ASPERGILLUS FLAVUS AND OTHER FUNGI ASSOCIATED WITH INSECT-DAMAGED FIELD CORN

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ABSTRACT

White and yellow ear corn collected from fields in Missouri and Illinois was examined for damage by corn borers, earworms, mites, stinkbugs, and sap beetles and for the presence of these insects. Kernels selected from insect-damaged and insect-free ears were examined for fungi. The combined incidence of *Aspergillus flavus* on damaged and undamaged corn varied from 0 to 10.6% depending on the county surveyed; the average incidence was 5.2%. A 6.3% incidence of *A. flavus* on kernels from ears having insect damage differed significantly from the 2.5% incidence on kernels from undamaged ears. *Fusarium, Rhizopus,* and *Syncephalastrum* also were observed more frequently on kernels from insect-damaged ears. Similar distributions of fungal genera were observed on kernels from ears damaged by earworms and corn borers and by mites and stinkbugs. *A. flavus* was isolated from 15% of the 195 insects collected from freshly harvested ears. The fungus was associated with a significantly higher proportion of earworms (37%) than corn borers (14%).

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Aspergillus flavus Link ex Fr. and *Aspergillus parasiticus* Speare, the generally accepted aflatoxin-producing species, have been isolated from or implicated as pathogens of a number of insects, including corn earworms (1), mites (2,3,4), grain moths (5), bud moths (6), subterranean termites (7,8), cabbage loopers (9), house flies (10,11), and corn borers (12,13).

The role of insect vectors in the infection and spread of *Aspergillus* spp. in corn has been examined (12,13). Taubenhaus (1) observed that infection of field corn by *A. niger* and *A. flavus* frequently accompanied corn earworm damage and that the fungi developed rapidly on immature ears. During the later stages of maturation, ear infection by the fungi remained localized in the insect feeding area and fully mature ears appeared immune to fungal infection. In a similar study, Christensen and Schneider (13) observed a significant relation between corn borer activity and the spread of fungal infections in corn.

Although insect damage may simply provide a fortuitous access for subsequent fungal infection (14), obligate relationships between insect vectors and fungal pathogens have been described (15). In a classic study of plant disease transmission by insects, Hansen and Davey (16) identified thrips and mites as the insects responsible for transmitting *Aspergillus* spores in a fungal rot of figs. Grain-infesting mites have also been implicated in the transmission of *Aspergillus* storage fungi in moldy grain (2).

Although these previous studies identified insects as a factor in the transmission of *Aspergillus* spp., none provide specific information on aflatoxin contamination of foods and feeds. In a field survey of peanuts in northern Transvaal, Aucamp (17) observed that Astigmatid mites were routinely associated with the underground portion of the plant. These mites were heavily contaminated with *A. flavus* spores both externally and internally. Under certain environmental conditions the mites disseminated the fungus by penetrating the peanut pods. His survey provided presumptive evidence that infection of peanuts by *A. flavus* is mediated by a mite vector.

The work reported here is part of a field survey undertaken to determine whether *Aspergillus flavus* and aflatoxin could be detected in preharvest field corn and whether the presence of specific insects, or obvious insect damage, could be related to the presence of specific fungi. In this portion of the survey, we compared the distribution of the most common fungal genera encountered on a limited sampling of kernels from insect-free ears and from ears damaged by specific insects. The fungi observed on a limited number of insects collected directly from the ears also were recorded.

MATERIALS AND METHODS

The study was divided into two phases: 1) a general survey that included 60 fields in Mississippi, New Madrid, Scott, and Stoddard Counties located in the bootheel of southeastern Missouri and 2) an intensive study of two fields in Scott County, Mo., and five fields located in White and Crawford Counties in southeastern Illinois. Samples from Soctt County, Mo., and from Illinois were exclusively white corn, whereas the remainder were yellow varieties. Missouri corn was examined during the first 2 weeks of August 1972, and Illinois corn was

sampled in the third week of August 1972. A total of 4,950 ears were gathered at random from the 62 fields in Missouri and the five fields in Illinois.

In the general survey, 60 ears were collected from each of 15 fields (two independent 30-ear samples/field) in each of the four counties—a total of 3,600 ears.

In the intensive study, 600 ears were collected from 10 sites (30 ears/site) in each of two Scott County, Mo., fields and 750 ears were gathered from five sites (30 ears/site) in each of five Illinois fields.

Immediately after harvest, sample ears were husked and insect damage was assessed by noting the presence of either the pest or its typical damage. Insect activity was grouped into the following categories: (a) Corn earworm (*Heliothis zea* Boddie), (b) corn borer—European (*Ostrinia nubilalis* Hubner); Southwestern (*Zeadiatraea grandiosella* Dyer), (c) stinkbug (Pentatomoidae) (18), (d) sap and picnic beetles (Nitidulidae), and (e) mites (Acarina) (19). Insects were collected from husked ears and stored in sterile vials for 1 to 2 weeks. They were subsequently plated on Czapek's solution agar, incubated at 28° C for 5 days, and examined for fungi.

After the sample ears had been dried at 60° C to 12 to 14% moisture, two representative ears were selected for each available insect-damage category from each sampling site within a field; 580 ears, each exhibiting overt damage by a single type of insect damage, were selected. Two kernels were removed from each ear; one from an insect-damaged zone and one from a damage-free region. From earworm- and corn borer-infested ears, an intact kernel immediately adjacent to overtly damaged kernels was selected; from stinkbug- and mite-damaged ears, kernels with characteristic puncturing or red streaking were used. Sap and picnic beetle damage was not assessed microbiologically since these insects cause no obvious damage to kernels. Kernels from the butt and tip of one apparently insect-free ear from each collection site served as controls.

TABLE I
Incidence of Insect Damage on Corn Ears[a] Collected in General Survey and Intensive Study of Missouri and Illinois Corn

Collection Area	Incidence (%)[b]					
	Corn Earworm	Corn Borer[c]	Stinkbug	Mite	Beetles	No Insect Damage
General						
Stoddard	19.7	12.9	2.4	23.5	1.5	47.5
Mississippi	19.4	34.1	5.3	29.9	3.0	33.2
New Madrid	26.6	25.2	5.2	37.4	4.9	26.6
Scott	18.2	10.1	3.8	3.2	1.0	65.4
Intensive						
Missouri	25.6	9.6	2.0	0.0	0.3	61.8
Illinois	5.0	17.4	7.2	0.0	2.0	70.7

[a] Yellow varieties in Stoddard, Mississippi, and New Madrid counties in Missouri; white varieties in Scott County, Mo., and entire intensive study.
[b] Percentages may total more than 100% as a result of several types of damage to one ear.
[c] Both Southwestern and European corn borer in Missouri; only European corn borer in White and Crawford counties, Ill.

Kernels were surface-sterilized by washing for 1 min in a 1% sodium hypochlorite (NaOCl) solution and rinsed twice in sterile distilled water. Kernels were plated, five per plate, on Czapek's solution agar adjusted to pH 5.5, incubated at 28°C for 5 days, and examined for fungal growth under a stereoscopic microscope.

RESULTS AND DISCUSSION

The incidence of insect damage observed in sample ears collected in these surveys is presented in Table I. Corn earworm damage, occurring primarily on

TABLE III
Distribution of Fungi on Corn Damaged by Specific Insects

Kernels		% Kernels							
Type of damage	No. examined[a]	Fungus-free	A. flavus group	A. glaucus group	Fusarium	Penicillium	Rhizopus	Syncephalastrum	All other fungi[b]
Earworm	304	60.2	7.2	7.2	6.5	3.3	6.2	0.7	12.4
Borer[c]	312	58.6	5.1	8.3	10.2	2.2	6.7	1.9	11.8
Stinkbug	126	74.6	6.3	4.8	5.5	2.4	3.2	3.2	6.3
Mites	98	63.2	7.1	9.2	3.1	3.1	4.1	3.1	14.3
Controls	320	77.5	2.5	7.4	3.1	3.1	0	3.7	4.0

[a]From each insect-damaged ear—one kernel from the damaged area and one from an undamaged area; from each insect-free control ear—one kernel from the tip and one from the butt.

[b]Includes: *Aspergillus* spp. other than *flavus* and *glaucus* groups. *Alternaria, Cladosporium, Helminthosporium, Trichoderma, Mucor, Nigrospora, Chaetomium, Paecilomyces, Olpitrichum,* and *Sepedonium.*

[c]Both Southwestern and European corn borer in Missouri; only European corn borer in Illinois.

TABLE IV
Fungi Associated with Insects Collected from the General Survey of Missouri Corn

Insect		No. of Insects Infected with				
Type	No. examined	A. flavus	Fusarium spp.	Rhizopus spp.	Penicillium spp.	Mucor spp.
Earworm	27	10	17	2	4	5
Borer (Southwestern and European)	96	14	40	10	5	13
Beetles (*Nitidulidae*)	15	1	9	3	0	3
Unidentified	57	4	29	11	0	12
Total	195	29	95	26	9	33
Percentage based on 195 insects		15	49	13	5	17

TABLE II
Fungi Associated with Corn Kernels from Ears Collected in Missouri and Illinois

Collection Area	Variety	No. Kernels Examined[a]				Per Cent Kernels Infected											
						Total				Aspergillus flavus				Aspergillus glaucus			
		ID Ears		IF Ears		ID Ears		IF Ears		ID Ears		IF Ears		ID Ears		IF Ears	
		ID	UD	T	B	ID	UD	T	B	ID	UD	T	B	ID	UD	T	B
General																	
Stoddard	Yellow	78	78	30	30	35	22	17	17	14.1	11.5	3.3	6.6	2.6	1.3	3.3	0
Mississippi	Yellow	81	81	27	27	53	48	26	31	8.6	8.6	0	7.4	7.4	3.7	3.7	7.4
New Madrid	Yellow	88	88	29	29	64	58	45	52	7.9	7.9	3.5	0	27.0	25.0	25.0	28.0
Scott	White	73	73	30	30	34	23	10	7	1.4	1.4	0	0	1.4	0	0	3.3
Intensive																	
Missouri	White	39	39	20	20	28	10	20	10	2.6	5.1	5.0	5.0	2.6	0	5.0	0
Illinois	White	61	61	24	24	18	13	13	7	0	0	0	0	3.3	1.6	8.3	4.2
Totals																	
General		320	320	116	116	47	39	24	27	8.1	7.5	1.7	3.4	10.3	8.1	7.8	9.5
Intensive		100	100	44	44	22	12	16	9	1.0	2.0	2.3	2.3	3.0	1.0	6.8	2.3
Survey totals		420	420	160	160	41	32	22	22	6.4	6.2	1.9	3.1	8.6	6.4	7.5	7.5

[a]Two kernels from each test ear: from each insect-damaged (ID) ear, one kernel from the damaged area and one from an undamaged (UD) area; from each insect-free (IF) control ear, one kernel from the tip (T) and one from the butt (B).
[b]Includes: *Aspergillus* spp. other than *flavus* and *glaucus* groups, *Alternaria, Cladosporium, Helminthosporium, Trichoderma, Mucor, Nigrospora, Chaetomium, Paecilomyces, Olpitrichum,* and *Sepedonium.*

Table II, *continued on next page.*

Table II, *continued*
Fungi Associated with Corn Kernels from Ears Collected in Missouri and Illinois

Collection Area	Variety	Per Cent Kernels Infected																			
		Fusarium				Penicillium				Rhizopus				Syncephalastrum				All Other Fungi[b]			
		ID Ears		IF Ears		ID Ears		IF Ears		ID Ears		IF Ears		ID Ears		IF Ears		ID Ears		IF Ears	
		ID	UD	T	B	ID	UD	T	B	ID	UD	T	B	ID	UD	T	B	ID	UD	T	B
General																					
Stoddard	Yellow	3.9	0	3.3	0	2.6	1.3	10.0	0	3.9	1.3	0	0	1.3	0	0	0	7.7	7.7	0	10
Mississippi	Yellow	22.2	8.6	3.7	7.4	2.4	1.2	0	0	8.6	7.4	0	0	6.2	6.2	14.8	11.1	12.3	18.0	3.7	0
New Madrid	Yellow	17.0	9.1	10.4	10.4	4.5	3.8	6.9	3.5	7.9	9.1	0	0	1.2	1.2	6.9	10.4	18.0	14.0	0	3.5
Scott	White	10.5	2.7	0	0	4.0	1.4	3.3	3.3	8.2	6.8	0	0	1.4	1.4	0	0	10.9	12.2	6.6	0
Intensive																					
Missouri	White	0	0	5.0	5.0	10.2	0	5.0	5.0	5.1	0	0	0	0	0	0	0	7.7	5.1	10.0	0
Illinois	White	3.3	0	0	0	3.3	0	0	0	4.9	0	0	0	0	0	0	0	4.9	11.5	8.3	8.3
Totals																					
General		13.4	5.3	4.3	4.3	3.4	1.9	5.2	1.7	7.2	6.2	0	0	2.5	2.2	5.2	5.2	12.5	13.0	2.6	3.4
Intensive		2.0	0	2.3	2.3	6.0	0	2.3	2.3	5.0	0	0	0	0	0	0	0	6.0	9.0	9.1	4.5
Survey totals		10.7	4.0	3.7	3.7	4.0	1.4	4.4	1.9	6.7	4.8	0	0	1.9	1.7	1.4	1.4	10.9	12.1	4.4	3.7

ear tips, was uniformly observed in general survey corn with an average incidence of 20.9%. In the intensive study, damage by this insect was detected five times as frequently on Missouri samples as on those from Illinois. Corn borer damage observed on the butt, tip, and central portion of test ears in the general survey showed an average occurrence of 20.6%. In the intensive study, evidence of corn borer activity was found about twice as frequently on test ears from Illinois as on those from Missouri. Only a limited number of ears showed stinkbug damage. Mite activity, identified by red streaking of kernels, was observed on 30.3% of the yellow corn but on only 3.2% of the white corn in the general survey and was completely absent in white corn from the intensive survey. Sap or picnic beetles or their larvae were observed only on ears damaged by borer or earworm; highest observed incidence (4.9%) occurred in New Madrid County. The number of insect-free ears varied considerably between the areas examined but white corn in general and Illinois white corn in particular showed the least insect activity.

Microbiological data from a limited sampling of damaged and undamaged kernels from insect-damaged and insect-free ears are summarized by county in Table II.

In the general survey, maximum fungal infection on both insect-free and insect-damaged ears was observed on yellow corn from New Madrid County. White corn from Scott County, Mo., in both the general and intensive studies, and from Illinois exhibited an overall low incidence of fungi. Maximum increase in incidence of fungi, as the apparent result of insect damage, occurred in corn from Mississippi and Scott Counties.

The distribution of fungi in general survey corn differed significantly (5% level) between the four counties, principally as the result of high proportions of (a) *Aspergillus flavus* in Stoddard, (b) *A. glaucus* group in New Madrid, (c) *Fusarium* and *Rhizopus* in New Madrid and Mississippi, and (d) *Syncephalastrum* in Mississippi.

Kernels from insect-damaged ears showed a distinctly higher occurrence (37%) of fungi than those from insect-free ears (22%); the greatest variation occurred in the incidence of *A. flavus, Fusarium,* and *Rhizopus*. There was little difference between fungal infection of kernels from butt or tip regions of insect-free ears.

Area differences in *A. flavus* occurrence were significant, ranging from 10.6% (23/116) in Stoddard County to zero in Illinois. The combined *A. flavus* incidence on kernels from all test ears from both the general and the intensive study was 5.2% (61/1160); 6.3% on kernels from insect-damaged ears, and 2.5% on those from insect-free ears. The difference between 6.3 and 2.5% is significant.

Essentially no variation in *A. flavus* incidence was observed between kernels from overtly damaged areas and those from undamaged areas of insect-damaged ears.

Data from both the general and intensive studies on the distribution of fungi on kernels of corn damaged by specific insects are compiled in Table III. Although 5.1% of the kernels from ears associated with corn borers yielded *A. flavus*, whereas 7.2% of those where corn earworm had been present yielded this fungus, on the basis of statistical chi-square tests, distribution of fungal genera did not vary significantly in relation to specific types of insect damage.

However, *A. flavus* was observed on 15% of a limited number of insects collected from damaged ears (Table IV). Although no insect species was specifically linked to a particular fungus, *A. flavus* was associated with a

significantly higher proportion of earworms (37%) than of corn borers (14%).

Observations indicate that insect damage results in increased fungal infection. Areas of highest insect incidence also showed the highest rate of fungal invasion. Overall fungal incidence was significantly higher in kernels from insect-damaged ears than from insect-free ears, with *A. flavus, Fusarium,* and *Rhizopus* being most clearly damage-associated.

The possibility that factors other than insect damage are operative is suggested by the similar incidences of *A. flavus, A. glaucus* group, and *Syncephalastrum* in kernels from both damaged and undamaged regions of insect-damaged ears and an overall 22% infection rate in intact kernels from ears diagnosed as insect-free.

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