
XL-808	2
XL-1214	1
XL-1295	1
EX-8914	1
85275	1
Funks	
G-4323	1
G-4522	2
G-4525	5
G-4574	4
G-4606-1	34
G-4636	2
G-4657	2
G-4689	7
G-4709	8
G-4733	3
G-4740A	20
G-4768-W	2
G-4787-W	4
G-4848-2	8
28753	4
29018	2
29092	1
29258	20
Golden Harvest	
H-2606	5
H-2660-W	8
H-2665-W	2
H-2666	3
H-2680	6
H-2686A	2
H-2740A	5
H-2745	1
H-2750	1
H-2655	3
Goldkist (Green)	
GK-695	1
GK-748	9
GK-875	2
GK-915	11
GK-925	1
GK-955	6
GK-1055	1
GK-1175	2
FFR-2325	1
Gutwein	
62	18
72	8
74	6
2875	5
2880	2
2910	22
MDM-2885	3
Jacques (Wilstar)	
JX-177	5
JX-179	2
JX-180	10
Jacques (Wilstar) cont'd.	
JX-227	1
JX-247	4
W-200	2
W-300	7
5555	7
6662	9
McCurdy	
72-24	3
72-44A	4
75-200	5
75-210	3
76-92	2
76-101	7
77-87	18
79-78	5
81-50	6
82-25	2
X-890	1
MSX-88	2
Northrup-King-McNair	
PX-72	1
PX-74	4
PX-83	1
PX-87	8
PX-664	2
PX-675	7
PX-707	1
PX-718W	4
PX-723	13
488	5
X-170	10
X-233	3
EXP-3232	2
P-A-G	
751	5
SX-17A	11
SX-98	14
SX-333	17
SX-346	3
SX-351	3
SX-373	5
262-193	5
Paymaster	
UC-8201	5
UC-9451	15
UC-9532	8
UC-9792	21
UC-9797	2
UC-9902	4
12052	12
12052A	1

Continued

TABLE 1 (CONTINUED). CORN HYBRIDS GROWN FOR 1-3 YEARS AT ONE OR MORE LOCATIONS IN ALABAMA, 1976-81

Hybrid ident. and years x location tested		Hybrid ident. and years x location tested		Hybrid ident. and years x location tested	
Pioneer		**RBA**		**Trojan**	
511-A	27	111	2	T-1120	2
519	3	122	1	T-1189	5
3009	20			T-1230	7
3040	8	**Ring Around**		TXS-113	1
3152	2	1401	2	TXS-116	3
3160	10	1504	6	TXS-119A	14
3179	5	1604	7		
3183	5			**USS AG**	
3184	14	2501	10	1515	15
3311	1	2502	20	2020	4
3320	12	2601	11	2315	17
3535	2	3602	3	2461	2
X-7227	1	3605-W	3	7501	2

by hand at harvest, placed in burlap sacks, labelled with plot numbers, and dried to approximately 14-16 percent grain moisture content. Grain was removed from cobs with motorized shellers, weighed, and the moisture content (range 14 percent ± 2 percent) determined. A 1-pound sample of shelled corn was then taken from each hybrid, placed in a paper sack, which was labelled, stapled, and sent to Auburn University. All samples were ground in a hammermill to pass a 1-mm screen and stored at 15.5 percent moisture and 95-104°F 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. In addition, 177 corn hybrids that were grown for only 1-3 years are listed in table 1.

Seed were supplied by seed companies for the agronomic trials, and the removal of old hybrids and the addition of new ones for testing were partly the decision of the seed producers. In the first 2 years of this study, a random selection of hybrids for analysis from several locations was made by E. L. Carden. However, after the severe aflatoxin outbreak of 1977, every hybrid grown at 12 locations was sampled and analyzed for several years. The number of hybrids times locations that were sampled and analyzed, table 1, varied from 1 to 34, with over 82 percent of the hybrids being tested less than 10 times; 31 hybrids were evaluated 10 to 34 times for aflatoxin.

The number of corn hybrid samples analyzed each year for aflatoxin were: 95 (1976), 86 (1977), 481 (1978), 668 (1979), 572 (1980), and 450 (1981) for a total of 2,352 analyses. Samples of corn were analyzed for aflatoxin by the Pons aqueous acetone method for cottonseed (29), as modified and described in AOAC Methods of Analysis section 26.052-26.060 in 1976 and 1977 (3). Analyses in 1978-81 were made by the fluorometric-iodine rapid screen method (FL-IRS) developed at Auburn by Davis and Diener (*11,12*) with one modification (*13*).

RESULTS

Data on the aflatoxin levels of 177 corn hybrids grown only 1-3 years at 12 locations are not presented, although the hybrids are identified in table 1. The pattern of data on aflatoxin occurrence of the hybrids in table 1 was essentially the same as that recorded in tables 2, 3, and 4. 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 Southeastern corn, they showed high levels (100-350 p.p.b) as did the other reported hybrids, tables 2, 3, and 4. In 1981, most hybrids had low levels of aflatoxin contamination (10-50 p.p.b.) at nearly every location, as did those listed in tables 2, 3, and 4.

Data from the aflatoxin analyses of 38 corn hybrids grown at 12 locations in at least 4 of the 6 years from 1976 to 1981 are presented in tabular form, tables 2-4, by regional location within Alabama. Table 2, 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 p.p.b., respectively, compared with aflatoxin levels that were much lower in 1976, 1978, 1979, and 1981. In 1976, when only 19 samples of 15 hybrids, table 2, were analyzed, only 1 sample from Brewton was heavily contaminated (200 p.p.b.), while samples of 2 other hybrids from Headland contained about 50 p.p.b. of aflatoxin B_1. In 1978, Headland was the only southern location which had samples appreciably contaminated, with 5 hybrids averaging 198 p.p.b., while 18 others were uncontaminated. In 1979, certain samples from Monroeville were thought to have been contaminated postharvest because of the high aflatoxin level that averaged 319 p.p.b. Only 3 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 19 p.p.b. in 66 hybrids at three locations (a trial was not carried out at Headland in 1981).

In 1977, corn was under severe drought stress and high insect infestation throughout the State, resulting in practically every hybrid planted at all locations being contaminated with aflatoxin at levels exceeding 100 p.p.b. In 1977, four samples from Monroeville averaged 2,500 p.p.b. and 6 hybrids from Brewton averaged 300 p.p.b. Armyworm infestation was so severe at the other two locations that samples were deemed worthless for experimental purposes. In 1980, 91 of 99 samples tested contained aflatoxin at levels exceeding 100 p.p.b., with a mean of 210 p.p.b. Drought (water stress) exacerbated the level of aflatoxin contamination as well as the incidence of contamination of all hybrids tested in 1980. Finally, the incidence of aflatoxin was high in 1981, but aflatoxin levels were low, averaging only 19 p.p.b. Both aflatoxin level and incidence were low in 1976, 1978, and 1979.

Table 3 presents the aflatoxin contamination data for 21 corn hybrids grown in five central Alabama locations (Camden, Camp Hill, Shorter, Marion Junction, and Prattville). Mean aflatoxin levels were comparatively high in 1977 and 1980 (47 and 195 p.p.b., respectively) compared with only 5 p.p.b. in 1978, 9 p.p.b. in 1979, and 28 p.p.b. in 1981. The high mean level of aflatoxin calculated for 1976 (170 p.p.b.) is an anomaly and should be disregarded as this was skewed by a single sample's value of 2,000 p.p.b. from Shorter, probably due to postharvest contamination. Generally, in the five central Alabama locations, the incidence of aflatoxin contamination was high in 4 of the 6 years (1976, 1977, 1980, 1981), but the mean level of contamination was many times higher in 1980 than in 1981 (195 vs. 28 p.p.b.).

Table 4 provides data on 22 corn hybrids grown in the three northern locations near Bella Mina, Crossville, and Winfield. Here, a high incidence and high mean level of aflatoxin contamination occurred in 1976 (413 p.p.b.), 1977 (66 p.p.b.), and 1980 (178 p.p.b.). Three highly contaminated samples (1,000-3,333 p.p.b.) from Crossville, believed to have resulted from postharvest contamination, distorted the data for 1976. Omitting these three samples from this one location, the mean aflatoxin level for 1976 was 21 p.p.b., similar to the low levels at other locations in 1978 (8 p.p.b.), 1979 (2 p.p.b.), and 1981 (24 p.p.b.). The incidence of aflatoxin-contamination was high in 1981 in the northern locations, as it was in the central and southern locations. Contamination was generally higher in hybrids grown at Crossville in 1980 than at the other northern locations.

Table 5 presents a summary of the means of the data on aflatoxin occurrence in Alabama in corn hybrids by region and year. The epidemic years of 1977 and 1980 are obvious from these data. It is also obvious that the problem was more severe in the southern locations than elsewhere in the State. In addition, the number of samples analyzed for each mean is indicated. Reasons for the notably smaller number of samples tested for aflatoxin in 1976 and 1977 are explained under materials and methods.

TABLE 2. AFLATOXIN CONTAMINATION OF CORN HYBRIDS GROWN 4-6 YEARS AT FOUR SOUTHERN ALABAMA LOCATIONS, 1976-81

Variety and location	Aflatoxin B_1 in p.p.b.					
	1976	1977	1978	1979	1980	1981
Coker 16						
Brewton	0	–	0	0	115	15
Fairhope	–	–	0	–	114	15
Monroeville	0	2,000	0	–	200	58
Headland	60	–	0	360	96	0
Coker 22						
Brewton	–	–	0	0	105	14
Fairhope	–	–	0	0	108	48
Monroeville	–	–	0	460	208	17
Headland	–	–	475	0	120	–
Coker 77B						
Brewton	–	–	0	0	250	20
Fairhope	–	–	0	0	360	18
Monroeville	–	–	5	480	199	8
Headland	0	–	0	42	375	–
DeKalb XL-80						
Brewton	–	–	0	0	105	17
Fairhope	–	–	0	–	102	19
Monroeville	0	–	0	300	200	5
Headland	–	–	0	0	75	–
DeKalb XL-394						
Brewton	0	–	0	0	105	–
Fairhope	–	–	0	–	108	–
Monroeville	–	–	0	70	260	–
Headland	–	–	0	0	106	–
Funks G-4507A						
Brewton	–	–	0	0	135	10
Fairhope	–	–	46	–	120	12
Monroeville	0	6,000	5	500	350	14
Headland	–	–	–	0	465	–
Funks G-4611						
Brewton	–	100	0	0	261	14
Fairhope	–	–	0	0	288	14
Monroeville	0	–	0	340	235	5
Headland	–	–	0	0	145	–
Funks G-4810						
Brewton	0	–	0	0	400	–
Fairhope	–	–	0	0	138	18
Monroeville	–	–	7	350	450	4
Headland	–	–	59	0	20	–

Continued

TABLE 2 (CONTINUED). AFLATOXIN CONTAMINATION OF CORN HYBRIDS GROWN 4-6 YEARS AT FOUR SOUTHERN ALABAMA LOCATIONS, 1976-81

Variety and location	Aflatoxin B_1 in p.p.b.					
	1976	1977	1978	1979	1980	1981
Funks G-4864						
Brewton	–	250	0	0	180	9
Fairhope	–	–	0	0	108	36
Monroeville	–	–	0	150	300	11
Headland	–	–	0	0	160	–
Funks G-4949A						
Brewton	–	–	0	0	103	20
Fairhope	–	–	0	–	102	42
Monroeville	–	–	0	390	172	23
Headland	–	–	0	0	160	–
Golden Harvest H2500						
Brewton	–	–	–	–	90	18
Fairhope	–	–	0	0	60	6
Monroeville	–	–	0	570	73	16
Headland	–	–	0	–	1,240	–
Golden Harvest H2775						
Brewton	–	–	–	0	–	9
Fairhope	–	–	0	0	117	10
Monroeville	0	–	–	750	–	4
Headland	40	–	0	0	–	–
McCurdy 67-14						
Brewton	–	–	0	0	175	11
Fairhope	–	–	0	0	101	20
Monroeville	–	1,000	4	90	196	12
Headland	–	–	0	0	310	–
McCurdy 84AA						
Brewton	–	–	0	0	252	10
Fairhope	–	–	–	–	350	10
Monroeville	–	–	0	370	225	–
Headland	–	–	0	0	405	–
McNair S-338						
Brewton	–	–	0	0	400	–
Fairhope	–	–	0	0	167	–
Monroeville	–	–	0	110	300	–
Headland	0	–	0	0	170	–
McNair X-300						
Brewton	–	200	0	0	108	–
Fairhope	–	–	–	0	106	–
Monroeville	–	–	0	560	255	–
Headland	0	–	0	0	140	–
Northrup King PX-79						
Brewton	–	–	0	0	110	–
Fairhope	–	–	18	0	120	21
Monroeville	–	–	0	150	190	–
Headland	–	–	150	0	255	–
Northrup King PX-95						
Brewton	–	–	–	0	125	10
Fairhope	–	–	0	0	33	32
Monroeville	–	–	–	120	196	28
Headland	–	–	0	0	265	–
Pioneer 3030						
Brewton	200	0	0	0	105	12
Fairhope	–	–	0	0	112	38
Monroeville	–	1,000	3	300	196	34
Headland	–	–	0	0	240	–

Continued

TABLE 2 (CONTINUED). AFLATOXIN CONTAMINATION OF CORN HYBRIDS GROWN 4-6 YEARS AT FOUR SOUTHERN ALABAMA LOCATIONS, 1976-81

Variety and location	Aflatoxin B_1 in p.p.b.					
	1976	1977	1978	1979	1980	1981
Pioneer 3145						
Brewton	–	–	0	0	109	10
Fairhope	–	–	0	0	150	38
Monroeville	0	–	0	220	165	30
Headland	–	–	0	0	240	–
Pioneer 3147						
Brewton	–	–	0	0	256	18
Fairhope	–	–	0	0	299	10
Monroeville	–	–	9	570	250	37
Headland	–	–	0	0	175	–
Pioneer 3368A						
Brewton	0	–	–	–	120	38
Fairhope	–	–	0	0	110	10
Monroeville	4	–	0	–	228	10
Headland	–	–	0	–	600	–
Pioneer 3369A						
Brewton	–	–	0	0	400	15
Fairhope	–	–	0	0	150	41
Monroeville	0	–	0	166	400	15
Headland	–	–	130	0	125	–
Ring Around 1501						
Brewton	–	1,000	0	0	208	14
Fairhope	–	–	0	0	238	18
Monroeville	–	–	10	330	260	14
Headland	–	–	175	1,300	–	–
Ring Around 1502						
Brewton	–	–	0	0	–	10
Fairhope	–	–	0	–	106	18
Monroeville	–	–	0	465	200	6
Headland	0	–	0	0	70	–
Trojan TXS-114						
Brewton	–	250	0	–	266	13
Fairhope	–	–	0	0	298	24
Monroeville	–	–	0	230	210	68
Headland	–	–	0	120	225	–
Aflatoxin mean in p.p.b. of samples/year	15	1,180	12	110	209	14
Total varieties evaluated/year						
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

TABLE 3. AFLATOXIN CONTAMINATION OF CORN HYBRIDS GROWN 4-6 YEARS AT FIVE CENTRAL ALABAMA LOCATIONS, 1976-81

Variety and location	Aflatoxin B_1 in p.p.b.					
	1976	1977	1978	1979	1980	1981
Coker 16						
Camden	–	–	5	0	120	11
Camp Hill	–	–	0	0	110	–
Shorter	–	–	38	0	–	–
Marion Junction	4	–	0	0	–	–
Prattville	–	–	–	0	70	34
Coker 22						
Camden	–	–	2	0	240	24
Camp Hill	–	–	0	0	277	–
Shorter	–	–	0	0	–	–
Marion Junction	–	–	–	0	185	10
Prattville	120	–	–	4	60	108
Coker 56						
Camden	–	20	1	–	118	12
Camp Hill	–	–	0	0	100	–
Shorter	2,000	–	27	0	–	–
Marion Junction	–	–	0	15	186	3
Prattville	–	–	–	0	69	–
Coker 77B						
Camden	–	200	–	0	266	18
Camp Hill	–	–	0	0	246	–
Shorter	–	–	–	0	–	–
Marion Junction	–	–	0	–	166	14
Prattville	–	–	–	0	302	152
Funks G-795-W-1						
Camden	–	0	0	0	320	18
Camp Hill	–	–	0	49	96	–
Shorter	–	–	0	0	–	15
Marion Junction	4	–	0	26	295	11
Prattville	–	–	–	10	80	45
Funks G-4507A						
Camden	–	–	2	0	360	20
Camp Hill	20	–	0	0	100	–
Shorter	–	–	22	0	–	–
Marion Junction	200	–	0	150	325	10
Prattville	–	–	–	0	163	45
Funks G-4611						
Camden	–	–	0	0	283	21
Camp Hill	–	–	0	41	300	–
Shorter	–	–	0	0	–	–
Marion Junction	–	–	28	20	170	4
Prattville	–	–	–	0	284	26
Funks G-4776						
Camden	–	–	0	0	160	–
Camp Hill	–	–	0	0	280	–
Shorter	–	–	0	0	–	–
Marion Junction	0	–	0	0	168	–
Prattville	–	–	–	0	146	–
Funks G-4810						
Camden	–	–	1	0	350	–
Camp Hill	0	–	0	0	190	–
Shorter	–	–	0	0	–	–
Marion Junction	–	–	0	3	460	30
Prattville	–	–	–	0	288	–

Continued

TABLE 3 (CONTINUED). AFLATOXIN CONTAMINATION OF CORN HYBRIDS GROWN 4-6 YEARS AT FIVE CENTRAL ALABAMA LOCATIONS, 1976-81

Variety and location	Aflatoxin B_1 in p.p.b.					
	1976	1977	1978	1979	1980	1981
Funks G-4949A						
Camden	–	60	0	0	155	12
Camp Hill	–	–	0	0	180	–
Shorter	–	–	25	0	–	–
Marion Junction	–	–	0	5	196	–
Prattville	–	–	–	0	50	55
Funks G-5945						
Camden	–	0	0	0	223	–
Camp Hill	0	–	0	30	150	–
Shorter	–	–	27	0	–	–
Marion Junction	0	–	0	0	–	–
Prattville	–	–	–	3	95	–
McCurdy 67-14						
Camden	–	–	0	0	115	19
Camp Hill	0	–	0	0	195	–
Shorter	200	–	0	0	–	–
Marion Junction	–	–	0	–	298	2
Prattville	–	–	–	0	180	48
McNair X-300						
Camden	–	–	10	–	115	–
Camp Hill	–	–	0	0	300	–
Shorter	0	–	0	0	–	34
Marion Junction	–	–	0	3	460	30
Prattville	–	–	–	0	288	–
McNair 508						
Camden	–	0	0	0	134	20
Camp Hill	–	–	9	28	160	–
Shorter	–	–	0	0	–	–
Marion Junction	–	–	0	40	300	–
Prattville	–	–	–	0	80	22
Pioneer 3145						
Camden	–	–	12	0	102	8
Camp Hill	–	–	9	0	225	–
Shorter	–	–	0	0	–	–
Marion Junction	–	–	0	0	193	45
Prattville	–	–	–	0	160	36
Pioneer 3147						
Camden	–	–	0	0	250	14
Camp Hill	0	–	0	0	290	–
Shorter	–	–	100	0	–	17
Marion Junction	–	–	0	30	196	26
Prattville	–	–	–	0	290	14
Pioneer 3368A						
Camden	–	–	0	0	45	12
Camp Hill	–	–	0	0	140	–
Shorter	4	–	0	0	–	–
Marion Junction	–	–	2	–	199	–
Prattville	–	–	–	–	0	20
Pioneer 3369A						
Camden	–	–	6	0	219	19
Camp Hill	–	–	0	0	98	–
Shorter	–	–	0	0	–	45
Marion Junction	–	–	–	0	38	16
Prattville	–	–	–	0	303	64

Continued

TABLE 3 (CONTINUED). AFLATOXIN CONTAMINATION OF CORN HYBRIDS GROWN 4-6 YEARS AT FIVE CENTRAL ALABAMA LOCATIONS, 1976-81

Variety and location	Aflatoxin B_1 in p.p.b.					
	1976	1977	1978	1979	1980	1981
Ring Around 1502						
Camden	–	–	1	0	125	11
Camp Hill	–	–	0	0	200	–
Shorter	–	–	5	0	–	16
Marion Junction	–	–	0	0	199	17
Prattville	–	–	–	0	110	33
Trojan TXS-114						
Camden	–	–	18	0	290	19
Camp Hill	–	–	0	0	310	–
Shorter	–	–	0	0	–	–
Marion Junction	–	–	5	350	–	13
Prattville	–	–	–	0	250	33
Trojan TXS-119						
Camden	–	–	0	24	135	18
Camp Hill	–	–	–	35	110	–
Shorter	–	–	–	0	–	–
Marion Junction	–	–	–	0	260	5
Prattville	–	–	–	24	50	120
Aflatoxin mean in p.p.b.						
of samples/year	170	46.7	4.6	9.0	196	28.2
Total varieties evaluated/year						
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

TABLE 4. AFLATOXIN CONTAMINATION OF CORN HYBRIDS GROWN 4-6 YEARS AT THREE NORTHERN ALABAMA LOCATIONS, 1976-81

Variety and location	Aflatoxin B_1 in p.p.b.					
	1976	1977	1978	1979	1980	1981
Coker 16						
Belle Mina	0	–	0	0	84	20
Crossville	–	1,000	0	0	112	–
Winfield	0	–	0	0	57	20
Coker 22						
Belle Mina	0	–	0	0	59	10
Crossville	2,000	–	0	0	140	–
Winfield	–	–	0	0	61	20
Coker 56						
Belle Mina	20	–	0	0	54	27
Crossville	–	–	0	0	122	–
Winfield	–	100	47	0	78	9
DeKalb XL-72-B						
Belle Mina	–	–	0	0	88	22
Crossville	–	–	–	0	160	–
Winfield	–	–	–	0	85	9
DeKalb XL-80						
Belle Mina	20	–	0	0	78	–
Crossville	20	–	0	0	190	–
Winfield	–	50	44	0	83	–

Continued

TABLE 4 (CONTINUED). AFLATOXIN CONTAMINATION OF CORN HYBRIDS GROWN 4-6 YEARS AT THREE NORTHERN ALABAMA LOCATIONS, 1976-81

Variety and location	Aflatoxin B_1 in p.p.b.					
	1976	1977	1978	1979	1980	1981
DeKalb XL-394						
Belle Mina	–	–	0	0	71	47
Crossville	1,000	60	0	0	200	–
Winfield	–	–	1	16	51	24
Funks G-795-W-1						
Belle Mina	–	0	0	0	200	11
Crossville	–	–	0	90	193	12
Winfield	–	33	0	0	200	13
Funks G-4507						
Belle Mina	–	–	0	0	268	15
Crossville	–	–	0	0	275	–
Winfield	100	–	0	0	300	15
Funks G-4611						
Belle Mina	0	0	0	0	228	25
Crossville	3,333	–	0	0	244	–
Winfield	0	0	0	0	288	22
Funks G-4747-W-1						
Belle Mina	–	–	–	–	75	–
Crossville	–	100	–	41	108	17
Winfield	–	–	–	–	–	–
Funks G-4810						
Belle Mina	–	0	0	0	214	10
Crossville	–	–	0	0	168	–
Winfield	–	0	0	0	210	23
McCurdy 67-14						
Belle Mina	–	–	0	0	90	36
Crossville	20	–	0	0	1,100	–
Winfield	–	20	228	0	64	20
McCurdy 84-AA						
Belle Mina	–	–	0	0	250	68
Crossville	–	–	–	0	264	–
Winfield	–	–	–	0	355	15
McNair X-300						
Belle Mina	20	0	0	0	86	–
Crossville	67	40	0	0	110	–
Winfield	–	–	22	0	90	–
Paymaster UC8951						
Belle Mina	–	0	0	0	228	19
Crossville	–	0	0	–	276	–
Winfield	–	–	–	0	266	33
Pioneer 3147						
Belle Mina	–	30	0	0	236	55
Crossville	–	–	32	0	110	32
Winfield	0	–	0	0	276	12
Pioneer 3369A						
Belle Mina	–	–	0	0	100	20
Crossville	–	–	0	0	55	–
Winfield	–	0	0	0	100	35
Ring Around 1501						
Belle Mina	–	–	0	0	244	26
Crossville	–	–	0	0	236	–
Winfield	–	–	–	0	288	27

Continued

TABLE 4 (CONTINUED). AFLATOXIN CONTAMINATION OF CORN HYBRIDS GROWN 4-6 YEARS AT THREE NORTHERN ALABAMA LOCATIONS, 1976-81

Variety and location	Aflatoxin B_1 in p.p.b.					
	1976	1977	1978	1979	1980	1981
Ring Around 1502						
Belle Mina	–	0	0	0	106	14
Crossville	–	–	0	0	137	25
Winfield	–	–	–	0	58	22
Ring Around 2602-W						
Belle Mina	–	–	–	0	–	–
Crossville	–	12	46	4	240	75
Winfield	–	–	–	–	–	–
Trojan TXS-114						
Belle Mina	–	0	0	0	268	17
Crossville	–	–	0	0	264	–
Winfield	–	–	–	0	288	8
Trojan TXS-115A						
Belle Mina	–	0	0	0	100	14
Crossville	–	–	–	0	127	–
Winfield	–	–	–	0	69	20
Aflatoxin mean in p.p.b. of samples/year	412.5	65.7	8.2	2.4	178.2	23.5
Total varieties evaluated/year						
Belle Mina	10	20	43	74	43	38
Crossville	12	18	36	76	86	14
Winfield	9	19	24	51	43	39

TABLE 5. AFLATOXIN CONTAMINATION OF CORN HYBRIDS BY REGIONS AND YEARS IN ALABAMA

Region of State and no. of sites	Aflatoxin mean in p.p.b.[1]					
	1976	1977	1978	1979	1980	1981
Southern (4)	16 (19)	1,180 (10)	12 (95)	110 (90)	210 (99)	19 (66)
Central (5)	32 (14)[2]	47 (06)	5 (77)	9 (99)	195 (81)	28 (53)
Northern (3)	20 (13)[3]	66 (22)	8 (51)	2 (62)	178 (63)	24 (41)

[1]Numbers in parenthesis are numbers of hybrids tested.
[2]One sample omitted because of apparent postharvest contamination.
[3]Three samples omitted because of apparent postharvest contamination.

DISCUSSION

Preharvest Contamination

Preharvest contamination of corn with aflatoxin generally occurs in the range of 20-150 p.p.b., although many samples show no visible infection. However, under stress conditions induced by drought, high temperatures, and insect infestations, preharvest aflatoxin contamination can, on rare occasions, reach high levels (1,000-2,000 p.p.b.). Preharvest contamination may occur when *A. flavus* colonizes corn silks during the first 2 weeks of silking and when it invades the developing kernels 4-13 days after pollination (*18,28*). Such infection is favored by high temperatures of 86-93°F (*19*). The effect of water stress (drought) apparently is to increase the amount of inoculum (spore loads) rather than plant susceptibility (*18,28,37*). In most years, only a relatively small number of kernels are contaminated, but they may contain high levels of aflatoxin. Alternately, infection may occur following direct inoculation of kernels injured by insects and carrying spores of *A. flavus*, which subsequently produces 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. Corn, other grains, peanuts, and other crops must be dried to safe storage moisture after harvest to prevent fungal deterioration. Safe storage moisture then must be maintained in facilities adequate to prevent moisture buildup and thus prevent fungus (mold) growth and subsequent toxin formation. Safe storage moisture in the case of corn is approximately 13 percent. Aflatoxin levels generally become high in postharvest-contaminated corn compared to preharvest-contaminated corn, frequently attaining levels of 2,000 to 5,000 p.p.b. of aflatoxin B_1 in Southeastern States.

In 1965, Auburn research 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 up to 8,000 p.p.b. of aflatoxin from postharvest contamination. High moisture corn was harvested before maturity and left undried in a closed bin for several days, after which it was diluted to a level of 150-200 p.p.b., which was apparently not detrimental when fed to 120-pound feeder pigs and lactating sows, but killed the more susceptible suckling pigs.

Genetic Control

In the investigation reported here, analysis of aflatoxin data for 6 years of over 200 corn hybrids grown throughout Alabama, tables 2, 3, and 4, indicates there was no resistance to aflatoxin formation in any hybrid tested. In 1977 and 1980, which were epidemic years with high levels of aflatoxin being common throughout the Southeast, all hybrids tested were contaminated with moderate to high levels of aflatoxin. In these 2 years, preharvest contamination resulted in levels high enough to cause serious problems for both corn and animal agribusiness (*14*,*27*). A considerable acreage of the corn crop in those years was plowed under because of the combined effect of low yield and preharvest aflatoxin 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 p.p.b. 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 5, except for the southern region in 1979. Aflatoxin contamination was high in all regions in 1977 and 1980, but it was highest in south Alabama. 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 (*42*), apparently believing 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 to be the major obstacle in developing a genetically resistant hybrid. Since the nature and mechanisms of resistance have not been elucidated, and since their results have not been consistently reproduced, it seems 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 primarily 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 the authors conclude that genetic resistance to aflatoxin contamination has not been demonstrated to exist in commercial maize hybrids, the important search for resistance should not be abandoned. Possible innovative and unique sources of resistance may be obtained through application of the tools of of biotechnology. For example, recent research (*31*) has demonstrated the presence of extrachromosomal elements, including double-stranded RNA (ds-RNA), in a nontoxigenic strain of *A. flavus*. When treated with cycloheximide (an antifungal antibiotic), 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* 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 (ds-RNA, DNA plasmids, or whatever) to corn or other higher plants via protoplast fusion or other techniques, we might create a source of resistance to aflatoxin formation for utilization by the plant breeder. Thus, the possibility of utilizing genetic resistance to aflatoxin contamination still exists.

SUMMARY

Aflatoxin contamination was determined for up to 215 corn hybrids grown at 12 locations in Alabama during 1976-81. In 1977, corn from the southern region of the State averaged 1,180 p.p.b. aflatoxin B_1, and that from the central and northern regions averaged 47 to 66 p.p.b., respectively. In 1980, each region averaged approximately 200 p.p.b. Levels of contamination were mostly insignificant in 1978, with regional averages ranging from 5 to 12 p.p.b. in the central and northern regions. Contamination levels in 1976 and 1981 were low, but relatively uniform throughout the State, ranging from 16 to 32 p.p.b. The principal conclusions from this investigation were 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.

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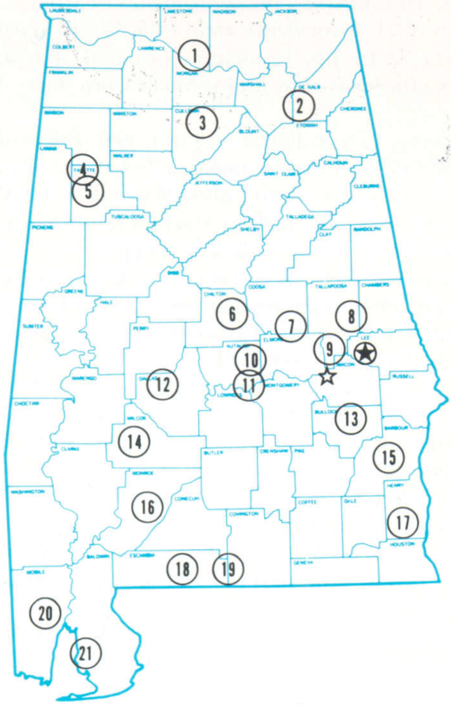
Alabama's Agricultural Experiment Station System
AUBURN UNIVERSITY

With an agricultural research unit in every major soil area, Auburn University serves the needs of field crop, livestock, forestry, and horticultural producers in each region in Alabama. Every citizen of the State has a stake in this research program, since any advantage from new and more economical ways of producing and handling farm products directly benefits the consuming public.



Research Unit Identification

⊛ Main Agricultural Experiment Station, Auburn.
☆ E. V. Smith Research Center, Shorter.

1. Tennessee Valley Substation, Belle Mina.
2. Sand Mountain Substation, Crossville.
3. North Alabama Horticulture Substation, Cullman.
4. Upper Coastal Plain Substation, Winfield.
5. Forestry Unit, Fayette County.
6. Chilton Area Horticulture Substation, Clanton.
7. Forestry Unit, Coosa County.
8. Piedmont Substation, Camp Hill.
9. Plant Breeding Unit, Tallassee.
10. Forestry Unit, Autauga County.
11. Prattville Experiment Field, Prattville.
12. Black Belt Substation, Marion Junction.
13. The Turnipseed-Ikenberry Place, Union Springs.
14. Lower Coastal Plain Substation, Camden.
15. Forestry Unit, Barbour County.
16. Monroeville Experiment Field, Monroeville.
17. Wiregrass Substation, Headland.
18. Brewton Experiment Field, Brewton.
19. Solon Dixon Forestry Education Center, Covington and Escambia counties.
20. Ornamental Horticulture Substation, Spring Hill.
21. Gulf Coast Substation, Fairhope.