Aspergillus flavus and Aflatoxin in Iowa Corn Before Harvest

Abstract. Aspergillus flavus *and aflatoxin were detected in ears of Iowa corn on plants before harvest in 1975. Presence of the fungus was associated with kernel injury caused by the second generation European corn borer. Amounts of aflatoxin* B_1 *in corn from a limited number of selected ears ranged from 1 part per billion to 1560 parts per billion with a mean of 430 parts per billion.*

Aspergillus flavus Link ex Fr. is the principal organism responsible for contamination of agricultural commodities by the carcinogenic metabolite aflatoxin B_1. Traditionally, *A. flavus* development has been considered to be a problem that affects cereal grains after harvest (*1*). However, field studies have shown that the fungus can infect seed before harvest if spores are introduced into the region of developing kernels (*2–4*).

Investigation of toxin occurrence in ears of field corn grown at geographically diverse locations demonstrated that corn from areas in the southern United States had significantly higher levels of toxin than did similar samples from the Midwest (*2*). Other studies supported the premise that growing conditions in the South were more conducive to *A. flavus* infection of corn and subsequent synthesis of toxin (*5*). A definitive examination of the field occurrence of *A. flavus* and aflatoxin was made in 1973 with corn from a region of South Carolina (*6*). Of the test samples, 60 percent contained kernels internally colonized by the fungus and 32 percent were contaminated with the toxin at levels exceeding 20 parts per billion, the action guideline of the Food and Drug Administration.

Corn being delivered to many country and terminal elevators is being examined for the bright greenish-yellow (BGY) fluorescence that is associated with aflatoxin in the seed (*7*). In October 1975, news accounts related an occurrence of BGY fluorescence in freshly harvested corn from Iowa (*8*, *9*). In response to the reports, we examined approximately 6000 ears of corn on standing plants in western Iowa. Of these, 20 had characteristic sporophores and spore heads of *A. flavus*, in all cases associated with feeding injury caused by larvae of the second generation European corn borer *Ostrinia nubilalis* (Hübner).

Extensive *A. flavus* development on a test ear is shown in Fig. 1A. The fungus is clearly associated with an insect damage track and a larger region of fungal growth on the butt end. On this ear, *A. flavus* was uniformly distributed along the damage track caused by the larva. Discolored kernels were routinely noted in zones of fungal development; many of these kernels exhibited BGY fluorescence (*7*).

The second generation European corn borer has become a widespread pest in the Corn Belt. During the 1940's and 1950's, many commercially grown hybrids were extremely susceptible to infestation by the first generation (*10*). However, corn breeders have incorporated factors that inhibit leaf feeding by the first generation and now many hybrids have a reduced susceptibility. Corn germ plasm with resistance to sheath feeding by the second generation borer has been more difficult to obtain, and this pest has increased. *Aspergillus flavus* inoculation studies indicate that maximum infection of developing corn occurs during the 20- to 40-day period after silking (*2*, *3*). Since the activities of the second generation larvae on ears overlap this time period, the insect becomes suspect as a major factor contributing to dissemination of the fungus in midwestern corn. In addition scattered areas of drought in western Iowa during July and August may have predisposed the developing ears to insect infestation and fungal infection.

Although *A. flavus* was invariably associated with insect damage, the tracks left by feeding larvae did not always exhibit *A. flavus*. Ears shown in Fig. 1, B through D, depict variation in the fungal colonization process. Figure 1B shows an ear with general *A. flavus* growth on the distal end, but the track extending from the tip has little or no sign of the fungus. Figure 1C exhibits limited *A. flavus* growth on an ear tip associated with another track that is free of fungus. The ear shown in Fig. 1D has a track in the middle region with *A. flavus* growth limited to the center of the insect damage.

Figure 1E shows the same ear as Fig. 1D with a row of kernels damaged by insects; the adjacent row of undamaged seed was removed. The borer larva has fed along the upper kernels with apparent penetration of the pericarp in most seeds along the track. The discolored kernels located in the center of the zone damaged by insects exhibited *A. flavus*, with uncontaminated areas on either side. Figure 1F shows another insect track extending from the tip about one-fourth the length of the ear. Conidial heads of *A. flavus* are apparent only on the tip end of the track.

Although the visual observation of the

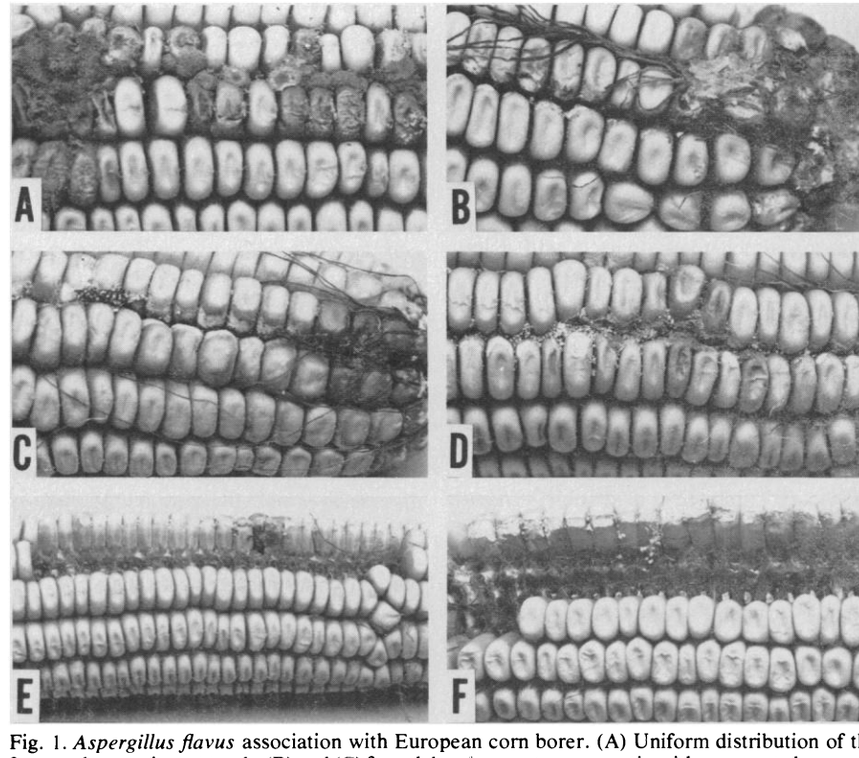

Fig. 1. *Aspergillus flavus* association with European corn borer. (A) Uniform distribution of the fungus along an insect track; (B) and (C) fungal development on an ear tip with apparent absence of the fungus in the area extending from the infected kernels; (D) insect track in the middle region of an ear with *A. flavus* growth restricted to the center of the damage; (E) ear D with a row of insect-damaged kernels removed; and (F) insect track from ear tip with *A. flavus* conidial heads apparent on kernels through the damaged zone.

Table 1. *Aspergillus flavus* infection of corn kernels from selected, freshly harvested ears. Kernels were selected from either an insect-damaged row or the adjacent, undamaged row. The surfaces of kernels were sterilized with 2 percent sodium hypochlorite for 2 minutes, rinsed twice with sterile water, placed on ME agar (malt extract, 3 percent; agar, 1.5 percent) in petri plates, incubated at 28°C, and examined under a microscope after 6 days.

Ear (Fig. 1)	Kernel row	Kernels examined (No.)	Kernels infected (No.)			Sterile kernels (No.)
			A. flavus	*Fusarium*	*Penicillium*	
B	Insect-damaged	25	2	25	6	0
B	Undamaged	25	0	18	2	7
C	Insect-damaged	17	11	14	0	3
C	Undamaged	17	0	0	0	17
D and E	Insect-damaged	24	13	9	0	4
D and E	Undamaged	22	0	1	0	21
F	Insect-damaged	23	23	2	0	0
F	Undamaged	20	0	16	4	0

greenish-yellow spore masses was definite evidence for the presence of *A. flavus*, the question remained of the extent of concealed mycelial proliferation throughout the ear. This problem was examined by determining the incidence of the fungus in selected kernels; a row of seed exhibiting insect damage was compared with undamaged kernels from the adjacent row. In addition, other predominant internal fungi of kernels sterilized on the surface were enumerated after 6 days of incubation on malt extract agar (*7*) at 28°C.

Since the four test ears depicted in Fig. 1 were considered representative of all those containing *A. flavus*, kernels from these ears were examined for fungi (Table 1). The most striking observation in this test was the exclusive association of the fungus that produces aflatoxin with kernels damaged by insects. Corn from ear 1B had a low incidence of *A. flavus* in kernels from the damaged row and a high occurrence of *Fusarium* in both rows. *Aspergillus flavus* on this ear was restricted to the ear tip with widespread *Fusarium* infection. *Fusarium moniliforme* Sheldon was the predominant *Fusarium* species on all ears (*11*). Seed from ear 1C exhibited a high incidence of *A. flavus* and *Fusarium* in the kernels damaged by insects, with no fungi in the adjacent row. Insect-damaged kernels of ear 1D-1E from the middle region of the ear exhibited considerable *A. flavus* and *Fusarium* infection, but seed in the adjacent row was essentially free of fungus. Ear 1F contained *A. flavus* in all damaged kernels; *Fusarium* was limited in damaged seed but its incidence was higher in the adjacent row.

Penicillium oxalicum Currie and Thom was observed on numerous ears in the field and in a limited number of test kernels (Table 1). A few kernels were infected with *Aspergillus clavatus* Desm. However, the three predominant fungi observed on the ears damaged by insects were *A. flavus*, *F. moniliforme*, and *P. oxalicum*.

Eleven test ears were shelled, and seed from individual ears was ground, extracted, and assayed for aflatoxin (*12*). Corn from all of the ears contained aflatoxin B_1, ranging in concentration from 1 part per billion to 1560 parts per billion, with a mean of 430 parts per billion. The reason for the great variation in toxin levels is not known, but it might be the result of differences in aflatoxin production capabilities of strains of *A. flavus*. Fifteen isolates of the fungus from ear 1F were transferred to the APA (aflatoxin-producing ability) medium (*13*); this qualitative test showed that five of the 15 produced aflatoxin.

Our observations of Iowa corn provide conclusive evidence for the infection of field corn by *A. flavus* in the Midwest and the production of aflatoxin in the seed before harvest. Infection by the fungus appeared to be exclusively associated with injury resulting from feeding of larvae of the second generation European corn borer.

E. B. LILLEHOJ
D. I. FENNELL, W. F. KWOLEK
Northern Regional Research Center, Agricultural Research Service, Peoria, Illinois 61604

References and Notes

1. C. M. Christensen, *Bot. Rev.* **23**, 108 (1957).
2. E. B. Lillehoj, W. F. Kwolek, E. E. Vandegraft, M. S. Zuber, O. H. Calvert, N. Widstrom, M. C. Futrell, A. J. Bockholt, *Crop Sci.* **15**, 267 (1975).
3. G. W. Rambo, J. Tuite, P. Crane, *Phytopathology* **64**, 797 (1974).
4. J. J. Taubenhaus, *Tex. Agric. Exp. Stn. Bull.* **270**, 3 (1920).
5. H. W. Anderson, E. W. Nehring, W. R. Wichser, *J. Agric. Food Chem.* **23**, 775 (1975); O. L. Shotwell, C. W. Hesseltine, M. L. Goulden, *Cereal Sci. Today* **18**, 192 (1973).
6. E. B. Lillehoj, W. F. Kwolek, G. M. Shannon, O. L. Shotwell, C. W. Hesseltine, *Cereal Chem.* **52**, 603 (1975).
7. D. I. Fennell, R. J. Bothast, E. B. Lillehoj, R. E. Peterson, *ibid.* **50**, 404 (1973).
8. D. Muhm, Des Moines *Register*, 22 October 1975, p. 1.
9. ——— and A. Jacobson, *ibid.*, 21 October 1975, p. 1.
10. T. A. Brindley, A. N. Sparks, W. B. Showers, W. D. Guthrie, *Annu. Rev. Entomol.* **20**, 221 (1975).
11. We thank J. J. Ellis for identification of *Fusarium moniliforme*.
12. Anonymous, *J. Assoc. Off. Anal. Chem.* **55**, 426 (1972).
13. S. Hara, D. I. Fennell, C. W. Hesseltine, *Appl. Microbiol.* **27**, 1118 (1974).

20 May 1976

Elevated Plasma Zinc: A Heritable Anomaly

Abstract. *An extremely high concentration of zinc in the plasma (hyperzincemia) was found in five out of seven members of one family and in two out of three second-generation individuals, an indication that the condition is heritable. The excess zinc in the plasma appears to be bound to serum proteins, with no apparent clinical symptoms or abnormalities.*

Numerous acquired disease conditions have been demonstrated to result in lowered concentrations of zinc in the plasma (or serum) (*1*, *2*). In addition, achrodermatitis enteropathica, a hereditary disease in which the zinc metabolism is altered, dramatically responds to oral zinc therapy (*3*). In contrast, few if any clinical conditions causing high concentrations of zinc in the blood have been reported. Although the effect of zinc toxicity on concentrations in the blood in humans has not been studied under controlled conditions, one report indicates that the increase in serum zinc was minimal even when toxic quantities of zinc were ingested (*4*). Zinc sulfate therapy can result in increased serum zinc concentrations, but this increase is usually less than twice baseline values and is transitory (*5*). With the above exceptions, we have found no clinical conditions that are characteristically accompanied by a sustained elevated zinc concentration.

During the course of screening patients for zinc in the plasma we noted that in a 28-year-old black male the concentration was extremely high (> 300 μg/100 ml). Initially we suspected that the high concentration in the plasma might be the result of toxic ingestion. However, subsequent blood samples from this subject over a 20-week period, as well as a study of his siblings, established that the increased concentration cannot be attributed to toxic ingestion (*6*).