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Aflatoxin Contamination of Corn in the Field

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Aflatoxin has been found in corn samples at all stages of development and maturity from the late milk stage until harvest. Insect damage was observed in 90% of the samples that showed bright greenish-yellow (BGY) fluorescence normally associated with the presence of aflatoxin in cotton seed and corn. A program of spraying insecticide, as recommended to sweet corn growers, reduced the incidence of worm damage an average of 97.7% and other insect damage an average of 92.1%. Stressed growing conditions, such as dense population of plants or reduced fertilization, appear to have a positive influence on the incidence of contamination by aflatoxin. The highest incidence of aflatoxin was found in the warmer, more humid growing regions of the country.

The subject of aflatoxin has become well known in the last 10 to 15 years. Studies conducted with animals have shown it to be an extremely toxic compound; however, the extent of toxicity to man is not as well understood. The major producers of the toxin appear to be the molds *Aspergillus flavus* and *Aspergillus parasiticus*. Diener and Davis (1969) report the presence of aflatoxin in bermuda grass, hay, soybean meal, oats, cassava, corn, peas, rice, soybeans, wheat, cow peas, sesame, sorghum, sweet potatoes, etc.

Christensen and Kaufmann (1968), Golumbic and Kulik (1969), and others have published studies relating aflatoxin to grain stored at high moisture and temperatures. This paper reports on studies conducted over a 3-year period, by the Quaker Oats Company, associating aflatoxin with corn before and during harvesting.

1971 STUDY

The first field survey was a result of low levels of aflatoxin appearing in by-product (feed) material from a corn mill in the spring of 1971. A careful survey and sampling program for in-plant contamination produced negative results from corn storage to finished products. Since no evidence of mold growth conditions was found in the plant, attention was directed to incoming corn. Was the corn contaminated at time of purchase or was it occurring during storage? A comprehensive field survey and sampling program was developed for the 1971 crop, covering essentially all the corn producing areas of the United States. Sampling started 6 weeks prior to harvest and continued through harvest and subsequent handling as long as identity could be maintained.

Arrangements were made with some of the farmers to let a small area of corn in the field stand 3 months beyond harvest. Periodic samples were taken during this time to determine if aflatoxin developed in corn after maturity in the field.

Sample Examination Procedure. The sample, either ear or shelled corn, was first subjected to inspection under "black light" (long-wave ultraviolet, 365 nm), for the characteristic bright greenish-yellow (BGY) fluorescence normally associated with the presence of aflatoxin in cotton fibers as reported by Marsh et al. (1969). Evidence indicates that the fluorescing substance in cotton fibers is formed by a heat-liable enzyme in the living plant that oxidizes kojic acid produced concurrently with aflatoxin by *A. flavus*. This method was adapted to corn and reported by Shotwell et al. (1972).

Kernels of corn and material that fluoresced under the black light were analyzed by the thin-layer chromatography (TLC) method as shown in Official Methods of AOAC (1970), to confirm the presence of aflatoxin. The duckling feed test, as reported by Sargeant et al. (1961), was also used in some of the early samples for confirmation of aflatoxin.

Results. Aflatoxin was found in the first field sampling when the corn was in the late milk stage. There appeared to be no additional aflatoxin development in the standing corn remaining in the field after harvest. Contamination was found in most growing areas but the highest incidence of aflatoxin was in the warmer, more humid growing regions of the country and followed the same geographical pattern as found in cottonseed and reported by Marsh and Taylor (1958).

Table I shows results of some field samples that were sieved over $^{18}/_{64}$ and $^{16}/_{64}$ in. round hole perforated (RHP) screens; the sized fractions were picked under ultraviolet light to remove the fluorescing material and analyzed by TLC method to confirm aflatoxin.

Table II shows the analytical results of a field sample where the corn was picked for fluorescing material before analyzing for aflatoxin, and an ear with several fluorescing kernels and the first and second rows of kernels surrounding the fluorescing kernels.

1972 STUDY

The objective of the 1972 program was to determine how and when the aflatoxin producing mold spores invaded the corn kernels. This study was confined to two geographical areas where field contamination had been found the previous year and the Quaker Oats Company Research Farm

The Quaker Oats Company, John Stuart Laboratories, Barrington, Illinois 60010.

Table I. Field Sampling Data

	ppb of aflatoxin
Sample A	
Whole raw corn	90
Pickings from above:	
1. Fraction, $^{18}/_{64}$ + $^{16}/_{64}$	123
2. Fraction, $^{16}/_{64}$	238
3. Damaged corn	1,466
4. 2 whole kernels that glowed	601,600
5. Breakage that glowed	14,250
Sample B	
Whole raw corn	12.5
Pickings from above:	
1. Fraction, $^{18}/_{64}$ + $^{16}/_{64}$	ND[a]
2. Fraction, $^{16}/_{64}$	40
3. 1 kernel that glowed	121,665
4. Damaged corn	88
5. Breakage that glowed	12,709

[a] None detected.

Table II. Field Samples

	ppb of aflatoxin
Whole corn before picking	23
Raw corn after hand picking under uv light	7
Fluorescing material picked from sample under uv light	20,252
Field Samples (Ear Corn)	
Glowing kernels from one ear	117,400
First circle of kernels around glowers	ND[a]
Second circle of kernels around glowers	ND[a]

[a] None detected.

near Barrington, Ill. Four or five fields were selected in each area and studied from time of planting until harvest. Sampling included: seed planted, soil at various depths at time of planting, growing corn plants, and soil at periodic intervals until harvest, with intensive sampling during flowering and ear formation stages. An isolated area of each field was treated weekly with an insecticide, beginning at silking stage and continuing until silks were brown, in an attempt to determine the cause and effect of insect damage and its relationship to aflatoxin contamination. In one area the insecticide used was DDT and in the other area Malathion, since these were the pesticides of choice in each area.

Ears of corn were punctured with a hypodermic needle to simulate insect damage; others were punctured and then covered with a paper bag to reduce air-borne mold spore penetration. In one area, a liquid inoculum containing aflatoxin producing *A. flavus* mold spores was injected into the kernels. The spores and method for preparation of inoculum were provided by Dr. C. W. Hesseltine of USDA Northern Regional Research Laboratory, Peoria, Ill. In addition, at the Quaker farm the inoculum was sprayed on the silks using a perfume sprayer. This was sprayed on green silks, green-brown silks, and brown silks. Base point of the ear inoculation (injection of liquid inoculum into shank just above ear node of stalk) was also performed at the Quaker Research Farm.

Results. Less than 5 ppb of aflatoxin was found in one seed corn sample and in the root system of two plants. The puncturing of ears with a hypodermic needle, puncturing and covering with a paper bag, spraying inoculum on the silks, and base point inoculations produced no aflatoxin. Spraying Malathion insecticide resulted in good insect control, but showed no relationship to aflatoxin formation. Control samples in this area indicated very low incidence of aflatoxin contamination. Spraying DDT resulted in some reduction in insect damage, but apparently the interval between sprayings was too long for good control. The concentration of the DDT solution appeared to be too strong, as some leaf browning and underdeveloped ear formation resulted, especially when plants were sprayed in the early silk and ear formation stage; consequently, very little correlation was observed between spraying DDT and aflatoxin formation.

Aspergillus flavus spores (10,000/g) were found in one of 24 soil samples when analyzed by the method supplied by Schroeder (1972). This field was not planted due to a change in the planting schedule.

In all geographical locations, direct injection inoculation of a liquid suspension of mold spores produced aflatoxin in nearly early kernel. There appears to be a 6–8 week period during the growth and maturity of the ear when the kernel is most susceptible to contamination by aflatoxin. This period is from approximately 2 weeks after flowering until the kernel has matured and dried to a moisture content of about 18 to 20%. For approximately 2 weeks after flowering, the kernel is mostly liquid. When inoculated, the liquid squirts out, the kernel dries up, and no aflatoxin is formed. When the kernel has matured and dried to approximately 18 to 20% moisture before inoculation, little or no aflatoxin will be produced. This is probably due to the sugar content being too low to allow mold growth as reported by Davis and Diener (1968), or the low moisture content. In Table III, it will be observed that inoculations performed 2, 3, and 4 weeks after flowering (13, 14, and 15 weeks after planting) produced the highest levels of aflatoxin. Although the results shown in this table are for white corn, the studies conducted on the Research Farm with yellow corn essentially duplicated these findings.

In Georgia, aflatoxin was found in random field samples of both white and yellow corn with no inoculations. It seemed apparent that insects might play an important role in the aflatoxin contamination of corn since nearly all aflatoxin was found in areas damaged by insects. A few kernels were found that appeared sound, without visible insect damage, but had BGY fluorescence and positive aflatoxin assay. Damage to the protective bran layer either by insect or mechanical means, during the high moisture stage of kernel development, may result in mold invasion and aflatoxin development in the field. Kernels have been observed with BGY fluorescence in a small area in the crown of the kernel. This suggests that mold spores may have entered through the scar where the silk was attached.

1973 STUDY

Taubenhaus (1920), reporting on a study of black and yellow molds of ear corn states, "The fact that *Aspergillus niger* and *A. flavus* are always associated with black and yellow molds of ear corn and broom corn in Texas would indicate that these organisms are the cause of the trouble". Later in the report he states "The ear mold of corn is caused by the fungus *Aspergillus niger* and this organism can only invade the ear during its milky stage and when it has been injured by the earworm". "The earworm and possibly other insects not only open the way to *Aspergillus* infection, but they are also carriers of the spores of these fungi".

This report, in addition to the seemingly important role played by insects in the contamination of corn in the field by aflatoxin in the 1972 study, led to the inclusion of a comprehensive insect control program in the 1973 study.

Table III. Inoculation Studies in Field in Southwest Georgia

Date	Weeks	Action	Black light	Aflatoxin, ppb
4/24/72	0	Planted white corn		
7/20/72	12	Inoculated first sample (N-1)		
7/27/72	13	Inoculated 2nd sample (N-2)		
		Sampled 1st inoculation	+	31,233
7/31/72	14	Sampled 1st inoculation (N-1)	+	297,907
		Sampled 2nd inoculation (N-2)	+	243,095
8/1/72	14	Inoculated 3rd sample (N-3)		
8/10/72	15	Sampled 1st inoculation (N-1)	+	139,520
		Sampled 2nd inoculation (N-2)	+	484,702
		Sampled 3rd inoculation (N-3)	+	39,510
		Sample of N-1 butt (not inoc.)	+	54,335
8/11/72	15	Inoculated N-4		
8/17/72	16	Sample N-1	+	290,030
		Sample N-2	+	567,764
		Sample N-3	+	629,036
		Sample N-4	+	331,922
8/18/72	16	Inoculated N-5		
8/24/72	17	Inoculated N-6		
		Sample N-1	+	416,500
		Sample N-2	+	161,500
		Sample N-3	+	398,500
		Sample N-4	+	610,500
		Sample N-5	+	48,800
		N-5 did not fluoresce until split open		
8/31/72	18	Sample N-1	+	439,346
		Sample N-2	+	298,485
		Sample N-3	+	562,377
		Sample N-4	+	338,657
		Sample N-5	+	44,032
		Sample N-6	+	71,124
		Samples N-5 and N-6 did not fluoresce until cut open		
		Fluorescence weaker on each successive week of inoculation		
9/1/72	18	Inoculated N-7		
9/6/72	19	Inoculated N-8 (H_2O 16.7%)		
		Sample N-1	+	159,500
		Sample N-2	+	346,500
		Sample N-3	+	620,000
		Sample N-4	+	316,500
		Sample N-5	+	76,000
		Sample N-6	+	97,000
		Sample N-7	+	30,000
9/14/72	20	Sample N-3	+	80,252
		Sample N-4	+	518,839
		Sample N-5	+	47,900
		Sample N-6	+	44,683
		Sample N-7	+	4,065
		Sample N-8	0	ND
9/15/72		Inoculated N-9		
9/20/72	21	Sample N-4	+	456,000
		Sample N-5	+	126,000
		Sample N-6	+	55,800
		Sample N-7	+	33,700
		Sample N-8	0	ND
		Sample N-9	0	ND
10/5/72	23	Sample N-6	+	611,047
		Sample N-7	+	7,642
		Sample N-8 regular	0	12
		Sample N-8 dry	0	40
		Sample N-9 regular	0	8
		Sample N-9 dry	0	13

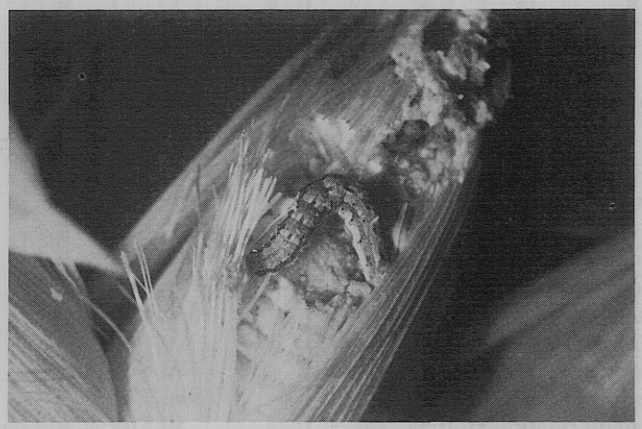

Figure 1. Earworm and damage to tip of ear; Funk 795 hybrid.

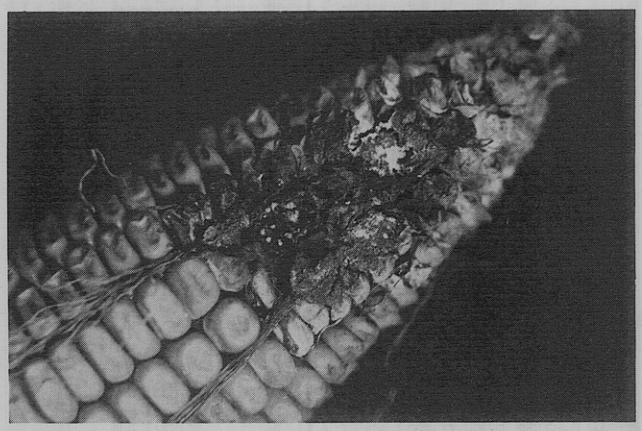

Figure 2. Shows same ear as in Figure 1 with worm and husks removed. Note fluorescence in worm damaged area.

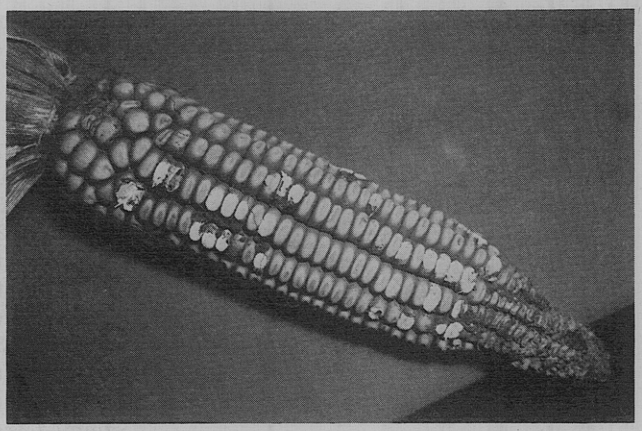

Figure 3. Natural contamination in ear from dense population control area; PAG-653 hybrid. Tops of kernels were removed to reveal fluorescence.

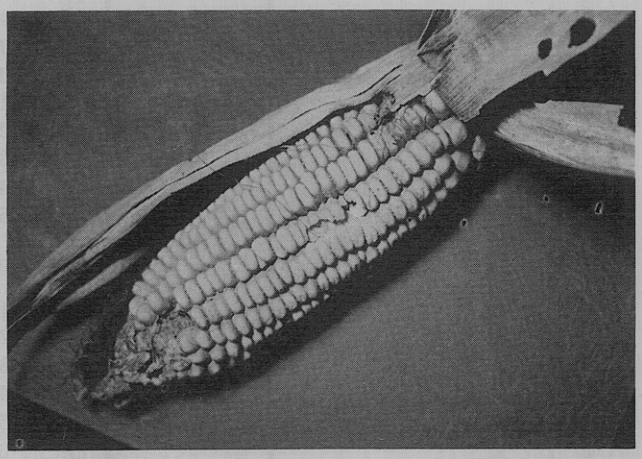

Figure 4. Natural contamination in worm damaged area. Ear from dense population area with 60 units of nitrogen side-dressing; PAG-653 hybrid.

Four hybrids were selected for planting. Three were popular hybrids grown in this area of Georgia (PAG 653, Coker 814, and Funk 795). Some incidences of aflatoxin were observed in two of these, PAG 653 and Coker 814 in 1972. The fourth hybrid planted was Opaque-2. Nagarajan and Bhat (1972) reported that work done in India showed Opaque-2 contained a natural inhibitor to aflatoxin production due to the presence of a protein of low molecular weight.

This study was conducted in an 11-acre field in southwest Georgia. It had been planted in cotton in 1972, peanuts in 1971, and white corn in 1970. Good farming practices of fertilization and weed control were employed prior to planting. Side dressing, 140 units of 32% liquid nitrogen, was applied 4 weeks after planting except for two test areas described later.

Other features of the testing program included inoculating the silks of PAG and Opaque-2 hybrids with both liquid and powder suspensions of a strain of *A. flavus* mold spores known to produce aflatoxin. Inoculations began as the silks emerged and continued every other day (inoculating different ears) until the silks were brown. This was an attempt to determine the possibility of the mold following the route of the pollen and entering the kernel via the silk.

Liquid inoculum was injected into the kernels of PAG and Opaque-2 hybrids to observe possible differences in susceptibility to mold growth and the production of aflatoxin. Inoculation began approximately 2 weeks after flowering and continued at weekly intervals until the corn had matured and dried to a moisture content of 25–26%.

The plant population of PAG hybrid was doubled (32,000 plants per acre, 38-in. row spacing) in one area and nitrogen side dressing reduced (60 units from 140 units) in part of this area and compared to a normal population area to study possible effects of stressed growing conditions and decreased fertilization on the incidence of aflatoxin contamination.

An additional test plot of each of the variables was sprayed with the insecticide "Gardona" (2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate, in 75% wettable powder form, manufactured by Shell Chemical Co., San Ramon, Calif.) to determine the extent of aflatoxin contamination possibly carried into the kernels principally by the earworm and fall armyworm. Spraying began as silks emerged and continued every other day until the silks were brown. This program has been recommended to sweet corn growers.

Control plots, involving normal farming practices, were located in each of the hybrid test areas. A total of 22 test plots were observed in this study. Each plot was isolated and identified with red tags located peripherally.

The field was planted in 24 row segments, alternating the hybrids with the exception of Opaque-2. There was only a sufficient quantity of Opaque-2 seed available to plant 12 rows.

Sampling. Soil samples were taken at planting and at 1 month and 2 months after planting at depths of 0–3, 3–6, and 6–9 in. to be analyzed for *A. flavus* mold spores. No *A. flavus* mold was found in any of the samples.

Sampling of four to six ears in each of the 22 test plots

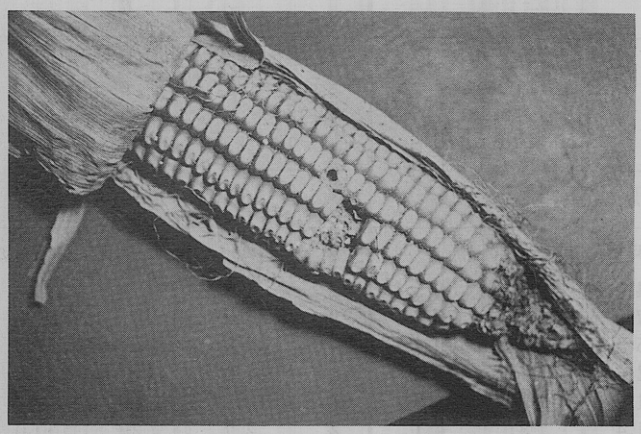

Figure 5. Natural contamination in worm damaged area; Funk 795 hybrid.

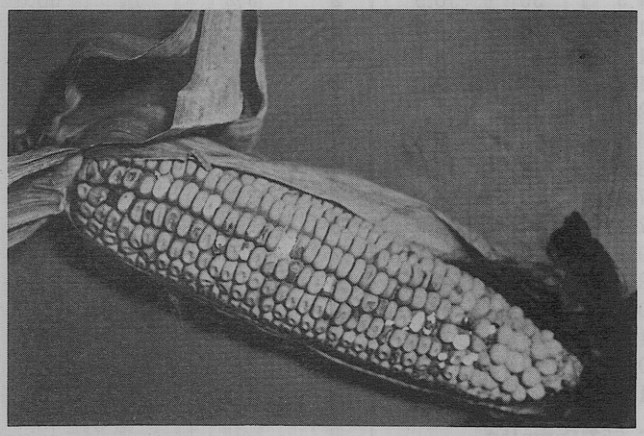

Figure 6. Natural contamination in insect damaged ear of Opaque-2 hybrid. Tops of kernels were removed to reveal fluorescence.

Figure 7. Shows different heights of corn as just entering tassel stage: Funk 795 on left, Opaque-2 center, and Coker 814 on right.

was started 3 weeks after flowering and continued at weekly intervals until the field was harvested, a total of ten sets of samples. Sixteen samples were taken from the harvester, representing the different hybrids and test conditions in the field.

Sample Evaluation. The noninoculated ears sampled each week were examined under long-wave ultraviolet light within 2 to 4 hr from time of pulling. Any ears with naturally occurring BGY fluorescence were photographed immediately. Five of the seven photographs (Figures 1–7) show the fluorescence observed at this time. Figure 1 shows an earworm in the same area as the fluorescing material in Figure 2. All samples were stored at 10–13° for 1 to 3 days until final evaluation was completed. Every ear was very carefully examined under long-wave ultraviolet light and normal fluorescent light. No additional BGY fluorescing material was found in any of the samples that had been examined and photographed previously. Specific samples were selected and assayed for aflatoxin, using the TLC method. Some of these results are shown in Table IV. A detailed record was kept of photographs taken and observations made during examinations of samples. From this record, the information was taken that is shown in Table V.

Table IV. Typical Aflatoxin Content of Selected Samples

Sample	Sample wt, g	ppb of aflatoxin
Seed corn planted, Opaque-2	200	None
Seed corn planted, Coker 814	200	None
Seed corn planted, Funk 795	200	None
Seed corn planted, PAG 653	200	11
Opaque-2 fluorescing kernels	10	69,347
Opaque-2 fluorescing husks	50	None
Opaque-2 BGY fluorescing kernels, inoculated	10	91,971
Opaque-2 BGY fluorescing kernels, inoculated	10	587,580
Opaque-2 pink & blue fluorescing kernels, inoculated	5	1,545,392
PAG, BGY fluorescing kernels, inoculated	10	461,887
PAG, blue fluorescing kernels	5	287,788
PAG, BGY fluorescing cob tip	5	None

The samples taken from the harvester were carefully examined under long-wave uv light. The fluorescing kernels were hand picked out and a percentage by weight calculated. This information is presented in Table VI. As observed in this table, this study does not support the theory that weeds growing in corn create enough of a stress condition to have a positive influence on the incidence of aflatoxin contamination.

Two sets of ear samples taken Sept 5th and Sept 12th were examined under long-wave uv light and all fluorescing kernels removed from the cob. The ears were shelled, the corn examined under uv light, and the fluorescing kernels removed. The corn was run through a set of rolls to crack open the kernels. This cracked corn was again examined under uv light and all fluorescing material removed. The picked material and the pickings were submitted to the Analytical Section for aflatoxin analysis. The results are shown in Tables VII and VIII. The results in these tables give an indication of the amount of aflatoxin contamination that can be removed by visual examination and picking under long-wave uv light. The highly contaminated samples will often have low levels of aflatoxin remaining after cracking the kernels open and carefully removing the fluorescing cracked corn. Lillehoj et al. (1973) report, "That not all greenish yellow fluorescence is a sign of aflatoxin. It could be a sign of something else that could be quite harmless". A USDA bulletin (1972) states that the fluorescence in corn is not stable and will fade in 4 to 6 weeks if exposed to ordinary light and more quickly with uv light. Both of these factors may influence the aflatoxin content of visually separated samples.

Table V. Evaluation of Ear Samples for Aflatoxin

Test area description	No. ears	Worm damaged		Other insect damaged		BGY fluorescence		BGY fluorescence in worm or insect damage	
		No.	%	No.	%	No.	%	No.	%
Opaque-2									
Opaque-2 control	62	40	64.5	35	56.5	2	3.2	1	50.0
Liquid inoc. of silks	81	55	67.9	61	75.3	29	35.8	24	82.8
Dust inoc. of silks	81	61	75.3	57	70.4	27	33.3	25	92.6
Injection inoc.	62	45	72.6	47	75.8	13	21.0[a]	13	100.0
Opaque-2 control, spray insect.	61	1	1.6	2	3.3	0	0	0	0
Liquid inoc., spray insect.	74	1	1.4	3	4.1	2	2.7	2	100.0
Dust inoc., spray insect.	78	1	1.3	4	5.1	1	1.3	0	0
Inject. inoc., spray insect.	58	1	1.7	13	22.4	0	0[a]	0	0
Summary Opaque-2 Samples									
Control, no inoc., no spray	62	40	64.5	35	56.5	2	3.2	1	50.0
Control, no inoc., spray insect.	61	1	1.6	2	3.3	0	0	0	0
Inoc., no spray	224	161	71.9	165	73.7	69	30.8	62	89.9
Inoc., spray insect.	210	3	1.4	20	9.5	3	1.4	2	66.7
Control high ground 60 units of N	63	33	52.4	17	27.0	2	3.2	2	100.0
Control high ground	58	27	46.6	5	8.6	1	1.7	1	100.0
Dense pop. control	60	47	78.3	14	23.3	4	6.7	3	75.0
Dense pop. control 60 units of N	59	40	67.8	9	15.3	8	13.6	7	87.5
Liquid inoc. silks	85	45	52.9	27	31.8	19	22.4	17	89.5
Dust inoc. silks	88	52	59.1	16	18.2	12	13.6	11	91.7
Injection inoc.	64	27	42.2	13	20.3	8	12.5[a]	7	87.5
Control high ground, spray insect.	60	0	0	0	0	0	0	0	0
Dense pop., spray insect.	61	1	1.6	4	6.6	1	1.6	1	100.0
Liquid inoc., spray insect.	91	0	0	3	3.3	2	2.2	2	100.0
Dust inoc., spray insect.	94	1	1.1	1	1.1	2	2.1	2	100.0
Summary PAG-653 Samples									
Control, no inoc., no spray	240	147	61.3	45	18.8	15	6.3	13	86.7
Control, no inoc., spray insect.	121	1	0.8	4	3.3	1	0.8	1	100.0
Inoculated, no spray	237	124	52.3	56	23.6	39	16.4	35	89.7
Inoculated, spray insect.	185	1	0.5	4	2.2	4	2.2	4	100.0
Coker 814									
Control low area	59	39	66.1	8	13.6	1	1.7	1	100.0
Control low area, spray insect.	61	2	3.3	0	0	0	0	0	0
Funk 795									
Control	72	55	76.4	12	16.7	6	8.3	6	100.0

[a] This fluorescence occurred in areas of the ear not inoculated. There was 100% fluorescence in inoculated areas of the ears.

DISCUSSION AND CONCLUSIONS

An interesting supplemental experiment was performed that demonstrates one possible way for aflatoxin producing mold spores to enter the corn kernel. The experiment consisted of removing corn earworms, in different stages of development, from the silks or ear, dusting them with a powder containing *A. flavus* mold spores, and returning them to their original place. Six worms were removed, estimating two in second instar and one in each of the other instars, dusted, and returned to the same position as originally found. These ears were sampled, one each week, beginning 10 days after dusting. In every ear except one, the worm had eaten into the kernels and BGY fluorescence was found around the worm damaged area. The exception was a worm estimated to be in the last instar. Indications are that when it was replaced in the ear, it immediately ate through the husk, dropping to the ground to pupate without damaging any additional kernels.

An additional experiment demonstrates the susceptibility of corn, before maturity, to invasion by mold once the bran coat is broken. This experiment was performed when the moisture of the corn kernels was approximately 45%. The husk was pulled down on one side of the ear exposing an area of kernels about 1.5 in. × 3 in. A boundary was drawn with a marking pen within this area and a liquid suspension of *A. flavus* mold spores applied to the kernels within the boundary without damaging the bran coat. The husks were returned to normal position and a small piece of masking tape applied to prevent the husks from opening. Three ears were treated in this manner. Three additional ears were treated in the same manner except that the kernels were scratched very lightly with the point of a knife blade before applying the inoculum.

One ear of each test was sampled each week beginning 1 week after inoculation and continued until the inoculated ears were depleted.

Table VI. Samples from Harvester

Hybrid	Sample no.	Field position	Crop condition	Sample wt, g	Fluorescing kernels wt, g	Fluorescing kernels, %	Ranking worst to best
PAG-653	1. Regular spacing	Top (south)	Weedy, stress	1388	2.3	0.165	1
	2. Regular spacing	Top (south)	Weedy	1516	0.8	0.052	6
	3. Regular spacing	Top (south)	Few weeds	1455	0.2	0.013	14
	1. Dense population	Central	Very few weeds	1450	1.6	0.110	3
	2. Dense population	Central	Very few weeds	1426	0.4	0.028	11
	1. Regular spacing	Central	Very few weeds	1450	0.3	0.020	13
Coker 814	1. Regular spacing	Top	Few weeds	1466	0.5	0.034	9
	2. Regular spacing	Top	Few weeds	1464	0.6	0.040	8
	3. Regular spacing	Top	Few weeds	1485	0.9	0.060	5
	1. Regular spacing	Bottom (north)	Very weedy	1354	0.6	0.044	7
	2. Regular spacing	Bottom (north)	Very weedy	1472	0.4	0.027	12
Funk 795	1. Regular spacing	Central	Few weeds	1385	0.4	0.028	11
	2. Regular spacing	Central	Few weeds	1417	1.1	0.077	4
	3. Regular spacing	Central	Few weeds	1371	1.9	0.138	2
Opaque-2	1. Regular spacing	Central	Several weeds	1270	0.4	0.031	10
	2. Regular spacing	Central	Several weeds	1264	0.1	0.007	15

Yield comparisons, 12 rows each hybrid full length of field

Hybrid	Yield, bushel
Coker 814	69.5
PAG-653 dense population	67.0
PAG-653 regular population	60.0
Funk 795	60.0
Opaque-2	38.0
Total	881.0 bushels for 11-acre field

The three ears that were inoculated without damaging the bran coat had a total of three kernels that showed BGY fluorescence. The three ears that had the bran coat scratched before applying the inoculum showed mold growth and fluorescence in every kernel damaged. The first week the mold was growing along the damaged area. The second week it had spread over a large area of the kernel, and the third week the mold had spread over the entire inoculated area of the ear.

With the background of two previous years study, and after examination of over 1500 ears taken during maturation, and 16 samples taken from the harvester this year, the following observations and conclusions were made. (A) Spraying insecticide reduced the incidence of worm damage by as much as 99.1% with an average of 97.7% reduction. Other insect damage was reduced by as much as 100% with an average of 92.1% reduction. (B) In noninoculated areas that were sprayed with insecticide, the incidence of BGY fluorescence was reduced by as much as 100% with an average of 96.2% reduction. (C) In ears that showed BGY fluorescence, 89.3% had either worm or insect damage. (D) There may be varietal differences in susceptibility to damage by insects and, thus, possible contamination by aflatoxin as indicated by insect damage and BGY fluorescence in this study. However, more definitive test work would be necessary to make this determination. (E) In noninoculated, non-insecticide-sprayed areas, the highest incidences of BGY fluorescence were generally found in areas of stressed growing conditions (dense population and/or low fertilization). This would indicate the possibility of a greater risk of aflatoxin contamination in corn grown under stress conditions. (F) The most susceptible period to invasion by *A. flavus* mold and production of aflatoxin in the growth and development of the corn kernel is from about 2 weeks after flowering until the corn has matured and dried to 20–25% moisture. (G) In approximately 90% of the samples where

Table VII. Picked, Shelled, Cracked Corn Samples

Sample	Aflatoxin, ppb	
	Sept. 5	Sept. 12
Control high ground	None	None
Control high ground insecticide	None	None
Control low ground	None	5–10
Control low ground insecticide	None	None
Liquid inoculated silks	None	224
Liquid inoculated silks insecticide	None	None
Dust inoculated silks	5	None
Dust inoculated silks insecticide	None	5
Dense population control	None	5–10
Dense population control insecticide	5	None
Opaque-2 control	5	5
Opaque-2 control insecticide	None	5
Opaque-2 liquid inoculated silks	167	386
Opaque-2 liquid inoculated silks insecticide	5	None
Opaque-2 dust inoculated silks	52	96
Opaque-2 dust inoculated silks insecticide	859	56
Opaque-2 injected inoculum	227	4837
Opaque-2 injected inoculum insecticide	None	3558
PAG, injected inoculum	None	None
Dense population control, 60 units of nitrogen	None	5
Control high ground, 140 units of nitrogen	None	None
Funk hybrid control	None	None

Table VIII. Fluorescent Pickings from Shelled, Cracked Corn Samples

Sample	Aflatoxin, ppb	
	Sept. 5	Sept. 12
Control low ground	No sample	1,018
Liquid inoculated silks	4,774	94,961
Dust inoculated silks	36,280	16,389
Dense population control	No sample	748
Dense population control 60 units of nitrogen	3,601	1,801
Opaque-2 control	10,125	No sample
Opaque-2 liquid inoculated silks	378,144	261,816
Opaque-2 dust inoculated silks	21,401	123,455
Opaque-2 injected inoculum	15,203	443,770
Opaque-2 injected inoculum insecticide	53	456,415
PAG injected inoculum	36,958	107,683
Funk hybrid control	15,982	No sample

aflatoxin was found, the bran coat had been broken. (H) Some kernels were found that appeared sound to visual examination but, when split open, revealed a strip of fluorescing material surrounding the germ. It is not known how the contamination occurred in these samples. (I) No evidence was observed in this inoculation study to indicate that Opaque-2 corn was any more resistant to aflatoxin contamination than the other hybrids; see Table V. Opaque-2 has a short, loose husk which permits easy access to insects, thus apparently making it more susceptible to contamination by aflatoxin. It is possible this specific test corn was not well suited to the growing area but was the only white Opaque-2 seed available. (J) The long, tight husks of Coker 814 and PAG 653 appeared to be a somewhat natural deterrent to the earworm, but a much greater deterrent to other insects. Once the earworm had eaten down through the silk to damage the ear, other insects followed this access and many times did additional damage to other parts of the ear, spreading BGY fluorescence along their trail.

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