Effect of Insect Damage to Maize Ears, with Special Reference to *Mussidia nigrivenella* (Lepidoptera: Pyralidae), on *Aspergillus flavus* (Deuteromycetes: Monoliales) Infection and Aflatoxin Production in Maize Before Harvest in the Republic of Benin

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J. Econ. Entomol. 91(2): 433–438 (1998)

ABSTRACT Maize infection by *Aspergillus flavus* Link and subsequent aflatoxin contamination as affected by insect damage to maize ears before harvest was studied with surveys in farmers' fields and in a field trial in the Republic of Benin, West Africa. The most important pest species was the lepidopteran earborer *Mussidia nigrivenella* Ragonot. Percentage of grain infected by *A. flavus* and of samples contaminated with aflatoxin, as well as the mean aflatoxin content of samples, increased with increasing borer damage. Ears with <2% insect damage had an average of 11.7 and 43.6 ppb of aflatoxin in 1994 and 1995, respectively. Ears in the highest damage class (i.e., > 10% damage) had an average aflatoxin of 514.6 and 388.2 ppb in 1994 and 1995, respectively. In 1994 only, coleopteran species such as *Sitophilus zeamais* Motschulsky and *Carpophilus* sp. significantly increased levels of aflatoxin in grain samples. In a field trial using *M. nigrivenella* infestation and *A. flavus* inoculation treatments, the presence of the insect feeding resulted in increased kernel infection and aflatoxin contamination. Artificial infestation with *M. nigrivenella* larvae increased aflatoxin content of maize by an average of 45 ppb, whereas inoculation with *A. flavus* spores increased the toxin level by 517 ppb. The significant interaction between infestation and inoculation indicated that higher levels of aflatoxin B1 were found when the fungus was associated with borers than with the fungus alone. *M. nigrivenlla* was the major field pest connected with *A. flavus* infection and subsequent aflatoxin production in preharvest maize in Benin.

KEY WORDS *Mussidia nigrivenella, Aspergillus flavus,* insect damage, maize ears, aflatoxin contamination, maize before harvest

VARIOUS FUNGI CAN infect maize, *Zea mays* L., grain before the grain is harvested (Anderson et al. 1975, Fennel et al. 1975, Marasas et al. 1979). The most common species is aflatoxin-producing *Aspergillus flavus* Link. Infection of maize by *A. flavus* and contamination by aflatoxin before harvest are well documented (Hesseltine et al. 1976, Lillehoj et al. 1977). Aflatoxins, toxic metabolites of the fungi *A. flavus* and *A. parasiticus*, are potent carcinogens which pose serious health hazards to humans and domesticated animals (Diener et al. 1987). Aflatoxin concentration allowed in foods and feeds is regulated in many developed countries and in some products, including maize, aflatoxin levels must be kept below 20 ppb (Stoloff et al. 1991).

Although fungal infection may also occur on undamaged kernels, physically damaged grains accumulate more aflatoxin than nondamaged grains (Diener et al. 1987). Insect damage to husks and grain enhance infection and level of aflatoxin contamination in preharvest maize (Fennel et al. 1975). Widstrom (1979) postulated that the involvement of insects in the fungal infection process and the subsequent production of aflatoxins is an important contributor to aflatoxin contamination of agricultural products. Although many studies have been carried out on the interaction between insects and the aflatoxin-producing fungus, *A. flavus*, no attempt has been made in tropical Africa to relate damage caused by maize pests to *A. flavus* infection and aflatoxin contamination.

In the Republic of Benin, West Africa, the most important ear pest of maize in the field is *Mussidia nigrivenella* Ragonot (Schulthess and Sétamou 1997, Sétamou et al. 1995). *M. nigrivenella* attacks maize in the field and continues to damage the grain the first 2 mo of storage. *A. flavus* infection can result in high aflatoxin contamination of maize before harvest in Benin (unpublished data). The objective of the current work was to elucidate the role of insect damage in the contamination of maize before harvest by *A. flavus* and aflatoxin.

Materials and Methods

Two country-wide surveys were conducted in 1994 and 1995, in the 4 different agroecological zones in the Republic of Benin: the coastal savanna with 2 growing seasons (April–July and September–November);

0022-0493/98/0433–0438$02.00/0 © 1998 Entomological Society of America

southern Guinea savanna with the same seasonal pattern as the coastal savanna but lower precipitation; northern Guinea savanna with 1 growing season (April to September); and the Sudan savanna with also 1 growing season from late May to September but dryer than the Sudan savanna. In coastal savanna and southern Guinea savanna, fields were monitored during the major season in July, and in northern Guinea savanna and Sudan savanna in October–November. In each zone, 20 fields in 1994 and 15 in 1995 were sampled, and within each field 20 ears were chosen. The fields were divided into 4 quadrants and within each quadrant 5 cobs were chosen randomly.

The on-station trial was conducted to study the interaction between *M. nigrivenella* and *A. flavus*. The trial was planted in 1994 during the 2 wk of May, at the Benin Station of the International Institute of Tropical Agriculture (IITA), Calavi, situated in the coastal savanna zone. The 90-d maize variety 'TZESR', an improved IITA streak resistant variety, was used. A factorial experiment was designed to study the main effects of both artificial infestation by *M. nigrivenella* and artificial inoculation with spores *of A. flavus*, and their interaction on aflatoxin levels. Treatments were whole plots, which consisted of 10 rows (8 m each). Treatments were arranged in a randomized block design with 3 replications. The planting distance was 80 cm between rows and 40 cm within rows. Treatments consisted of control (T0) plots treated with an insecticide to prevent ear damage; artificial infestation with *M. nigrivenella* (T1); artificial inoculation with *A. flavus* spores (T2); and both artificial infestation and inoculation (T3 = T1 × T2). In T0, cypermetrin (Cymbush 3 E [emulsifiable]) was sprayed at the rate of 1.5 kg [AI]/ha, fortnightly between 5 and 9 w after planting. The insecticide was sprayed using an Electrodyn (Imperial Chemical Industries PLC, England) sprayer held such that the Bozzle was down wind and slightly behind the user. The Bozzle was positioned between the 2 crop rows next to the user. Whilst spraying, the user walked at a normal speed (1 m/s). Two rows were sprayed at a time, keeping the nozzle 40 cm above the top of the crop. Both infestations and inoculations were conducted the same day at 9 w after planting. The T1 and T3 plots were artificially infested by placing 5 first-instar *M. nigrivenella* onto the silks of the ears using a camel's-hair brush. In the T2 and T3 plots, ears were inoculated through the silk channel using a syringe. The tip opening of the needle was inserted into the silk channel to inject 1 ml of an *A. flavus* suspension (1×10^6 spores ml^{-1}). In the T3 treatment, the infestation with *M. nigrivenella* was done immediately after the inoculation. At harvest, 20 ears were sampled randomly from the 8 central rows, avoiding the 2 plants at each end of the row. For both the surveys and field trial, the cobs were dissected immediately after harvest. The percentage of grains damaged and the number of insects feeding on ears were recorded per lepidopteran species, and for coleopteran species only the number of insects found was considered because their damage level at this period was <1% of the total kernels. The lepidopteran species infeodated to ears such as *M. nigrivenella* and *Cryptophlebia leucotreta* Meyrick start their damage from the tip of the ear. *C. leucotreta* larvae penetrate the kernels and feed inside without noticeable frass; whereas, *M. nigrivenella* larvae produce a lot of silky frass on the kernels which is easily detected because they bore into the kernels. The stem borers *Sesamia calamistis* Hampson and *Eldana saccharina* Walker also feed on kernels but their type of damage differs significantly from that of the ear borers. *S. calamistis* eggs are laid on the inner surface of the husks and newly emerged larvae penetrate the ears and start feeding on the surface of the kernels. These larvae are often hidden by the frass they produce. *E. saccharina* larvae collected on ears generally emigrate from the stem to the ear. Hence, damage caused by this borer goes from the rachis to the kernels with abundant wet frass rejected on the surface of the kernels.

The ears were then put in paper bags and brought to the laboratory for further analysis.

The ears were oven dried for 3 d at 45°C and then shelled. Five kernels per ear were selected randomly, surface disinfected for 1 min in 3.5% NaOCl, rinsed twice in sterile distilled water, and plated on sterile filter paper (10 cm in diameter) (Whatman No. 1). Plates were incubated at 25° C for 5–7 d. All cultures that developed from the kernels were identified directly on the plates. Percentage of kernel infection per fungal species was based on the total number of kernels plated per field or treatment. The remaining kernels from each field or treatment were mixed and ground in a Romer Mill (Romer Laboratories, Union, MO) and assayed for aflatoxin by the method of Thomas et al. (1975).

Total aflatoxins (B1, B2) as x = ppb of sample and number of insects were transformed to log $(x + 1)$, and all percentages were arcsine transformed before analyses. Analyses were performed with SPSS (Norusis and SPSS 1993). A correlation analysis was done to establish relationships between survey variables. For each year, data were analyzed separately. Analysis of variance (Zar 1974) was conducted to study the effect of treatments in the on-station experiment.

Results

Four lepidopteran species, *M. nigrivenella*, *Cryptophlebia leucotreta* Meyrick, *Sesamia calamistis* Hampson and *Eldana saccharina* Walker, and 3 coleopteran species, *Sitophilus zeamais* Motschulsky, *Carpophilus* spp. F. and *Cathartus quadricollis* Guerin were found on the maize just before harvest. For both years, percentage of fields and of ears within field infested by the various pests is presented in Table 1.

Sesamia calamistis Hampson was commonly encountered in maize fields but on a few ears only thus grain damage by *S. calamistis* was low. For *C. leucotreta* Meyrick, both percentage of fields and plants infested were relatively high but because the larvae are small, damage by this insect was minor. The sugar cane borer, *E. saccharina* Walker, was encountered in ≈8% of the fields and average grain damage was 0.3% during

Table 1. Insect infestation, mean *A. flavus* infection, and mean aflatoxin levels (ppb) in preharvest maize in 1994 and 1995 in Benin across 4 agroecological zones

Variable	Lepidoptera				Coleoptera			*A. flavus*	
	M. nigrivenella	*S. calamistis*	*E. saccharina*	*C. leucotreta*	*S. zeamais*	*Carpophilus* spp.	*C. quadricollis*	Infection	Aflatoxin
				1994					
% fields infested[a]	97.5	61.3	7.5	82.5	27.5	40.0	13.8	85.0	41.3
% ears infested[b]	45.0	5.8	0.6	18.0	10.0	9.2	2.0	74.0	—
Mean[c]	4.7	1.1	0.2	0.8	0.8	0.2	0.0	20.6	59.4
				1995					
% fields infested	70.0	35.0	7.0	93.3	35.0	37.0	21.7	60.0	31.7
% ears infested	42.0	3.8	0.5	34.0	7.6	9.5	5.8	56.0	—
Mean	3.6	0.4	<0.1	0.5	0.2	0.3	<0.1	19.5	46.2

[a] Percentage of fields infested by each insect species, infected by *A. flavus* and contaminated by aflatoxin.
[b] Percentage of ears infested by each insect species and infected by *A. flavus*.
[c] Mean percentage of kernels damaged by insect, percentage of kernel infected by *A. flavus* and aflatoxin detected in samples.

both years. The predominant lepidopteran species was *M. nigrivenella*, which was found in most fields and infested 45 and 42% of the cobs in 1994 and 1995, respectively. Across both years, the number of *M. nigrivenella* accounted for 80% of the total Lepidoptera found. Mean grain damage caused by this cob borer was 4.7% in 1994 and 3.6% in 1995, accounting for 67–75% of the total insect damage in the 2 yr.

Coleopteran species were also routinely found in maize fields, with *Carpophilus* spp. the most frequently encountered and *C. quadricollis* the least common species. For all species, ear infestation was low and average grain damage by coleopteran pests invading maize before harvest was between 0 and 0.8%.

Aspergillus flavus was found in a high percentage of the fields with an average kernel infection of ≈20% in each year. Of the fields infected by *A. flavus*, 48.6 and 52.8% were contaminated by aflatoxins in 1994 and 1995, respectively. Only aflatoxins B1 and B2 were detected in the samples and of these, B1 was at the highest concentration. The mean concentrations of positive samples were 141.5 ppb in 1994 and 145.9 ppb in 1995.

Correlations between insect pest infestation variables with the percentage of kernels infected by *A. flavus* are shown in Table 2. For both years, *M. nigrivenella* damage was significantly correlated with *A. flavus* infection and aflatoxin contamination. Likewise, percentage ears infested per field by the borer and the mean number of larvae recovered per infested ear were significantly correlated with grain infection level and aflatoxin concentration of maize. *S. calamistis* was correlated with *A. flavus* infection but was not significantly associated with aflatoxin production,

Table 2. Correlations between *A. flavus* infection, aflatoxin contamination, and various insect damage levels in preharvest maize in Benin

Variable	1994			1995		
	A. flavus[a]	B1[b]	B2	*A. flavus*[a]	B1[b]	B2
M. nigrivenella						
% ears infested	0.30**	0.43**	0.27*	0.28*	0.27*	0.14
% grains damaged	0.35**	0.36**	0.12	0.42**	0.54**	0.25*
No. borer/ear	0.24*	0.36*	0.15	0.15	0.30*	0.13
S. calamistis						
% ears infested	0.02	0.09	0.11	0.26*	0.14	0.09
% grains damaged	0.12	0.14	0.19	0.26*	0.19	0.10
No. borer/ear	0.27*	0.20	0.12	0.25*	0.14	0.07
E. saccharina						
% ears infested	−0.02	−0.05	0.03	—	—	—
% grains damaged	0.22*	0.06	0.11	—	—	—
No. borer/ear	−0.04	−0.11	−0.04	—	—	—
C. leucotreta						
% ears infested	0.03	−0.04	0.00	0.06	0.09	0.01
% grains damaged	−0.07	−0.07	−0.06	0.11	0.04	0.12
No. borer/ear	−0.28*	−0.16	−0.28*	−0.14	−0.26*	−0.19
S. zeamais						
% ears infested	0.05	0.17	0.22*	0.04	0.18	0.23
Carpophilus sp.						
% ears infested	0.19	0.22*	−0.08	0.11	0.10	0.07
Coleopteran spp.						
% grains damaged	0.17	0.27*	0.32*	0.14	0.16	0.37*

—, Correlation not calculated because pest densities were too low. *r* values marked with * and ** have *P* values <0.05 and <0.01, respectively.
[a] Percentage of grains damaged by all insect species and % kernels infected by *A. flavus* were arcsine transformed before analysis.
[b] Aflatoxin (B1 and B2) were $\log(x + 1)$ transformed before analysis.

Table 3. *A. flavus* infection and aflatoxin B1 production as a function of percentage ear damage because of insects on maize before harvest in the Republic of Benin, 1994 and 1995

Ear damage class	No. of fields	Mean %		Aflatoxin, ppb[a]	
		Grain damage	*A. flavus* infection	mean	% fields
		1994			
0–2	22	1.1	17.8	43.6	27.3
2–4	19	3.0	20.7	54.3	47.4
4–10	30	5.9	22.8	74.9	43.3
>10	9	12.6	50.9	514.6	66.7
		1995			
0–2	31	0.4	8.2	11.7	17.0
2–4	13	2.7	19.3	54.4	46.0
4–10	12	7.0	15.8	48.3	50.0
>10	4	14.3	29.8	388.2	50.0

[a] Average ppb of aflatoxin in contaminated samples in ppb.

Table 4. Means of *M. nigrivenella* damage, *A. flavus* infection (%) and aflatoxin concentrations (ppb) of treatments in the 1994 on-station experiment followed by *F* values of the factorial effect

Treatment	*M. nigrivenella* damage	*A. flavus*, %	Aflatoxin B1, ppb
T0 (control)	2.1	28.0	5.0
T1 (insects)	8.5	37.3	52.8
T2 (*A. flavus*)	4.2	76.5	525.0
T3 (insects + *A. flavus*)	8.7	88.3	566.7
Factorial effect			
F values			
Insect infestation	25.5**	2.8NS	11.6**
A. flavus inoculation	1.7NS	78.4**	91.5**
Interaction	1.2NS	0.03NS	10.3*

T0, control; T1, infested with *M. nigrivenella*; T2, inoculated with *A. flavus*; T3, infested with *M. nigrivenella* and inoculated with *A. flavus*; *, Significant at $P < 0.05$; **, significant at $P < 0.01$; NS, nonsignificant.

whereas ear damage caused by *E. saccharina* was significantly correlated with grain infection only in 1994. Mean number of *C. leucotreta* was negatively correlated with *A. flavus* infection and aflatoxin levels in maize.

The number of coleopteran species generally was not significantly related to grain infection ($P > 0.05$) but their damage level was significantly and positively correlated ($P < 0.05$) with the level of aflatoxin B2 content of samples during both surveys, and with aflatoxin B1 levels only in 1994 (Table 2). *C. quadricollis* had no impact on *A. flavus* infection nor aflatoxin contamination ($r = 0.09$, $n = 80$ in 1994 and $r = 0.11$, $n = 60$ in 1995).

The classification of fields according to *M. nigrivenella* damage levels and the corresponding toxin levels recovered from grains are shown in Table 3. During both years, *A. flavus* infection and aflatoxin accumulation tended to increase with insect damage to ears. Few infected kernels and little aflatoxin were recovered from ears that were lightly damaged by insects. Contaminated ears that had between 2 and 4% grain damage had an average aflatoxin B1 content of 54 ppb; >50 % of the samples in this damage class contained no aflatoxin, however. In 1994 and 1995, plants with average borer damage >10% had average aflatoxin B1 levels of 514.6 and 388.2 ppb, respectively.

In the on-station experiment (Table 4), plots artificially infested with *M. nigrivenella* had a significantly higher percentage of grain damage than control plots, but not with infection of *A. flavus*. Likewise, artificial inoculation with fungal spores significantly increased percentage of grain infected at harvest, and both infestation and inoculation significantly increased aflatoxin B1 concentrations by an average value of 45 and 517 ppb, respectively. The interaction infestation × inoculation was significant for the level of aflatoxin B1, thus more aflatoxin B1 is accumulated in maize in the presence of *M. nigrivenella*. In the mixed treatment (T3), inoculation had no effect on ear damage, indicating that *A. flavus* has no effect on survival of *M. nigrivenella* larvae. The effect of infestation on *A. flavus* infection was not significant but, on a per-ear basis, a positive relationship was found between percentage of grain damaged by *M. nigrivenella* and percentage of grains infected by *A. flavus* ($r = 0.40^*$, $n = 120$) in the T0 and T1 treatments.

Discussion

These results show that insects help *A. flavus* invade maize before harvest and are correlated with the subsequent contamination of grain by B aflatoxins. However, the importance of insects in aflatoxin contamination may vary according to their mode of action on the ears. For example, Widstrom et al. (1975) showed that the European corn borer, *Ostrinia nubilalis* (Hübner), was more effective than the fall armyworm, *Spodoptera frugiperda* J. E. Smith, in producing conditions that are conducive to high levels of aflatoxin production in grain. In the current study, increase in *A. flavus* infection and aflatoxin levels of samples was mainly due to *M. nigrivenella*. As early as 1914, this borer was mentioned as one of the chief pests attacking grain maize in Southern Nigeria (Lamborn 1914). The borer usually damages maize from the tip of the kernels (Moyal 1988), where natural infection of *A. flavus* occurs, and thus could easily promote the spread of the fungus. It frequently eats a channel through a whole line of seeds (Moyal and Tran 1991), and thus breaks the testa of grains which constitutes the natural barrier to *A. flavus* growth. Also, because of their numbers, *M. nigrivenella* larvae tend to be highly destructive, and thus favor establishment and spread of *A. flavus* in grain.

Other ear-feeding lepidopteran species were less strongly associated with *A. flavus* infection or aflatoxin levels. This may have been because of the generally low pest infestations encountered in the surveys. In the rainforest and forest/savanna transition as well as in the midaltitudes of most other western African countries, high numbers of the pyralid *E. saccharina* and the noctuid *Busseola fusca* Fuller, and to a lesser extent *S. calamistis*, are commonly found feeding in the ear (Bosque-Pérez and Mareck 1991, Gounou et al. 1994, Cardwell et al. 1997) often destroying it in the

milk stage of ear development. *E. saccharina* often feeds on the debris on the soil before penetrating into the stem and later into the ear. Thus, it is likely *E. saccharina* can be an important vector of the fungus when there is a high *E. saccharina* pest density. *C. leucotreta* generally feeds only on the silk and on the tip grains, which may explain why it was not associated with fungal infection.

The positive correlation of both *S. zeamais* and *Carpophilus sp.* damage and *A. flavus* infection and aflatoxin levels, despite low associated feeding damage, suggests that these insect pests may also act as vectors of the fungus. Beti et al. (1995) reported that the maize weevil enhances growth of *A. flavus* by increasing the area susceptible to fungal growth. *S. zeamais* was reported to contribute significantly to increased *A. flavus* infection on corn ears by transporting spores and damaging corn kernels (McMillan et al. 1980b). Infestation by both beetles is associated with *M. nigrivenella* damage on maize ears (Schulthess and Sétamou 1997). Before pupating, this borer produces an exit hole through which storage beetles enter the ear. McMillan (1987) also observed that nitidulid beetles such as *Carpophilus* spp. are attracted to damaged ears, including those caused by ear worms and the European corn borer. They are also found feeding preferentially on moldy ears (Wicklow 1989). Hence, these beetles may infest previously damaged or *A. flavus*-infected ears on which they will disperse fungal spores. In our field trial, artificial infestation with *M. nigrivenella* larvae had no effect on fungal infection. This was likely because of generally low ear damage as well as of relatively high natural infestation by *M. nigrivenella* (i.e., ≈50% of total borer population). The survey suggested that the relationship between damage and fungal infection is nonlinear, and ear damage must be considerable (i.e., >10%) to have an effect on infection rates. Field observations show that high damage is mostly associated with older large larvae, which produce exit holes (F. S., unpublished data). Also, the inoculum introduced was sufficient to ensure establishment of the fungus on >75% of the grain. Thus, any further effect of the borer may not have contributed significantly to grain infection. Percentage of *A. flavus* infection between insecticide-protected and artificially infested plots was not significantly different, indicating that the borer was not the source of grain infection but only dispersed spores already present on the grains.

The fungus did not affect the damage level by *M. nigrivenella*, indicating that the fungus has no biocidal action. In a laboratory study, Sétamou (1996) showed that diet containing spores of *A. flavus* did not adversely affect growth and development of *M. nigrivenella*. The effect of the fungus and its metabolites on insect biology varies depending on the insect species (McMillan et al. 1980a). Although some insects are sensitive to the presence of *A. flavus* in their diets, others are resistant. Dowd (1992) reported that resistant insects feeding on *A. flavus*-contaminated substrates without an adverse effect can vector the fungus. This shows the importance of the relatively unknown earborer *M. nigrivenella* in the invasion of ears by *A. flavus*. The negative relationship between *C. leucotreta* variables and *A. flavus* and the toxin levels suggests that the borer may be susceptible to *A. flavus*.

The current study suggests that one of the most important factors determining the levels of aflatoxin found in preharvest maize in Benin is the extent of damage caused by *M. nigrivenella*. The rapid increase of aflatoxin B1 noticed at grain damage level >10% suggests an exponential effect of borer damage on aflatoxin accumulation. Wicklow (1991) and Widstrom (1979) reported that maize is particularly vulnerable to *A. flavus* infection when pressure from ear-infesting insects is high. Likewise, McMillan et al. (1987) showed that maize ears inoculated with *A. flavus* only sustained significantly lower aflatoxin amounts than ears from plots inoculated with *A. flavus* and supplemented with insects. One method to reduce aflatoxin contamination could be the prevention of insect damage. Because of the cryptic behavior of *M. nigrivenella*, insecticides are not recommended (Moyal 1988, Sétamou et al. 1995). Thus, discarding heavily damaged ears (>10% of the grains) at harvest should reduce the aflatoxin in samples.

Acknowledgments

We thank Gbelissanon Pascal, Koube Maximilien, and Hotegni Josephine for technical assistance, and C. Lomer and W. Meikle for review. This research was supported by the Deutsche Gesellschaft für Technische Zusammenarbeit, Federal Republic of Germany.

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Received for publication 24 March 1997; accepted 24 October 1997.