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Aspergillus Species Colonization of Termite-Damaged Peanuts in Parts of West Africa and Its Control Prospects

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ABSTRACT

A survey of farmers' peanut fields in Benin showed that *Aspergillus flavus* Link ex Fries infection was influenced by *Microtermes* (Isoptera: Termitidae) damage. Field trials conducted at the ICRISAT station and a farmer's plot in Mali on termite damage and *Aspergillus* spp. infection showed that peanut plots treated with carbofuran at planting and supplemented with chlorpyrifos at 40 days after planting (dap), or treated with only chlorpyrifos at 40 dap, significantly reduced pod damage by the termite *M. lepidus* Sjöstedt. and colonization by *A. flavus*. The cultivar ICG 10946 which had significantly more *A. flavus* ($P < 0.05$) also had a higher percentage of damaged pods compared with the other cultivars at the on-station trials. The least infected cultivar in all the trials was 47-16 which also had a comparatively lower percentage of termite-damaged pods, although this was not significant. However, termite damage was correlated with *A. flavus* percentage colonization.

Key Words: Groundnut, *Microtermes* spp., pod damage, pod scarification.

Peanut (*Arachis hypogaea* L.), a leguminous plant rich in protein and oil, is grown predominantly in the semi-arid tropics of many developing countries (FAO, 1996). Production in West Africa showed a continuous decline from 1966 to 1985, followed by a period of expansion between 1985 and 1995 due to the cultivation of more available land (Debrah and Waliyar, 1997). In the West African subregion, peanut is exported to earn foreign exchange. It is also sold locally for the manufacture of cooking oil and various domestic uses and thus generates income for farmers.

One of the important factors that contributed to the decline of foreign exchange earnings in peanut exportation was the problem of pod contamination by aflatoxins. These are highly carcinogenic mycotoxins produced by the *Aspergillus* group fungi (Diener *et al.*, 1982). These fungi produce aflatoxin B_1, the most potent naturally occurring carcinogenic substance known, causing liver damage to animals, including humans (CAST, 1979). The consistent association of insect damage with sporulation of *A. flavus* Link ex Fries suggests that an important role is played by insects in aflatoxin contamination (Widstrom, 1979). The insects carry *Aspergillus* spores which come in contact with the attacked pods. Furthermore, the presence of spores and mycelium of the fungus on the surface of kernels is likely to facilitate invasion of kernels by fungi that are injured by insects. In some West African countries, termites and other soil pests are major causes of peanut pod damage and associated with *Aspergillus* infection and aflatoxin contamination (McDonald and Hackness, 1963, 1964; McDonald *et al.*, 1964; McDonald, 1969; Johnson and Gumel, 1981; Pollet and Decleret, 1987; Umeh, 1997). Termite damage is observed either as scarification or direct penetration of pods (Johnson *et al.*, 1981; Wightman and Wightman, 1994, Umeh, 1997), both of which favor fungal infection. *Aspergillus* infection and aflatoxin contamination of pods are favored by drought and high soil temperatures that are characteristic of the later part of the cropping season in many peanut production regions (Hill *et al.*, 1983; Blankenship *et al.*, 1984; Cole *et al.*, 1985; Diener *et al.*,

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1987). These conditions also are known to increase termite attack in the semi-arid tropics.

Aflatoxin contamination of peanuts has been recognized as a serious problem for more than two decades in West Africa. Despite numerous studies, no strategies exist for minimising their contamination, through for example, reducing the soil-borne insect pests that contribute to the problem. There are few control options available to resource-poor farmers whose awareness of the dangers of aflatoxin contamination of peanut generally is limited. The present study therefore had the objectives of (a) assessing the level of *Aspergillus* colonization in relation to peanut damage by termites in farmers' fields, and (b) reducing *Aspergillus* colonization by insecticide applications and use of peanut cultivars less susceptible to termite attacks.

Materials and Methods

Survey of Farmers' Peanut Fields in Benin. Twenty-three peanut fields belonging to farmers in Kandi, Ndali, and Savé areas of Benin (situated between latitudes 8° and 12°S) planted to a 90-d cultivar locally called Moto or to the 120-d cultivar 69101 (Manga) were surveyed during harvest of the 1997 cropping season. Fifteen plants were uprooted along transect lines within an area of 1600 m^2 situated in the middle of each farm. Plants were examined externally for termite damage. Pods of uprooted plants were removed, examined for termite damage (classified as scarified or perforated), and put into paper bags. The pods were dried under atmospheric conditions and shelled. Samples of 50 seeds were taken per field for *Aspergillus* spp. infection studies.

Field Trial on Seed Infection by* Aspergillus *spp. at Samanko in 1996. A termite-infested field at the ICRISAT station at Samanko (latitude 12° 31'S) in Mali was planted to 10 peanut cultivars of 90-120-d maturity—namely 28-206, ICGS 11, 47-10, ICG (FDRS) 4, ICG (FDRS) 10, 55-437, J11, 47-16, JL 24, and ICG 10946. Each cultivar was planted in plots treated with 5 kg ai/ha carbofuran (Furadan®) and in nontreated plots. The dimension of each plot was 4 × 4 m. The experiment was planted in a randomized complete block design with four replications. Each plot was eight rows wide with 50 cm between rows and 25 cm between plants within the rows. Single superphosphate at 100 kg/ha was applied to each plot at the time of land preparation.

At the appropriate harvesting dates, 10 plants were harvested at random from the inner rows of each plot. The number of pods scarified or perforated by *Microtermes* spp. was recorded. The pods were dried and samples of 50 seeds were taken from each plot for the assessment of percentage *Aspergillus* spp. colonization.

On-Station and On-Farm Termite Damage Trials in 1997. Termite-infested plots in ICRISAT station at Samanko and in a farmer's plot at Kita (latitude 12° 50'S) in Mali were used for 1997 trials. The same plot dimensions and plant spacing as in the 1996 trials were maintained. The layout was a split plot design with the insecticide treatments as main plots and cultivars as subplots. The treatments included the following: (a) carbofuran granular formulation at 5 kg ai/ha at planting, (b) chlorpyrifos (Dursban®) EC at 480 mL ai/ha at 40 dap, (c) carbofuran at 5 kg ai/ha at planting + chlorpyrifos at 480 mL ai/ha at 40 dap, and (d) no insecticide treatment (control plots).

Three of the cultivars which were less susceptible to termite damage in the 1996 trials [55-437, 47-16, and ICG (FDRS)10], and two relatively susceptible cultivars (ICG 10946 and J11) were used for the Samanko and Kita trials, respectively. J11 was used only for the Kita on-farm trial due to insufficient seeds of ICG 10946. At harvest, 10 randomly selected plants were uprooted per plot and the number and percentage of pods attacked by termites were recorded.

Laboratory Determination of Peanut Fungal Invasion of the Farmers' Plots and Field Trials. To determine the percentage of *Aspergillus*-infested seeds in the survey and field trials, 50 seeds per field or per treatment (in the trials) were distributed in three petri dishes lined with filter paper after being sterilized in 1% sodium hypochlorite solution for 30 sec. The petri dishes were maintained humidified for 6 d. Records of the number of seeds infested by *Aspergillus* spp. per field or treatment were recorded at the end of 1 wk of incubation under average temperature and relative humidity of 25 C and 65%, respectively. Identification of *Aspergillus* spp. was based on conidial color by using identification keys.

ANOVA was computed for all data ($P = 0.05$) associated with *Aspergillus* spp. infections in the field trials. The means of significant tests were separated using the Duncan Multiple Range Test. *Aspergillus* spp. colonization was correlated with termite pod damage.

Results

Survey of Farmers' Fields for* Aspergillus *spp. Colonization. During the survey of peanut farms in Benin, 40% of the fields observed were partly harvested. Uprooted plants were heaped into rows in the fields for the pods to dry. External observations made on sampled plants showed that 5 to 31% were attacked by *Microtermes* spp. in each farm. Termites also damaged peanut pods. The percentages of damaged pods (scarified and perforated by *Microtermes*) per farm ranged between 12.2 and 35.8% (Table 1). Seven out of 23 fields had low percentages of pods colonized by *Aspergillus* (0-5%), while four fields recorded substantial levels of pod colonization (> 23%). Laboratory bioassays showed that seeds were predominantly colonized by *A. flavus*, with a lower occurrence of *A. niger* Van Theighem (Table 1). A positive correlation ($r = 0.6$; $df = 21$; $P < 0.002$) was found between *A. flavus* percentage and termite damage (a summation of the percentages of scarified and perforated pods). No correlation was found between termite damage and *A. niger* infection, or separately between the various categories of pod damage (pod scarification or perforation) and the identified *Aspergillus* spp.

On-Station and On-Farm Trials on Termite Damage of Pods and* Aspergillus *spp. Infection. In all trials, termite damage of the pods was by *M. lepidus* Sjöstedt., whereas damage by *Odontotermes* spp. (Isoptera:Termitidae) was negligible. Substantially high percentages (> 30%) of seeds were colonized by *A. flavus* in plots of the cultivars ICG 10946 (both treated and nontreated) and nontreated 28-206 in the 1996 trial. Apart from JL 24 (treated and untreated), untreated ICG (FDRS) 10 and 47-10 colonization of the seeds of other treated or nontreated cultivars seldom exceeded 23%.

Table 1. Colonization of termite-damaged peanut pods of cultivar Moto and Manga by *Aspergillus* spp. in farmers' fields in Benin.

Survey area	Peanut cultivars	Pods scarified by termites	Pods perforated by termites	Total damaged pods	Seeds with *A. flavus*	Seeds with *A. niger*
		%	%	%	--------%	---------
Kandi						
1	Moto	18.0	6.0	24.0	7	6
2	Moto	5.3	13.0	18.3	18	9
3	Moto	14.4	17.7	32.1	24	0
4	Manga	9.6	19.0	28.6	4	2
5	Manga	8.9	20.8	29.7	28	10
6	Manga	27.0	6.9	33.9	19	2
7	Manga	0	14.7	14.7	1	0
8	Moto, Manga	3.6	21.7	25.3	26	25
Ndali						
1	Moto, Manga	12.8	16.2	29.0	13	4
2	Manga	8.7	10.0	18.7	2	0
3	Manga	6.2	11.1	17.3	7	1
4	Manga	0	18.5	18.5	2	0
5	Manga	15.0	16.2	31.2	17	0
6	Manga	11.7	21.3	33.0	10	10
7	Manga	2.4	24.4	26.8	7	5
8	Manga	10.2	25.6	35.8	32	18
Savé						
1	Moto	10.0	5.8	15.8	14	8
2	Manga	10.7	11.5	22.2	10	6
3	Moto	15.0	3.0	18.0	18	3
4	Moto	11.2	14.0	25.5	4	5
5	Moto	4.6	7.9	12.2	4	2
6	Moto	10.0	5.5	15.5	6	4
7	Moto	12.5	12.5	12.5	3	0
Mean ± SE		9.87 ± 1.29	14.05 ± 1.37	23.41 ± 1.56	12 ± 1.96	5.2 ± 1.3

However, 47-16, ICG (FRDS) 4, and treated cultivars of 47-10, ICG (FRDS) 10 and J11 had comparatively lower percentages of seeds colonized by *A. flavus*, while 55-437 had the lowest percentage colonization (Table 2). *Aspergillus flavus* percentage colonization was weakly correlated to the percentage of pods damaged by termites ($r = 0.43$; df = 18; $P < 0.05$) in 1996. The invasion of the seeds by other fungi such as *A. niger* and *Fusarium* spp. was negligible.

Insecticide application, cultivar, and interactions of insecticide and cultivar significantly ($P < 0.05$) reduced *Aspergillus* spp. colonization of seeds in the various treatments at Samanko in 1997. Seeds from pods of cultivar ICG 10946 that were most damaged by termites (Fig. 1) also were significantly more colonized than the other cultivars (Table 3) at Samanko, while the least damaged cultivar 47-16 (Fig. 1) was less colonized by *Aspergillus* spp. at Kita. Observation of selected visibly undamaged pods in the field trials of Samanko and Kita showed relatively lower seed colonization by *A. flavus* compared to those from damaged samples, with a lower colonization of 47-16 and 55-437, and significantly higher colonization of ICG 10946 cultivar at Samanko compared to other cultivars (Table 3). *Aspergillus flavus* colonization of J11 was only significantly higher than that of 47-16 at Kita. Peanut seeds from plots treated with carbofuran + chlorpyrifos at Samanko that were observed to be less damaged by termites (Fig. 2) were significantly less colonized by *A. flavus* than seeds from the control plots and carbofuran-treated plots (Table 4). Seeds from plots treated only with chlorpyrifos at 40 dap also were significantly less colonized by *A. flavus* than those from the control plots at Samanko. Insecticide effects on *A. niger* colonization were not significant. Termite damage (scarified and perforated pods) was positively correlated ($r = 0.58$; df = 46; $P < 0.001$) with *A. flavus* occurrence in 1997. The correlation between termite pod damage and *A. niger* seed colonization was low and nonsignificant. A separate analysis of the correlation between *Aspergillus* spp. percentage colonization and the percentage of pods scarified or perforated by termites was not significant.

Discussion

The high pod damage caused by termites in farmers'

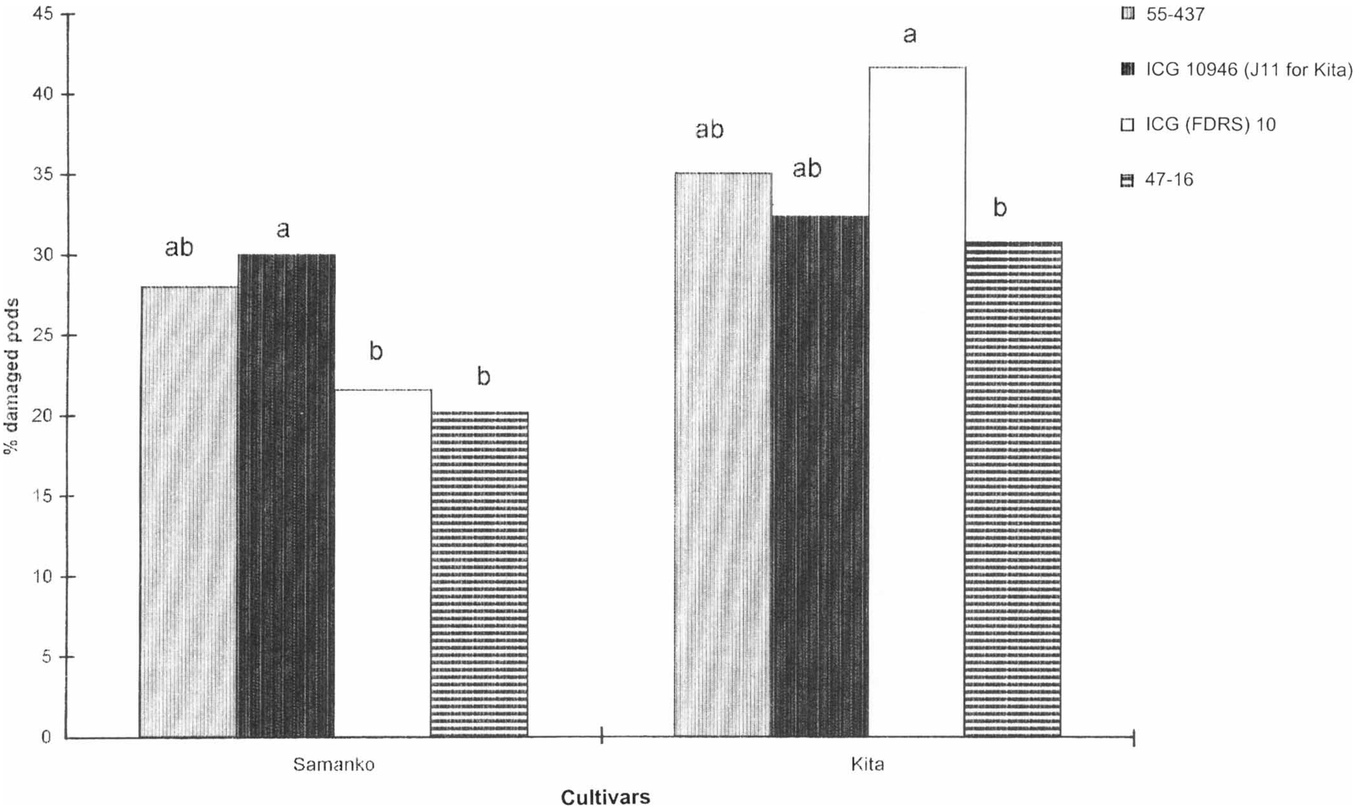

Fig. 1. Cultivar effects on termite damage of peanut pods in field trials at Samanko and Kita during the 1997 cropping season.

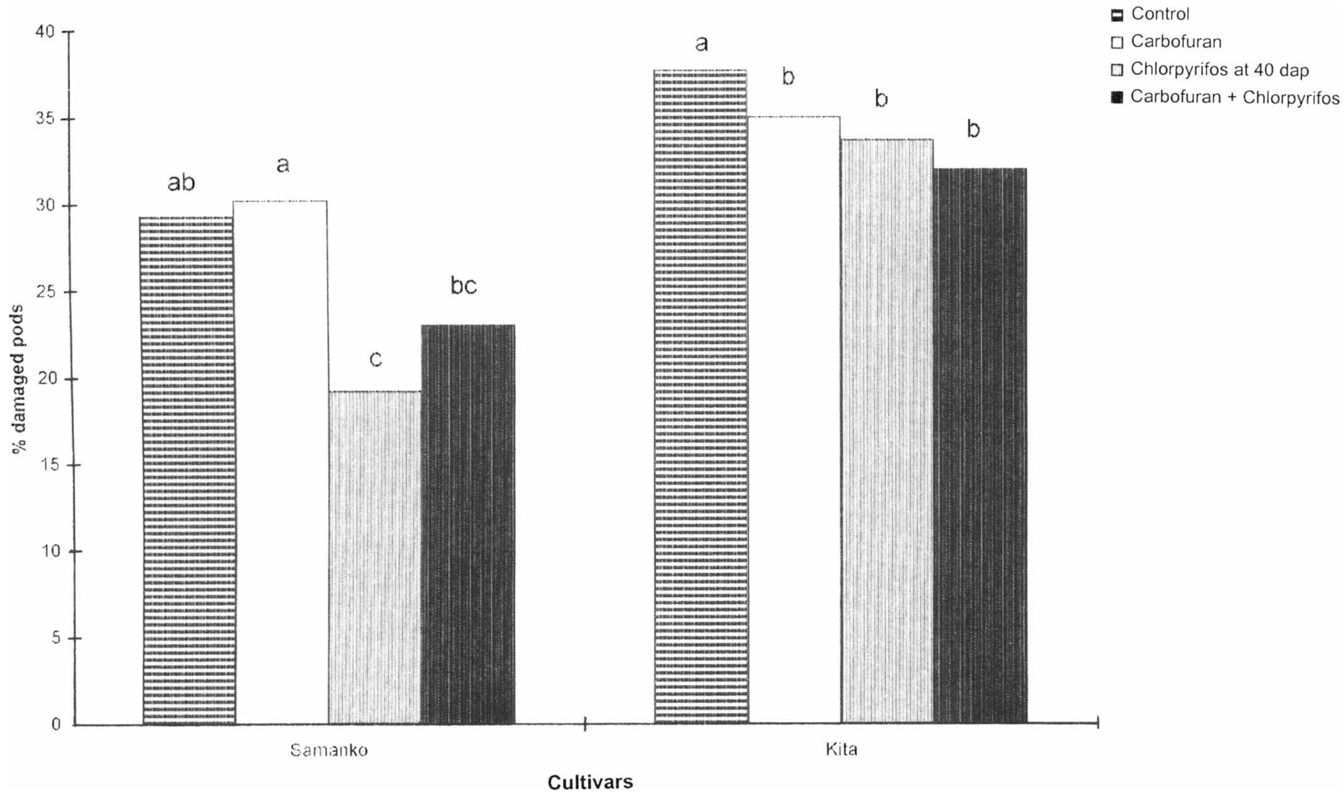

Fig. 2. Effect of insecticides on termite damage of peanut pods in field trials at Samanko and Kita during the 1997 cropping season.

fields in Benin (up to 35%) may have contributed to the high *A. flavus* population colonization observed in bioassays because the percentage of damaged pods was positively correlated with *A. flavus* percentage colonization. The latter species was more predominant than *A. niger* which showed a relatively low infection of peanut seeds and no correlation with termite damage. The farmers' practice of heaping uprooted peanut plants in the fields for pods to dry, as observed during the survey in Benin, also may have contributed to high termite infestation and damage because these heaped plants may have acted as food reservoirs for termite colonies (Umeh and Ivbijaro, 1997) and subsequent colonization by *Aspergillus* spp. Interviews with farmers in the surveyed areas (V. C. Umeh *et al.*, unpub. data) indicated termite damage has been a perennial problem. Incidences of more than 50% pod damage have been recorded on some farms. There is likely to exist a relationship between termite damage and *A. flavus* colonization because it has been established that insects physically move conidia adhering to their bodies onto plant parts during feeding (Urban *et al.*, 1987). Reports indicated that, although damage is not a prerequisite for *A. flavus* formation, the incidence of *A. flavus* and levels of aflatoxin contamination were higher in damaged kernels than in sound mature kernels (Hill *et al.*, 1983; Blankenship *et al.*, 1984; Bowen and Mark, 1993).

Table 2. Cultivar and insecticide effects on *A. flavus* infection of termite-damaged peanut pods in Samanko during 1996 in an on-station field trial.[a]

Cultivars	Treatment	*A. flavus* colonization	Damaged pods
		%	%
ICG 10946	None	46.3 a	31.5 b
ICG 10946	Carbofuran	34.7 ab	27.5 b
28-206	None	34.3 ab	5.0 j
JL 24	None	28.7 bc	40.0 a
47-10	None	26.8 bcd	28.5 b
JL 24	Carbofuran	26.0 bcd	22.5 cde
ICG (FDRS) 10	None	25.7 b-e	30.0 b
ICGS 11	Carbofuran	23.0 b-e	23.0 cde
ICGS 11	None	22.3 b-e	23.5 cd
47-16	None	14.3 cde	1.0 k
47-10	Carbofuran	13.0 cde	12.5 h
28-206	Carbofuran	12.0 de	8.5 hi
47-16	Carbofuran	11.7 de	1.0 k
ICG (FDRS) 10	Carbofuran	11.0 de	18.5 fg
55-437	Carbofuran	10.7 de	10.0 h
ICG (FDRS) 4	None	10.0 de	16.0 g
J11	None	10.0 de	25.0 bc
ICG (FDRS) 4	Carbofuran	8.0 ef	17.5 g
J11	Carbofuran	7.0 ef	25.5 bc
55-437	None	5.7 f	21.0 ef
Mean		19.05	19.4
SE (±)		2.57	2.41

[a]Means in the same column followed by the same letters are not significantly different at P = 0.05.

Table 4. Insecticide effect on *Aspergillus* spp. colonization of termite-damaged peanut pods in 1997 on-station trials at Samanko and on-farm trials at Kita.

Treatment	Seeds with the indicated *Aspergillus* spp.[a] (data from 50 seed)			
	A. flavus		*A. niger*	
	Samanko	Kita	Samanko	Kita
	%		%	
Control	20.7 a	12.5 a	8.4 a	8.5 a
Carbofuran	20.1 ab	12.1 a	7.0 a	6.5 a
Chlorpyrifos at 40 dap	15.2 bc	10.7 a	7.1 a	5.7 a
Carbofuran + chlorpyrifos	14.7 c	11.7 a	10.0 a	5.2 a
Mean	17.67	11.7	8.12	6.4
SE (±)	0.89	0.44	0.59	0.60

[a]Mean percentages of colonization in the same column followed by the same letters were not significantly different (P = 0.05).

Table 3. Effect of cultivar and termite damage to pods on *Aspergillus* spp. colonization in on-station trials at Samanko and on-farm trials at Kita in 1997.

Cultivar	Seeds with the indicated *Aspergillus* spp. (data from 50 seed)[a]							
	A. flavus				*A. niger*			
	Samanko		Kita		Samanko		Kita	
	Damaged	Undamaged	Damaged	Undamaged	Damaged	Undamaged	Damaged	Undamaged
	%				%			
55-437	14.7 b	0 b	13.7 a	6 a	6.7 b	0 b	8.3 a	0 b
ICG 110946	22.7 a	8 a	—	—	12.6 a	6 a	—	—
J11	—	—	13.7 a	8 a	—	—	7.2 a	6 a
ICG (FDRS) 10	17.4 b	4 b	16.0 a	6 a	7.3 b	4 ab	9.2 a	2 b
47-16	15.9 b	2 b	3.7 b	2 b	6.6 b	0 b	1.2 b	0 b
Mean	17.67	3.5	11.7	5.5	8.3	2.5	6.4	2
SE (±)	0.79	0.92	1.17	0.79	0.85	0.86	0.95	0.84

[a]Mean percentages of colonization in the same column followed by the same letters were not significantly different (P = 0.05).

In the 1996 on-station field trial, termite damage partly contributed to higher incidence of *A. flavus* in some cultivars as evidenced by the positive correlation obtained between pod damage and *A. flavus*. However, since no additional insecticide treatment was applied after carbofuran was administered at planting, most of the termite control achieved in terms of reduced pod damage may be attributed to low susceptibility of the cultivars to termite attack. An additional insecticide application may have further reduced termite damage and clearly expressed the effect of cultivar on *A. flavus* colonization. The correlation between termite damage and *A. flavus* colonization in 1996 was therefore low.

In the subsequent trials conducted in 1997 at the various sites (on-farm and on-station trials), with additional insecticide and application periods, the results indicated that the correct timing of insecticide application reduced termite damage and contributed to lower *A. flavus* invasion of seeds. The application of chlorpyrifos at 40 dap, or carbofuran at planting + chlorpyrifos at 40 dap, significantly reduced colonization of seeds by *A. flavus* in the on-station trial compared to the nontreated control. Chlorpyrifos has enough residual activity (Mack *et al.*, 1991) to reduce termite damage and consequent infection by aflatoxigenic fungi from the time of application through pod formation and maturity. The same effectiveness was observed in the on-farm trial (farmers' plot) except that the percentages of colonization were not significantly different and probably due to differences in environmental factors such as rainfall and the time of harvesting. However, a positive correlation was established between the percentage of pods damaged by termites and *A. flavus* colonization in this study. This relationship did not exist when the various categories of pod damages—i.e., percentages of scarified or perforated pods were correlated separately with *A. flavus* colonization, probably due to low colonization observed compared to the situation where percentage damage was a summation of all categories of pod damage. Bowen and Mack (1993) observed a positive correlation between pod scarification by the lesser corn stalk borer *Elasmolomus lignosellus* (Zeller) and *A. flavus* contamination in four trials. Although *A. flavus* can attack visibly undamaged kernels, increased incidence is enhanced by insect injury (Hill *et al.*, 1983; Lynch and Wilson, 1991) that allows easier penetration of fungal pathogens into pods and seeds. This was similar to observations made in the present study whereby pod injury was caused by termites. Insects have been noted as one of the major causes of pod or kernel injury and have been directly implicated with carrying propagules of *A. flavus* which are deposited on a suitable substrate (Diener *et al.*, 1987). Further investigations will be needed to increase the choice of insecticides and cultivars and to establish treatment thresholds for *Microtermes*-infested peanuts in the West African subregion.

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