ASPERGILLUS FLAVUS PRESENCE IN SILKS AND INSECTS FROM DEVELOPING AND MATURE CORN EARS

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ABSTRACT

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In efforts to determine the origin of the inoculum responsible for *Aspergillus flavus* infection of corn before harvest, silks and insects from developing and mature ears of two corn hybrids grown at six locations were examined for the presence of the fungus. Incidence in silks varied by hybrid, location, and state of maturity. *A. flavus* occurred in at least one silk sample from each location and was encountered more frequently on silks from mature ears than on those from immature ears collected at silking. From 1200 test ears, 3442 insects were collected. Distinct interactions were observed between the number and type of insects and the location, hybrid, and sampling time. Insects, grouped in five broad categories, showed a relatively uniform presence of *A. flavus* ranging from 1.7 to 3.1%. Dissemination of the fungus was not related to the activities of a specific insect. Overall, 52% of *A. flavus* isolates from silk and 32% of those from insects produced aflatoxin in a qualitative test. Aflatoxin was detected in concentrations ranging from 1 to 61 ppb in mature corn of both hybrids at five of the six locations.

Surveys in the field and studies on controlled plots have shown that *Aspergillus flavus* can infect corn and produce aflatoxin before harvest (1–8). Preliminary investigations have identified *A. flavus* propagules associated with a significant number of insects (9). Examinations of the relation between insect activity, *A. flavus* infection, and aflatoxin contamination have implicated several insects, including: European corn borer, *Ostrinia nubilalis* (Hübner); fall armyworm, *Spodoptera frugiperda* (J. E. Smith); corn earworm, *Heliothis zea* (Boddie); and rice weevil, *Sitophilus oryzae* (Linn) (7–10). Although these studies provided evidence for an association between insect damage and the presence of *A. flavus* on preharvest corn, no definitive cause-effect relation between a specific insect and the fungus could be established.

A 1974 study in South Carolina and Florida compared *A. flavus* susceptibility of corn hybrids adapted to growth in the southern U.S. with the susceptibility of those not adapted (11). In this test, developing ears were inoculated by introducing *A. flavus* spores into the silk bundle. At both locations, toxin levels were lower in the hybrids adapted to the South. Apparent differences in susceptibility to the fungus may have reflected known differences in husk protection from insects responsible for the introduction and dispersal of fungal

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inoculum in the kernel region of developing ears. The study indicated that the process of *A. flavus* infection in the field depends on: 1) the availability of a fungal inoculum, 2) transmission of the spores into the ear, 3) initial infection of developing kernels by the toxin-producing fungus, and 4) dispersal of inoculum throughout the ear from the primary infection site. Although all four steps in the infection process may be functionally dependent on insect intermediaries, airborne fungal spores deposited early on corn silks might provide the original inoculum source.

Variations in the aflatoxin-producing potential of *A. flavus* strains isolated from nature may relate to insect transmission and successful infection of developing corn. Schroeder and Boller (12) and Mehan and Chonan (13) isolated the fungus from several field crops. The incidence of toxin-producing isolates ranged from 35% (rice) to 96% (peanuts). Apparently, differences in the substrate or other environmental factors exert a selective effect. Elucidation of the selectivity mechanism might explain the process of *A. flavus* development in agricultural crops.

The work reported in this paper was carried out to determine: 1) the role of silks as a repository for *A. flavus* spores on corn grown at diverse locations in the U.S., 2) the entomogenous origin of the *A. flavus* inoculum responsible for infection of corn before harvest, and 3) the difference between two distinctive hybrids in *A. flavus* associations with silks and insects on developing ears.

MATERIALS AND METHODS

Two hybrids (A and B) were selected for the study: Hybrid A with a relatively light open husk, adapted to the corn belt; and Hybrid B, with a tight heavy husk, adapted to the southern U.S. Duplicate test plots were planted in Georgia, South Carolina, Texas, Missouri, Illinois, and Iowa (Table I). At silking and at postphysiological maturity, samples of 12 ears per plot were collected from each hybrid at each location for microbiological examination of silks. Unshucked ears were individually bagged in paper bags, securely sealed, placed in a forced-draft oven at 60° C for 7 days, and shipped to the Northern Regional Research Center. Microbiological tests were carried out by transferring 5-cm segments of exposed silks from each ear to petri plates containing *Aspergillus* differential medium (ADM) (14). Plates were incubated at 28° C for 3 days and examined for *A. flavus* (14).

TABLE I
Planting and Silking Dates at Various Locations

Hybrid	Experiment, Ga.	Florence, S.C.	College Station, Tex.	Columbia, Mo.	Peoria, Ill.	Ames, Iowa
			Planting Dates			
A and B	4/28	4/9	4/19	5/14	5/9	5/15
			Silking Dates			
A	6/25	6/15	6/21	7/22	7/21	7/25
B	7/2	6/25	6/28	8/6	8/4	8/27

Determination of *A. flavus* presence in insects was made from 50 unshucked ears/location (25/replication) collected at 30 days after silking and at maturity. These ears also were bagged individually when collected, securely sealed, dried, and sent to Peoria. On arrival at NRRC, all insects from each test ear were collected, identified, and placed in a sterile vial. Aggregated insects from each ear were shaken for 2 min in a 2% sodium hypochlorite solution, rinsed twice in sterile water, and placed individually on ME agar (malt extract, 3%; agar, 1.5%). Plates were incubated at 28°C for 5 days and examined under a dissecting microscope for the presence of *A. flavus*.

Conidia from colonies developed on the insect bodies were streaked on Czapek's agar plates (15) and incubated at 24°C for one week. Conidia from individual heads in colonies representative of the predominant growth habit were transferred to slants of aflatoxin-producing ability (APA) medium (16). Toxin production was determined by examination of the slant cultures under long-wave uv light for the characteristic blue-white fluorescence (16).

After insect collection, test ears were shelled, ground, combined by replication, and blended for 15 to 30 min in a Twin Shell blender (PK-LB-6948). Ground samples were assayed for aflatoxin by the technique described in the Official First Action of the Association of Official Analytical Chemists (17). Quantities of aflatoxin present in the extracts were determined on thin-layer chromatographic (tlc) plates coated with 0.5 mm Adsorbosil-1. Plates were developed with water:acetone:chloroform (1.5:12:88 v/v/v), and fluorescent aflatoxin B_1 spots were measured densitometrically.

RESULTS

The number of ears with silks bearing *A. flavus* varied by hybrid and location (Table II). Differences in occurrence of the fungus between sampling times (silking and maturity) were conspicuous in Hybrid A at 4 of the 6 locations; silk from mature corn usually showed a higher incidence of *A. flavus*. Of the *A. flavus* isolates obtained from these silks, 52% produced aflatoxin in the qualitative test.

A total of 3442 insects were examined from 1200 test ears; 1236 insects were obtained from ears collected 30 days after silking and 2206 from mature ears. The

TABLE II
***Aspergillus flavus*-Positive Silk Bundles from Corn Ears[a]**

Location	Hybrid A		Hybrid B		Totals	
	Full silk	Mature	Full silk	Mature	Full silk	Mature
Ga.	1	5	9	8	10	13
S.C.	0	7	0	0	0	7
Tex.	6	5	2	1	8	6
Mo.	6	5	0	4	6	9
Ill.	0	5	1	3	1	8
Iowa	0	13	5	8	5	21
Totals	13/144	40/144	17/144	24/144	30/144	64/144

[a]24 Ears/location/sampling time.

insects were grouped into five categories: 1) beetle larvae (23.5%); 2) beetle adults (39.7%); 3) stored grain insects (30%); 4) corn borer, corn earworm, and miscellaneous caterpillars (5.7%); and 5) others (1.1%). Beetle larvae and adults were predominantly sap beetles, but also included limited numbers of ladybird, striped cucumber, flea, and leaf beetles, and adult corn rootworm. Stored grain insects were: rice weevils, Angoumois grain moth pupae, confused flour beetles, saw-tooth grain beetles, and square-necked grain beetles. Insects in the "Others" category included Diptera, Hemiptera, Neuroptera, and Psocoptera.

Numbers of insects in the five categories are summarized by location and variety in Table III. Distinct interactions were observed among the number and type of insects, location, hybrid, and sampling date.

More insects were collected from mature ears of both hybrids than from those sampled at 30 days after silking at all southern locations, with the exception of Hybrid A in Texas. Increases were attributed to beetle adults and stored grain insects in Georgia, to stored grain insects in South Carolina, and to beetle larvae and adults in Texas. The lower total insect population observed on Hybrid A in Texas resulted from the almost complete absence of beetle larvae at maturity. In Georgia and South Carolina, overall insect incidence was higher on Hybrid A than on Hybrid B, probably reflecting resistance to insect infestation conferred by the tight heavy husk of the latter hybrid. At southern locations, the number of beetle larvae observed on both corn varieties was higher at maturity than at 30 days past silking, except in South Carolina- and Texas-grown Hybrid A. Beetle adults were also encountered in greater numbers at maturity on both hybrids in Georgia and Texas, but were more numerous at 30 days past silking in Hybrid A in South Carolina. Stored grain insects, apparently a serious problem at maturity in Georgia and South Carolina, occurred in only limited numbers in Texas. Insects of the corn borer-corn earworm category were more common in Texas on both hybrids than at other locations. Insect infestation of sample ears of both hybrids was much reduced in the Midwest and Missouri and declined in all categories between 30 days past silking and maturity.

The presence of *A. flavus* in insects collected from test ears appeared to be relatively uniform in the five designated categories. The largest group of insects collected, corn sap beetles (>60% of total) carried the fungus in 3.1% of the larvae and 2.8% of the adults. *A. flavus* was present in 1.7% of the stored grain insects, 2.0% of the corn borers/corn earworms, and 2.8% (1/36) of the "other insect" category. Overall, the fungus was found in 87 (2.5%) of the 3442 insects. The distribution of *A. flavus*-containing insects by location was: Georgia, 4; South Carolina, 19; Texas, 50; Iowa, 0; Illinois, 1; Missouri, 10. Although *A. flavus* occurred in a wide range of insects, the limited number of insects carrying the fungus at some locations precludes statistical comparisons. However, the number of *A. flavus*-infected insects was significantly higher on mature corn (82/87) than on corn collected at 30 days past silking. Of the 50 *A. flavus*-infected insects from mature Texas-grown Hybrid B, 46 were beetles. However, beetles also represented the predominant (95%) insect on this set of ears.

The following percentages of isolates from insects in the five categories gave positive results in the qualitative test for aflatoxin presence: beetles and beetle larvae, 20%; stored grain insects, 67%; corn borer/corn earworm, 50%. The single isolate from the "Other" insect category was a toxin producer. Of the 87 *A. flavus* isolates obtained from insects, 32% demonstrated the ability to produce

TABLE III
Distribution of Insects, Number Infected with *A. flavus*, and the Aflatoxin-Producing Capabilities of Isolates

Location	Corn: Hybrid	Corn: Sampling time[a]	Insect Number: Beetles[b] Larvae	Insect Number: Beetles[b] Adults	Insect Number: Stored grain insects[c]	Insect Number: CB and CEW	Insect Number: Other[d]	Insect Number: Total observed	Insect Number: Total *A. flavus* infected	Number of *A. flavus* Isolates Producing Aflatoxin[e]
Ga.	A	1	49	111	2	2	0	164	0	0
		2	66	423	464	0	0	953	4	1
	B	1	0	8	6	11	1	26	0	0
		2	32	143	64	0	2	241	0	0
S.C.	A	1	270	100	1	3	0	374	0	0
		2	28	29	353	5	2	417	19	11
	B	1	12	69	0	6	0	87	0	0
		2	19	92	116	2	2	231	2	0
Tex.	A	1	75	4	1	38	0	118	0	0
		2	1	70	5	8	2	86	0	0
	B	1	18	90	6	31	0	145	0	0
		2	96	117	6	13	4	236	50	8

Mo.	A	1	3	0	4	6	0	13	2	2
		2	0	2	1	2	2	7	2	2
	B	1	9	5	3	0	0	17	2	2
		2	0	4	0	2	2	8	4	1
Ill.	A	1	88	23	3	4	0	118	1	0
		2	0	1	0	0	1	2	0	0
	B	1	43	64	0	23	2	132	0	0
		2	0	2	0	0	11	13	1	1
Iowa	A	1	0	7	0	11	3	21	0	0
		2	0	0	0	0	0	0	0	0
	B	1	0	1	0	20	0	21	0	0
		2	0	0	0	10	2	12	0	0
Totals			809	1365	1035	197	36	3442	87	28
with *A. flavus*			26 (3.1%)	38 (2.8%)	18 (1.7%)	4 (2.0%)	1 (2.8%)		2.5%	

[a] 1 = 30 days past silking; 2 = maturity.
[b] Predominantly corn-sap beetles.
[c] Rice weevils, Angoumois grain moth pupae, confused flour beetle, saw-tooth grain beetle, and square-necked grain beetle.
[d] Miscellaneous Diptera, Hemiptera, Neuroptera, and Psocoptera.
[e] Qualitative fluorescence test on aflatoxin-producing ability (APA) medium (14).

aflatoxin.

Aflatoxin assays showed that test corn from the six locations contained no detectable toxin at 30 days after silking. Mature Illinois corn was also toxin-free. However, aflatoxin was found in mature corn from the remaining five locations (Table IV). Aflatoxin was detected in both hybrids at concentrations ranging from 1 to 61 ppb. Very low levels of aflatoxin were detected in Hybrid A from Iowa and Missouri, and in Hybrid B from Georgia.

DISCUSSION

Limited examination of silks from developing ears showed that *A. flavus* occurred in at least one of the samples from all six locations. Although the test did not provide a quantitative determination of the number of *A. flavus* propagules on the silks, our results clearly showed that the fungus can be found readily on corn grown at diverse locations. The *A. flavus* propagules on silks could have fallen out of the airborne spora or early insect activity might have carried conidia of the fungus to the silks from soil, decaying vegetation, or from stored grain.

Conidia of *A. flavus* present on the silks at the full-silk stage of development could provide inoculum for infection of developing kernels. If this hypothesis is correct, some mode of transmission of the fungal spores from the silks into the kernel area of developing ears is required. Insects appear to be likely vectors.

The critical observation in this study was the detection of *A. flavus* in surface-disinfected insects from five of the six locations. The fungus was widely distributed between the five major insect categories. The incidence of *A. flavus* was relatively low in insects at both sampling times but was significantly higher at maturity than at 30 days after silking. Absence of *A. flavus* in Iowa samples may reflect an insufficient number of insects since only 53 insects were obtained from that location. However, although only 45 insects were collected from Missouri ears, 8 yielded the fungus.

Assuming that the surfaces of insects were effectively sterilized by the treatment used, the 87 *A. flavus*-contaminated insects apparently had ingested the fungal propagules prior to removal from test ears. Although internal presence of the fungus probably is not essential to its dissemination by insects, it would represent a distinct advantage for *A. flavus* infection of corn ears before harvest, since frass from the insect may provide an ideal environment for spore germination. Insect-inflicted damage to the pericarp of developing kernels would

TABLE IV
Aflatoxin B_1[a] in Mature Corn Grown at Six Locations

Hybrid	Location					
	Ga.	S.C.	Tex.	Mo.	Ill.	Iowa
	Aflatoxin (ppb)					
A	0	61	13.5	1	0	1
B	1	2.5	18	0	0	0

[a]Averages from 50 ears/hybrid/location.

provide the fungal mycelium with easy entry into the seed.

The apparent internal presence of *A. flavus* in collected insects raises questions regarding the pathogenicity of the fungus to insects. Although the role played by toxins in insect pathology has not been established unequivocally (18), the death of some *A. flavus*-infected insect species has been attributed to toxins (19–22). Toxicity of the fungus to insect hosts might be expected to correlate with synthesis of aflatoxin. The aflatoxins, particularly B_1, have been shown to have a chemosterilant effect on the eggs, and to have delayed and reduced development of larvae of several insect species (23). However, since only 1/3 of the isolates obtained from insects in this study produced aflatoxin, it is apparent that insect-borne strains of *A. flavus* are heterogeneous for this characteristic.

Variation in aflatoxin production in corn could reflect: 1) inherent differences in the ability of *A. flavus* isolates to synthesize the toxins, and 2) environmental effects on interrelations among *A. flavus,* insect vectors, and developing corn. Little or no aflatoxin was found in test ears from Illinois, Iowa, Missouri, and Georgia. Increased levels of toxin and highest populations of *A. flavus*-containing insects were observed in corn from South Carolina and Texas. The results provide further evidence for an insect-*A. flavus* association in establishment of infection by the fungus in developing corn. However, the presence of *A. flavus* in a broad range of insects on corn ears before harvest suggests that dissemination of fungal inoculum is not related to the activities of a specific insect.

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