AFLATOXIN INCIDENCE AND ASSOCIATION WITH BRIGHT GREENISH-YELLOW FLUORESCENCE AND INSECT DAMAGE IN A LIMITED SURVEY OF FRESHLY HARVESTED HIGH-MOISTURE CORN

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ABSTRACT

Freshly harvested corn (40–45% moisture) from the 1972 crop was examined for *Aspergillus flavus*-induced bright greenish-yellow (BGY) fluorescence and for aflatoxin. Sample ears were screened for insects that could provide access for fungal infection. After the ears had been dried and shelled the corn was cracked, examined under an ultraviolet light, and extracted for aflatoxin assay. Samples from 5% of the test ears exhibited BGY fluorescence while those from 2.5% of the test ears contained aflatoxin B-1 in excess of 20 ppb. Essentially all of the toxin-containing samples were BGY-positive. Aflatoxin was detected in significantly more earworm-damaged samples than in those with no insect damage. Dramatic differences in BGY fluorescence incidence, aflatoxin occurrence, and insect damage were observed between test areas. No aflatoxin-positive samples were found in the seven fields of southern Illinois corn and in about two-thirds of 62 fields of southeastern Missouri corn. There was no association between recorded agronomic practices followed in the test fields and subsequent aflatoxin contamination.

Current awareness of possible hazards associated with *Aspergillus flavus* contamination of foods and feeds originated with the discovery in the early 1960's that the fungus could produce a group of carcinogenic metabolites called aflatoxins (1). Previous studies had shown that toxin-producing strains of *A. flavus* could be isolated from field corn suspected of producing a toxic response in swine and cattle, but the poison was not characterized (2,3). Surveys of corn carried out during the past years disclosed only a limited incidence of aflatoxin contamination (4-6). A seizure by the Food and Drug Administration of aflatoxin-tainted white corn meal in 1971 (7) indicated the need for definitive information on conditions predisposing corn to infection by *A. flavus* and aflatoxin formation.

Aspergillus and *Penicillium* species are considered storage fungi since they ordinarily are not a problem on grain in the field but develop on seed after harvest (8,9). Growth of these fungi is not restricted, however, to harvested grain since they have been found in field corn (10,11). *Penicillium* spp. have been identified as common fungi (3.3% occurrence) isolated from corn kernels removed from the plant and plated directly on a nutrient medium; incidence of *A. flavus* group ranked second with a 0.6% occurrence (11). In 1972, investigators from the Quaker Oats Company demonstrated a limited incidence of aflatoxin in field corn (12).

The succession of fungi growing on seed is largely determined by moisture conditions; representatives of the *A. glaucus* group generally predominate at 13

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to 15% moisture, but above 15% other fungi appear, including *A. flavus*, *A. ochraceus* Wilh., and *A. versicolor* Vuill. (8,9). Lopez and Christensen (13) examined the invasive capacity of *A. flavus* on corn that had been conditioned to various moisture levels. They found that the fungus did not develop on seed below 17.5% moisture but grew readily at 18.5% and above.

In 1920 Taubenhaus (14) carried out extensive studies in Texas on the susceptibility of developing corn to *A. flavus* infection. He observed that the fungus appeared on the tips of ears, particularly erect ears that caught and retained moisture during the growing season. In artificial inoculation trials, *A. flavus* invaded kernels during the milk stage but not at maturity; susceptibility of corn to *A. flavus* infection was determined by the stage of maturation of the grain. Taubenhaus also examined the role of insect vectors in the infection and spread of fungi in corn (14). Development of certain *Aspergillus* species was observed at the site of insect damage, particularly that caused by corn earworm *Heliothis zea* (Boddie); these insects routinely carried many fungal species, including *A. flavus*. Later studies implicated mite vectors in contamination of peanuts with aflatoxin-producing strains of *A. flavus* (15). These findings suggest that arthropods contribute significantly to fungal infection of raw food and feedstuffs.

Broad screening for aflatoxin occurrence has been hampered by the complexity of procedures required for chemical identification and measurement of the toxin. The analytical work load can be reduced in many cases by initial application of a rapid ultraviolet (uv) light procedure as a presumptive test. This procedure detects a characteristic bright greenish-yellow (BGY) fluorescence broadly associated with the presence of aflatoxin (16). The method does not detect aflatoxin directly; the BGY fluorescence is emitted by other metabolites of *A. flavus* (16,17).

In this study our objective was to acquire information on the presence of BGY fluorescence and aflatoxin in freshly harvested corn. Corn from southern Illinois and southeastern Missouri was surveyed for: a) incidence of BGY fluorescence and insect damage in freshly harvested corn (40-45% moisture) on the ear; b) incidence of BGY fluorescence and aflatoxin in freshly harvested corn after drying, shelling, and cracking; and c) agronomic practices that might be related to fungal infection and toxin formation.

MATERIALS AND METHODS

The study was divided into two phases: The first, a general survey, including 60 fields in four diverse test regions (area 1-4) of a 2,000-square mile area in southeastern Missouri and the other, an intensive survey of two fields in the general survey area (area 5) and 5 fields in two 5-square mile regions in southern Illinois (area 6). In the general survey, collection of 60 ears from each of 15 fields (30 ears from each of two locations per field) from each area yielded a total of 3,600 ears; 30 ears per sampling site provide an 80% probability of observing positive results, assuming a 5%-sample ear incidence of BGY or aflatoxin. In the intensive study, 600 ears were collected from 10 sites (30 ears per site) in each of two fields in area 5, and 750 ears were gathered from 5 sites (30 ears per site) in each of 5 fields in area 6; this sampling provided a 95% probability that positives

would be detected, assuming a 1% incidence of BGY or toxin. Corn was examined during the first 3 weeks of August 1972. Corn was sampled 4–6 weeks earlier than farmers routinely began harvesting the crop. Samples from areas 4, 5, and 6 were exclusively white corn; whereas, the remainder were yellow varieties.

Corn was examined as follows: a) each ear was tagged and information recorded on an individual ear basis; b) ears were husked, insect damage was recorded, and insects were collected; c) husked ears were examined under uv light; d) ears were dried at 60° C to 12–14% moisture; e) dried corn from each ear was shelled, cracked, and examined under uv light; f) BGY-positive samples were extracted individually maintaining ear identity; BGY-negatives were composited by origin and insect damage with subsequent extraction of representative fractions; and g) extracts were assayed for aflatoxin.

Moisture determination of sampled corn was carried out on a field basis. Six ears from each field were randomly selected, a 200-g sample was hand shelled from these ears, and the shelled sample was subsequently dried at 103° C for 72 hr. Moisture percentages were calculated on a wet weight basis.

Activity of corn insects and mites was assessed by the actual presence of the pest or its characteristic damage. Five groups of insects or mites were recorded: corn tip complex, largely corn earworm, *Heliothis zea* (Boddie); corn borer, European, *Ostrinia nubilalis* (Hubner) and southwestern, *Zeadiatraea grandiosella* (Dyar); stinkbug, *Pentatomidae*; sap and picnic beetles, *Nitidulidae*; and mites, *Acarina*. Stinkbug damage was identified by the distinctive puncture lesions on caps of individual kernels, whereas mite activity was indicated by red streaks on kernels (18).

Microbiological tests were carried out by placing portions of plant material directly on water agar. Petri plates were incubated at 28° C and examined under a stereoscopic microscope after 5 and 10 days.

Aflatoxin analyses were made on each BGY-positive sample and on blended aggregates of BGY-negative material from each field. The assay technique was essentially the one described by Dantzman and Stoloff (19); 50 g of ground corn was extracted with 250 ml of chloroform and 25 ml of water on a reciprocal shaker for 30 min with subsequent filtration, drying of the extract with anhydrous sodium sulfate, and vacuum concentration. Aflatoxin in the extracts was estimated by development on thin-layer chromatographic plates (16) and comparison with reference standards. Aflatoxin B-1 and B-2 in sample extracts were verified by cochromatography of corn extracts with B-1 and B-2 reference standards.

RESULTS

BGY Fluorescence and Insect Damage

General Survey. A large number of husks and silks emitted a greenish-yellow fluorescence under uv light. However, the husk-silk fluorescence appeared to be slightly different from that routinely associated with *A. flavus* contamination (16,17). Microbiological testing of fluorescent husks and silks showed a limited incidence of *A. flavus*; it was tentatively concluded that the fungus was not necessarily responsible for development of the emission.

Results of the initial screening appear in Table I. Fluorescence was detected on 11.2% of husked ears with significant variation between fields. Incidence in area 1 was about one-half that observed in the other test regions. Overall, approximately 10% of the husked ears exhibited fluorescence only on immature seed at the ear tip. Therefore, about 1% of the ears had fully developed kernels that fluoresced. However, BGY occurrence in the shelled and cracked samples of 10.0% (area 1) and 14.2% (area 2) was significantly higher than the incidence of fluorescence detected on the middle and butt portions of intact ears from areas 1 (1.4%) and 2 (1.0%); this difference was unanticipated since shelling was expected to eliminate most of the immature tip kernels and consequently their contribution to sample fluorescence. Apparently, some of the test kernels exhibited BGY fluorescence only after cracking.

Corn borer damage in tip and butt kernels was identified as the most frequent expression of insect activity (Table I). There were significant differences between test regions in the incidence of corn borer activity; corn from areas 1 and 2 exhibited two to three times as much damage as samples from areas 3 and 4.

Corn earworm damage, largely on ear tips, was observed on 20.9% of the samples. Earworm distribution was more uniform than corn borer dispersion;

TABLE I
Incidence of Bright Greenish-Yellow (BGY) Fluorescence, *Aspergillus flavus*, Insects, and Insect Damage in a General Corn Survey

Observation	% Ears Observed in Area				Mean	Variation[a]	
	1	2	3	4	%	Area	Field
Ear Fluorescence (kernel)							
Tip	5.0	11.5	10.6	13.9	10.3	*	**
Middle	0.0	0.6	0.2	0.9	0.5	NS	NS
Butt	1.4	0.4	0.2	0.1	0.3	*	*
Total	6.4	12.5	11.0	14.9	11.2	*	*
Cracked corn fluorescence	10.0	14.2	1.5	0.6	6.6	**	NS
Corn borer[b]							
Cob only	4.8	1.5	1.3	1.0	2.1	**	NS
Tip kernels	10.8	11.6	4.7	4.9	8.1	**	NS
Middle kernels	1.4	1.6	0.7	1.4	1.4	NS	NS
Butt kernels	10.3	4.1	3.1	2.0	4.8	**	*
Total[c]	34.1	25.2	12.9	10.1	20.6	**	**
Corn earworm[b]							
Silk	1.1	3.3	0.4	1.1	1.6	**	*
Cob only	8.7	12.3	8.1	8.0	9.4	NS	NS
Ear tip	9.6	11.0	11.2	9.1	10.3	NS	**
Total	19.4	26.6	19.7	18.2	20.9	NS	**
Other pests							
Beetles	3.0	4.9	1.5	1.0	2.7	**	NS
Mites	29.9	37.4	23.5	3.2	23.7	**	**
Stinkbugs	5.3	5.2	2.4	3.8	4.2	*	NS
Insect-free	33.2	26.6	47.5	65.4	43.1	**	**

[a]NS = not significant; * = significant at 5% level; ** = 1% level.
[b]Damage site on sampled ears.
[c]Includes ears with damage observed on tip, middle, butt, and multiple combinations. The combinations are not presented independently but included in the total.

TABLE II
Incidence of BGY Fluorescence and Insect Damage in the Intensive Corn Survey

Observation	Incidence, %[a]	
	Area 5	Area 6
Ear fluorescence (kernel)		
Tip	5.4	27.7 **
Middle	0.4	0.1 NS
Butt	0.2	0.1 NS
Total	6.0	27.9 **
Cracked corn fluorescence	1.7	0.0 **
Corn borer[b]		
Cob only	3.7	7.7 **
Tip kernels	2.7	5.9 **
Middle kernels	0.7	0.9 NS
Butt kernels	1.6	1.0 NS
Total[c]	9.6	17.4 **
Corn earworm[b]		
Silk	5.2	0.0 **
Cob only	8.8	3.7 **
Ear tip	11.6	1.3 **
Total	25.6	5.0 **
Other pests		
Beetles	0.3	2.0 **
Mites	0.0	0.0
Stinkbugs	2.0	7.2 **
Insect free	61.8	70.7 **

[a]NS = not significant. Significant variation: * = 5% level, ** = 1% level.
[b]Damage site on sampled ears.
[c]Includes ears with damage observed on tip, middle, butt, and multiple combinations; the combinations are not presented independently but included in the total.

TABLE III
Incidence of BGY Fluorescence and Aflatoxin in the General and Intensive Corn Surveys

Survey Area	Number of Sample Ears	Incidence[a]	
		BGY positive	Aflatoxin B-1 >20 ppb
General			
1	900	84	39
2	900	133	73
3	900	14	5
4	900	6	3
Total	3,600	237	120
Intensive			
5	600	12	6
6	750	0	0
Total	1,350	12	6

[a]Incidence of aflatoxin is based on BGY-positive samples and does not include the nonfluorescing aggregates that contained the toxin.

differences between the four areas were not significant. In area 2, corn earworm activity occurred on 26.6% of the ears. Only limited incidence of beetles and stinkbugs was observed, but mite damage was detected on 23.7% of the ears. An interesting aspect of mite damage distribution was the low level (3.2%) in white corn from area 4. Of all the samples examined, more than one-half had some insect damage. Considerable variation was observed between fields and test regions in numbers of insect-free samples.

Intensive Survey. Results are presented in Table II. Incidence of kernel fluorescence in area 6 corn was significantly higher than that observed in area 5 corn but essentially all fluorescence was confined to immature seed at the ear tip. Ultraviolet examination of dried, cracked corn disclosed interesting differences; none of the area 6 seed exhibited the characteristic BGY emission, whereas 1.7% of the area 5 corn was positive.

Corn borer damage was more common in area 6 corn, but the incidence of corn earworm was approximately five times greater in area 5 corn. The intensive study of white corn demonstrated a complete absence of mite damage; this observation corroborated results obtained from examination of white corn in the general survey. Significantly more insect-free ears were found in area 6 (70.7%) than in area 5 (61.8%); this difference was largely an expression of reduced earworm levels in area 6.

Aflatoxin Assay

A total of about 5,000 individual corn samples were collected in both surveys. Shelled, cracked corn from 5% of the test ears was BGY positive. Earlier observations of BGY fluorescence and aflatoxin in cracked corn had shown that aflatoxin-contaminated corn exhibited the uv-induced emission, whereas nonfluorescing seed was usually toxin-free (16). Preliminary tests were carried out to determine the feasibility of reducing the number of aflatoxin determinations by testing aggregates of nonfluorescing samples. Assay of 25 randomly selected BGY-negative samples yielded no aflatoxin positives; this result provided some assurance that aflatoxin occurrence in non BGY fluorescing corn was infrequent. The 4,700 nonfluorescing samples were composited by sampling site and insect occurrence into 282 samples composed of corn from 2 to 30 ears. Aggregates were blended and representative samples tested for aflatoxin. Six samples (2%) contained aflatoxin B-1 above 20 ppb; the toxin-positive aggregates were randomly distributed on an area and insect damage basis, and all were from fields yielding 1 or more aflatoxin-positive ears.

Fluorescing cracked-corn samples were individually extracted and assayed for aflatoxin. Aflatoxin assays of BGY-positive samples are given in Table III. Approximately 3.3% of the general survey ears, but less than 1% of the intensive survey ears, contained aflatoxin B-1 in excess of 20 ppb. About 2.5% of all the sample ears contained toxin above 20 ppb. The overall occurrence of aflatoxin-contaminated ears must have been somewhat higher since we observed that corn from six (2%) of the nonfluorescing aggregates contained the toxin. No aflatoxin was detected in single ear or bulked samples from area 6, and white corn from areas 4 and 5 also exhibited a distinctly lower aflatoxin incidence than yellow corn from two of the three other general survey areas.

About 50% of all BGY-positive cracked-corn samples contained aflatoxin B-1 in excess of 20 ppb. None of the aflatoxin-contaminated samples contained levels

between 10 and 20 ppb but the toxin may have been present in the remainder of samples at levels below the detection limit of the test (~10 ppb). Because aflatoxin G-1 and G-2 were not found in contaminated corn, we concluded that *A. flavus* rather than *A. parasiticus* Speare (1) was responsible for the toxin elaboration.

Variation between fields in incidence of aflatoxin (60 ears per field) was highly significant. The number of positive samples varied from 0 (28 fields) to a high of 16 out of 60 ears in 1 test field; 12 fields had 3 to 7 ears positive and the remaining test fields had 1 or 2 aflatoxin-positive ears. The 6 aflatoxin-positive samples of composited nonfluorescing corn did not change toxin occurrence on a field basis since aflatoxin was exclusively observed in aggregates obtained from fields that also yielded individual ears of BGY and aflatoxin-positive corn. Of the BGY-positive ears, the proportion showing aflatoxin did not vary between fields or test areas.

Agronomic Practices Associated with General Survey Fields

During collection of samples for the general survey, corn growers were queried about agronomic practices associated with the test fields. Pre-emergence Atrazine® was the most common chemical treatment. Irrigation was employed routinely in areas 2 and 3 but not in areas 1 and 4. Almost all the yellow corn was a widely grown single-cross variety and the white corn was one of two double-cross varieties. Planting dates for each of the four areas ranged from April 3 to April 14. Dates of planting varied significantly between test regions. Moisture determinations carried out at the time of the survey demonstrated that the corn was relatively uniform in maturity ranging from 41.2 to 43.4% moisture. At this moisture level, corn was fully dented but not yet physiologically mature (20). Rainfall throughout the test region was generally higher than average but temperatures were normal.

TABLE IV
Association of Corn Earworm with other Pests or Aflatoxin in Corn from the General Survey

Pest or Aflatoxin	Corn Earworm Incidence[a] %
Corn borer	
Absent	22.9
Present	15.6**
Beetles	
Absent	21.0
Present	33.3**
Mites	
Absent	23.7
Present	13.9**
Stinkbug	
Absent	21.7
Present	13.8*
Aflatoxin ≥ 20 ppb	
Absent	21.0
Present	30.0*

[a]* = Significant association at the 5% level; ** = significant association at the 1% level.

Statistical Associations

Associations between all possible pairs of characteristics reported in Tables I and II were examined by chi-square tests (21). This statistical procedure is used to compare proportions of samples possessing a certain characteristic in the presence or absence of another recorded property. Table IV shows significant associations between corn earworm and other insects or the presence of aflatoxin. These data reveal negative associations between the presence of earworm and: a) corn borer; b) mites; or c) stinkbug. However, beetles were positively related to earworm damage. Incidence of aflatoxin-positive corn was significantly higher on ears showing earworm damage than on those without it.

Equations for relating BGY incidence of ground grain on a field basis to planting date, initial moisture level, corn borer, corn earworm, and mites yielded multiple correlations in the range of 0.4 to 0.6. Generally, the correlation was statistically significant. However, there was no clear evidence for selecting a particular combination of variables in an estimating equation for predicting BGY fluorescence. There were no associations between the agronomic practices followed in the test fields and subsequent fluorescence of the husked ear, or the shelled, cracked corn from sample ears.

DISCUSSION

Aflatoxin contamination of sample corn could have occurred: a) in the field; b) in the time between sample collection and drying of the corn to 12-14% moisture; or c) both in the field and during the survey procedure. Observed insect damage would appear to provide access routes for mold penetration of corn ears in the field. Transmission of *A. flavus* propagules by insect vectors appears likely.[3] However, visual examination of insect-damaged areas on freshly harvested corn did not reveal overt manifestations of *A. flavus* infection.

Possible aflatoxin production between picking and drying to 12-14% moisture requires consideration of the time elapsed between sample collection and attainment of sufficiently high temperature in the dryer to prevent growth of the fungus (44° to 46° C) (1). Sampled corn was processed and placed in the dryer within 8-12 hr after removal from stalks in the field. Test ears were uniformly dispersed on racks in a 60° C forced-draft oven for drying to 12-14% moisture. However, temperature of the samples was not carefully monitored during drying and, conceivably, the corn did not reach 44° to 46° C during the initial 24 hr. Therefore, sample ears may have been maintained under conditions conducive to fungal development for 2-3 days immediately after harvest. Presumably, additional fungal growth and aflatoxin formation would most likely occur in kernels already invaded by *A. flavus*.

The third alternative proposes that aflatoxin contamination of the corn surveyed represents the summation of *A. flavus* activity in the field and during handling and drying after harvest. Our information does not preclude this possibility.

Although our study did not delineate the specific time that aflatoxin was elaborated in the corn samples, we made several salient observations: Corn harvested at 40-45% moisture either contained aflatoxin while in the field or was

[3]Fennell, D. I., Kwolek, W. F., and Lillehoj, E. B. *Aspergillus flavus* and other fungi associated with insect-damaged field corn. In preparation.

highly susceptible to *A. flavus* proliferation and aflatoxin formation during the initial post-harvest period; incidence of aflatoxin in white corn was significantly lower than in yellow corn; occurrence of aflatoxin in corn was not uniformly distributed in the test area; aflatoxin incidence was correlated with corn earworm damage; no agronomic factors were associated with aflatoxin formation in corn; fluorescence of immature kernels at the tips of intact ears was not exclusively associated with BGY fluorescence of dried, shelled, cracked corn; and BGY fluorescence of dried, shelled, cracked corn was associated with the presence of aflatoxin although not all of the samples with fluorescing corn contained measurable quantities of the toxin.

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