Enhanced Infection of Peanut, *Arachis hypogaea* L., Seeds With *Aspergillus flavus* Group Fungi Due to External Scarification of Peanut Pods by the Lesser Cornstalk Borer, *Elasmopalpus lignosellus* (Zeller)[1]

Robert E. Lynch* and David M. Wilson[2]

ABSTRACT

The relationship between injury by the lesser cornstalk borer (LCB), *Elasmopalpus lignosellus* (Zeller), and invasion of peanut, *Arachis hypogaea* L., pods and seeds by species of the *Aspergillus flavus* group (*A. flavus* Link and *A. parasiticus* Speare) were studied under laboratory and field conditions. In the laboratory, LCB larvae were an excellent vector of an *A. parasiticus* color mutant (ATCC 24690) to all developmental stages of peanut pods. Fungal invasion and aflatoxin concentration in seeds were higher in immature pods (stage 2-3) than in more mature pods (stage 4-6). Contamination of seeds with ATCC 24690 was directly related to the extent of pod injury by larvae of the LCB. In field studies, over 50% of the LCB larvae collected from peanut were naturally contaminated with species of the *A. flavus* group. The planting date and harvest date of peanut had little influence on the incidence of fungal contamination of pods and seeds, or on aflatoxin content in seeds. However, increased pod injury by the LCB significantly increased the percentage of seeds infected with species of the *A. flavus* group. Seeds in pods with only external scarification from larval feeding had a significantly higher percentage of *A. flavus* group infection than seeds from uninjured pods. Therefore, infection and contamination of visibly uninjured seeds with aflatoxigenic fungi were enhanced by external injury to peanut pods by the LCB.

Key Words: *Aspergillus flavus*, peanut, lesser cornstalk borer, pod injury, peanut insects.

Product quality is of utmost importance to the peanut industry in the U.S. since the majority of the peanuts produced are used for human consumption. One of the major quality concerns of the industry is reducing contamination of

[1]Joint contribution of the USDA-ARS and the University of Georgia, Agricultural Experiment Station. The research was supported in part by USDA, State, Hatch, Peanut Collaborative Research Support Program, and Georgia Agric. Commodity Commission for Peanuts funds allocated to the USDA and/or to the Georgia Agricultural Experiment Stations.

[2]Supervisory Research Entomologist, Insect Biology and Population Management Research Laboratory, USDA-ARS, and Professor, Plant Pathology Department, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793-0748.

*Corresponding author.

peanut with aflatoxin, toxins produced by *Aspergillus flavus* Link and/or *A. parasiticus* Speare (henceforth the *A. flavus* group, unless the species was determined). Methods recently have been developed and evaluated for removing or reducing aflatoxin contaminated peanuts via belt screening that removes loose shelled kernels, immature pods, and foreign material (8, 23).

Penetration of peanut pods by insects may enhance invasion of pods by *A. flavus* group fungi and the formation of aflatoxin in seeds before harvest, after digging, and during storage (3, 6, 9, 10, 18-21, 24, 31). Aflatoxin concentration in seeds from pods injured by insects can be 30-60 times greater than aflatoxin concentration in seeds from uninjured pods (3, 9, 10, 31). Furthermore, insect injury to peanut pods may result in aflatoxin contamination in seeds under conditions that do not favor high aflatoxin content in seeds from uninjured pods (3, 5, 10).

Termites, *Microtermes thoracalis* Sjostedt and *Odontotermes* spp., in Asia and Africa and the lesser cornstalk borer (LCB), *Elasmopalpus lignosellus* (Zeller), in the U.S. have most often been associated with injury to peanut pods prior to harvest. Preharvest injury may lead to increased invasion of pods by species of the *A. flavus* group, and to subsequent aflatoxin contamination (5, 18-21, 28, 29). Two types of injury to peanut pods by both termites and the LCB have been described, i.e., pod scarification and pod penetration (11, 13). Injury to peanut pods by LCB larvae is greater on immature pods, pods in stages 1-3 (35), and often results in pod penetration (15). As pods reach stage 4, their mesocarp develops structural rigidity, and injury by the LCB is primarily external scarification without pod penetration. Similarly, pod scarification by termites usually occurs late in the growing season and is restricted to the more mature pods (11). Furthermore, conditions that favor injury to peanut pods by both termites and the LCB (11, 12, 15, 17), i.e., drought and high soil temperatures, are similar to conditions that favor invasion of pods by species of the *A. flavus* group and aflatoxin formation in seeds (3, 4, 10, 25, 26).

The interrelationship between an LCB infestation and increased infection of peanut pods by species of the *A. flavus* group was first suggested by Ashworth and Langley (1). Dickens *et al.* (5) showed increased contamination of seeds with *A. flavus* and increased aflatoxin content as a result of "typical LCB damage" and speculated that "the LCB may transport *A. flavus* spores through the pod to ideal sites of infection where the LCB feeds on the kernel." Widstrom (34) noted that "soil insects... and their relationship to the aflatoxin problem have not received as much attention as might be expected in view of the fact that the seriousness of the aflatoxin problem in feeds was first recognized with peanuts." However, none of the published reports concerning pod injury by soil insects provide definitive information of the interrelations between extent of pod injury by insects and invasion of pods and seeds by the fungus or aflatoxin formation.

Research reported here was designed to determine the interrelationships among peanut pod injury by the LCB, pod infection by species of the *A. flavus* group, and seed contamination with aflatoxin. Specifically, we examined the role of the LCB as a vector of an *A. parasiticus* color mutant, the relationship between extent of pod injury by the LCB in the field and contamination of pods and seeds with the *A. flavus* group, and the subsequent contamination of seeds with aflatoxin.

Materials and Methods

Laboratory Studies

Laboratory tests were conducted to determine the efficiency of LCB larvae in disseminating a mutant of *A. parasiticus* (ATCC 24690) that could be readily identified by its red-brown conidia (36). ATCC 24690 is quite competitive in nature and produces all of the four known aflatoxins. Treatments were arranged in a split-split plot with stages 2-6 of peanut pod development as whole plots, contamination of LCB larvae with ATCC 24690 versus no contamination of LCB larvae as the subplot, and level of pod injury, i.e., uninjured, externally scarified, and penetrated, as the sub-subplot. Each experiment was designed in a randomized complete block with 6 replications.

Peanut plants with pods in developmental stages 2 to 6 were pulled in the field, placed in plastic bags, taken to the laboratory, and refrigerated at 4.4 C until use with 1-2 hrs. Pods with the entire peg attached were removed from the plants, classified by stage of development (35), and rinsed for ca. 3 min in 1% sodium hypochlorite and then in distilled water. The pegs of three pods of the appropriate stage were inserted through the rubber cap of a floral Aqua-pic containing distilled water. Tests were conducted in 25-cm-diameter clear plastic dishes. The bottom of each dish was divided into 5 equal sections and three 1.7-cm-diameter holes were drilled in the bottom of the dish for each section to accommodate the Aqua-pics, each containing three pods of a designated stage, were randomly assigned to a section and inserted through the holes in the dish. The Aqua-pics and pods were then covered with sterilized sand.

LCB larvae used in the test were from a laboratory colony (16) maintained at the Insect Biology and Population Management Research Laboratory. Two tests were conducted, the first with 7-day-old larvae and the second with 10-day-old larvae. For the contaminated treatment, 25 larvae were placed in a petri dish containing 10 mL of a 1 x 10^5 spore/mL suspension of the *A. parasiticus* color mutant; uncontaminated larvae were treated with distilled water. Larvae from a petri dish were then removed from the water, placed on the surface of the sand in the larger dishes, and the dish was covered with a lid and placed in an incubator maintained at 26.7 C, 75% RH and a 16 hr light-8 hr dark photoperiod.

After 10 days, pods in each dish were removed and rated for injury by larvae of the LCB on a 0-3 scale where 0 = uninjured,.1 = external scarification, 2 = pod penetration, and 3 = pod contents partially consumed. LCB larvae were removed from each dish by sifting the sand through a 40-mesh sieve. The number of larvae/dish was recorded and the larvae and pods were frozen for later analysis for *A. parasiticus* contamination. Pods were rinsed in water, placed in 0.5% sodium hypochlorite for 5 min, and aseptically shelled for analyses for infection by species of the *A. flavus* group and for contamination with aflatoxin. Presence of the fungus was determined by placing the pods, seeds, or larvae on malt extract agar containing 10% NaCl by weight, incubating the dishes for 7 days at 30 C, and observing the presence of the red-brown conidia of ATCC 24690. Aflatoxin content of seeds was determined by HPLC (32).

All data were analyzed by analysis of variance (27). Percentage data were transformed to arcsine $\sqrt{\%}$ and aflatoxin data were transformed to log (aflatoxin +1) for analysis. Significantly different means were separated by using Waller-Duncan (30) k-ratio t-test at k=100 and P=0.05 for multiple comparisons, or by using the protected least significant difference analysis for paired comparisons (30).

Field Studies

Field experiments were conducted on the Belflower Farm, Coastal Plain Experiment Station, near Tifton, GA in 1983 and 1985 to determine the interactions among peanut planting dates, harvest dates, and insect injury to pods, infection of pods and seeds by species of the *A. flavus* group, and contamination of seeds with aflatoxin. Certified Florunner seed were planted at ca. 120 kg/ha in Tifton loamy sand (fine, loamy, siliceous, thermic Plinthic Paleudults). Plots were 6.1 m in length and 8 rows wide with 81 cm between rows and were treated before planting for weed control with benefin (N-Butyl-N-ethyl-alpha, alpha, alpha, trifluoro 2, 6-dinitro-p-toluidine) at 1.25 kg ai/ha and vernolate (S-Propyl dipropylthiocarbamate) at 2.24 kg ai/ha. Prior to complete plant emergence, all plots were treated for weed control with alachlor [2-chloro-2',-6'-diethyl-N-(methoxymethyl) acetanilide] at 3.36 kg ai/ha and naptalan (N-1-Naphthylphthalmic acid) + dinoseb [2-sec butyl 4,6- dinitrophenol (alkanolamine salts)] at 3.36 + 1.68 kg ai/ha, respectively, as recommended by the Georgia Extension Service. All plants were sprayed for leafspot control with chlorothalonil (Tetrachloroisophthalonitrile) at 2.48 l ai/ha on 10-14 day intervals beginning ca. 40 days after plant emergence.

The experiments were designed in a randomized complete block with a split-split plot arrangement where treatments were replicated 5 times with planting dates (April 4, April 25, May 16, and June 6, 1983, and April 22, May 13, and June 3, 1985) as whole plots; harvest dates (September 1, 8, and 15, 1983 and September 5, 26, and October 21, 1985) as subplots; and injury to pods by the LCB (uninjured, externally scarified, and penetrated) as sub-subplots. Pods were visually separated into the various injury categories at harvest and frozen for later analysis for mycofloral growth on hulls and seeds and aflatoxin contamination in seeds. All data were analyzed by analysis of variance (27). Percentage data were transformed to arcsine $\sqrt{\%}$ and aflatoxin data were transformed to log (aflatoxin +1) for analysis. Significantly different means were separated using Waller-Duncan (33) k-ratio t-test at k=100.

A total of 78 LCB larvae was collected from peanuts grown in the field by pulling plants and searching for larvae in silken tubes attached to pods or in the loosened soil. Larvae were placed individually in vials, taken to the laboratory, surface sterilized in 1% sodium hypochlorite for 1 minute, rinsed in water, and frozen for later analysis. The presence of the *A. flavus* group fungi was determined by placing individual larvae on malt extract agar and incubating as previously described.

In 1990, the relationship between external injury to peanut pods by LCB larvae and pod infection by species of the *A. flavus* group was substantiated by collecting LCB-scarified pods and uninjured pods from a peanut field shortly after plants were inverted. The experiment was designed in a randomized complete block with treatments of externally scarified pods versus uninjured pods replicated 10 times, i.e., pods collected at 10 different locations in the field. The presence of species of the *A. flavus* group and aflatoxin was determined as previously described. Percentage infection data and aflatoxin content were converted as previously described, subjected to analysis of variance, and significantly different means were separated using the protected least significant difference analysis for paired comparisons (30).

Results and Discussion

Laboratory Studies

No significant interactions between stage of peanut pod development and LCB contamination with the *A. parasiticus* mutant were noted for pod injury ratings, numbers of LCB larvae recovered, or percentage of larvae contaminated with the color mutant. Stage of pod development significantly influenced pod injury by the LCB and the number of larvae that were recovered (Table 1). Injury to pods was significantly greater for pods in stage 2 than for pods in stages 3-6, and significantly greater for pods in stage 3 than for pods in stages 4-6, similar to results that were previously reported (15). Likewise, significantly more LCB larvae were recovered from immature pods, stages 2 and 3, than were recovered from more mature pods, stages 4-6. Contamination of LCB larvae with ATCC 24690 did not influence pod injury ratings or the number of LCB larvae recovered at the end of the test. Also, stage of pod development did not affect the percentage of larvae contaminated with the *A. parasiticus* mutant. Thus, the contamination of larvae with ATCC 24690 was highly successful without adversely affecting the ability of larvae to injure peanut pods.

Stage of pod development did not affect the percentage of pods infected with ATCC 24690 (Table 2), indicating that LCB larvae visited all pods equally, and that the larvae vectored the fungus equally well among all stages of peanut pods. However, stage of pod development did influence the percentage of seeds infected with ATCC 24690. More seeds from stage 3 pods were infected with ATCC 24690 than seeds from stage 4-6 pods, and more seeds from stage 2, 4, and 5, pods were infected with ATCC 24690 than seeds from stage 6 pods (Table 2). In the laboratory, the LCB larva was an excellent vector of the ATCC 24690 mutant to all developmental stages of peanut pods, resulting in the contamination of over 95% of the pods and almost 62% of the seeds with the fungus.

Table 1. Laboratory evaluation of lesser cornstalk borer (LCB) larvae for dissemination of an *A parasiticus* color mutant to peanut pods in different stages of development.[a]

Variable	Pod injury rating[b]	No. LCB recovered	% LCB with A. parasiticus color mutant[c]
Stage of Pod Development[d]			
2	1.1a	12.6a	50.0a
3	0.7b	12.9a	46.5a
4	0.5c	7.0b	29.8a
5	0.5c	4.1c	28.3a
6	0.5c	6.6bc	38.8a
Larval Contamination with A. parasiticus			
+	0.6a	9.7a	82.9a
-	0.7a	8.0a	0.9b

[a]Means within a column for each variable followed by the same letter are not significantly different (k = 100, P = 0.05) using Waller-Duncan k-ratio t-test (33) or the protected least significant difference analysis for paired comparisons (30).

[b]Pod injury rated on a scale of 0 to 3, where 0 = no injury, 1 = external pod scarification, 2 = pod penetration, and 3 = pod contents partially consumed.

[c]Percentage data transformed to arcsine $\sqrt{\%}$ for analysis.

[d]Peanut pod developmental stages as described by Williams and Drexler (32).

LCB larvae preferred immature peanut pods, i.e., stage 3 or earlier, which resulted in a greater level of contamination with the fungus in seeds from immature pods. Sander *et al.* (26) found that seeds in immature pods were colonized by the *A. flavus* group fungi and contaminated with aflatoxin more often than seeds from more mature pods. Dorner *et al.* (7) reported that the increased frequency of aflatoxin contamination in immature seeds may be related to reduced phytoalexin production as water activity declines in seeds. LCB larvae may assist the decline in water activity in seeds and.or affect other resistance mechanisms associated with peanut pods or seeds through increased attraction to immature pods, increased injury to immature pods, creation of a favorable environment for fungal growth, and the excellent ability of larvae to vector the fungus. This hypothesis is further substantiated by a significantly increased aflatoxin content in seeds from stage 3 pods than in seeds from all other pod stages. LCB injury to immature pods (stages 1-3) is characterized by both external feeding and pod penetration, while injury to more mature pods (stages 4-6) is characterized primarily by external feeding on the pod exocarp which results in scarification (15). Thus,the increased percentage of infection and the increased aflatoxin concentration in seeds from stage 3 pods are probably related to increased pod injury, and the lower percentage infection and aflatoxin concentration in seeds from more mature pods are probably related to decreased LCB injury as the mesocarp develops structural rigidity in stage 4.

The percentage of pods and seeds infected with ATCC 24690 and the concentration of aflatoxin in seeds varied with pod injury class (Table 2). Peanut pods that were penetrated by LCB larvae had a higher percentage of pods contaminated with ATCC 24690 than pods that were uninjured or only externally scarified. The percentage of seeds infected with the mutant was directly related to the extent of pod injury; seeds that were partially consumed by the LCB had a

Table 2. Dissemination of an *A. parasiticus* color mutant in the laboratory by lesser cornstalk borer larvae and development of aflatoxin in seeds of peanut.[a]

Variable	% Pods with *A. parasiticus* color mutant[b]	% seeds with *A. parasiticus* color mutant[b]	Total aflatoxin (ppb)[c]
Stage of Pod Development[d]			
2	49.4a	36.6ab	4.9b
3	58.8a	45.8a	9.2a
4	53.7a	26.2b	1.6b
5	49.0a	25.3b	0.1b
6	46.0a	14.3c	0.1b
Larval Contamination with *A. parasiticus*			
+	96.4a	61.9a	4.1a
-	10.3b	1.9b	2.3a
Pod Injury Class[e]			
0	51.5b	23.0c	1.6b
1	47.6b	29.3c	1.8b
2	60.0a	47.0b	6.3a[f]
3	54.5ab	65.6a	--

[a]Means within a column for each variable followed by the same letter are not significantly different (k = 100, P = 0.05) using Waller-Duncan k-ratio t-test (33) or the protected least significant difference analysis for paired comparisons (30).

[b]Percentage data transformed to arcsine $\sqrt{\%}$ for analysis.

[c]Aflatoxin concentration converted to Log 10 (aflatoxin + 1) for analysis.

[d]Peanut pod developmental stages as described by Williams and Drexler (32).

[e]Pod injury rated on a scale of 0 to 3, where 0 = no injury, 1 = external pod scarification, 2 = pod penetration, and 3 = pod contents partially consumed.

[f]Classes 2 and 3 combined for aflatoxin analyses.

significantly higher percentage infection with the fungus than seeds from pods with a lesser degree of injury, and seeds from pods that were penetrated by LCB larvae had a higher percentage infection than seeds from uninjured or externally injured pods.

The percentage of seeds infected with ATCC 24690 was influenced by a significant stage of pod development x LCB larval contamination with the *A. parasiticus* mutant interaction. ATCC 24690 was isolated from less than 3% of the seeds where larvae had not been contaminated with the fungus, and no significant differences were noted in the contamination level with regard to pod injury class. Conversely, high levels of seed contamination occurred where larvae were contaminated with ATCC 24690. The percentage of seed contamination with ATCC 24690 was comparable for penetrated pods (91.2% a) and pods with partially consumed seeds (87.5% a), but was significantly higher for seeds from both penetrated pods and partially consumed seeds than for uninjured (44.0%c) and externally injured pods (61.5% b). Likewise, seeds from externally injured pods had a significantly higher level of contamination with the mutant than seeds from uninjured pods. Thus, in the laboratory, removal of exocarp from peanut pods by LCB larvae without pod penetration was sufficient to enhance seed contamination with ATCC 24690.

Aflatoxin content of peanut seeds was not affected by LCB larval contamination with ATCC 24690, but it was affected by stage of pod development and by pod injury class (Table 2). Aflatoxin content was significantly higher in seeds from stage 3 pods than in seeds from other pod stages. Also, aflatoxin content was significantly higher in seeds from penetrated pods than in seeds from either uninjured or externally scarified pods.

Field Studies

Neither planting date nor harvest date had a significant influence on the percentage of pods or seeds contaminated with species of the *A. flavus* group, or on the total aflatoxin content of seeds in either 1983 or 1985 (Table 3). However, the extent of pod injury significantly influenced the percentage of pods and the percentage of seeds contaminated with species of the *A. flavus* group. In 1983, peanut pods that had been penetrated by LCB larvae had a significantly higher percentage of pod and seed contamination with the fungus than uninjured pods, or pods that had been injured only externally. Likewise, peanut seeds from pods that only had been injured externally had a significantly higher percentage of infection with *A. flavus* group fungi than seeds from uninjured pods. Seeds from pods that had been penetrated by LCB larvae also had a significantly more aflatoxin than seeds from either uninjured or externally injured pods. Hill *et al.* (10) also noted an increased percentage of *A. flavus* group fungi in injured seeds, and noted that only seed from injured peanut pods contained more than a trace of aflatoxin in treatments that were not conducive to aflatoxin formation.

A significant planting date x harvest date interaction affected the percentage of pods contaminated with species of the *A. flavus* group in 1983. This interaction was due to differences between the percentage of uninjured pods contaminated with the fungus and the percentage of LCB-penetrated pods that were contaminated with species of the *A. flavus* group. No significant differences in the percentage contamination were noted between uninjured pods harvested September 1 and those penetrated by LCB larvae. However, LCB-penetrated pods harvested September 8 and September 15 had a significantly higher percentage contamination than uninjured pods.

Results from the 1983 and 1985 field study were similar, but conditions were less favorable in 1985 for peanut infection with species of the *A. flavus* group, and for aflatoxin formation (Table 3). In 1985, pod injury had a significant effect on the percentages of pods and seeds contaminated with *A. flavus* group fungi. The percentage of pods and seeds contaminated with the *A. flavus* group was significantly higher for pods that had been penetrated by LCB larval feeding than these percentages for uninjured or externally injured pods.

The percentage of pods contaminated with the *A. flavus* group was affected by a significant interaction among planting dates x harvest dates x pod injury classes (Table 4). This interaction was attributed to a lack of significant differences among pod injury classes in the percentage of pods infected with the fungus for peanuts planted May 13 and harvested September 26, compared with significant differences among all injury classes in the percentage of infected pods for peanuts planted June 3 and harvested September 5. Conversely, significant differences were noted in the percentage of fungal infected pods only between penetrated pods versus externally injured or uninjured pods for all other plating date x harvest date combinations.

Over 50% of the LCB larvae collected from peanut in the field were naturally contaminated with fungi of the *A. flavus* group. Other insects, especially those that injure corn, have been shown to be naturally contaminated with, and thus serve as a carrier of *A. flavus* group fungi (14, 22). Mites of the genera *Caloglyphus* and *Tyrophagus* also have been

implicated as possible carriers for fungi that result in aflatoxin contamination in peanuts (2).

In 1990, the percentage of pods and seeds infected with *A. flavus* group fungi was significantly greater for LCB-scarified pods (pods = 55.6%; seeds = 27.0%) than for uninjured pods (pods = 17.7%; seeds = 3.6%). These data further substantiate the hypothesis that external scarification of peanut pods by LCB larvae exacerbates infection of seeds without the necessity of pod penetration by the insect. However, seeds from neither uninjured nor externally injured pods in the 1990 test were contaminated with aflatoxin.

Several authors (3-5, 10, 28, 29) have noted a relationship between insect injury to peanut pods and increased seed infection with fungi of the *A. flavus* group. However, without exception, these reports considered insect injury as seed injury resulting from feeding by LCB larvae that had penetrated the pod and fed on the seed(s). However, LCB injury to peanut pods includes not only pod penetration and seed injury, but external scarification by larvae feeding on the exocarp of more mature pods (15).

Data presented here show that the LCB is an excellent carrier of an *A. parasiticus* color mutant and that it vectors the fungus equally well to all developmental stages of peanut pods in the laboratory. Seeds injured in immature pods by LCB feeding had a higher percentage of *A. parasiticus* and aflatoxin contamination than seeds from more mature pods. Also, the percentage infection in seeds in the laboratory increased with an increase in the extent of injury to pods by LCB feeding.

Field conditions during this study were less than optimum

Table 3. Influence of planting date, harvest date, and injury to pods by larvae of the lesser cornstalk borer on the incidence of peanut contamination with *A. flavus* group fungi and aflatoxin content of seeds.[a]

	1983			1985		
Variable	% Pods with *A. flavus* group[b]	% Seeds with *A. flavus* group[b]	Total aflatoxin (ppb)[c]	% Pods with *A. flavus* group[b]	% Seeds with *A. flavus* group[b]	Total aflatoxin (ppb)[c]
Planting Date[d]						
1	87.4a	54.3a	4.9a	4.6a	6.0a	0.0a
2	87.6a	44.9a	2.7a	7.7a	6.3a	0.0a
3	92.2a	47.7a	3.1a	10.7a	3.7a	0.0a
4	92.4a	44.5a	8.4a	--	--	--
Harvest Date[e]						
1	89.4a	45.4a	3.5a	12.9a	7.5a	0.0a
2	88.1a	49.1a	1.5a	8.6a	6.3a	0.0a
3	92.1a	49.0a	3.7a	6.4a	3.7a	0.0a
Pod Injury Category[f]						
1	87.1b	36.8c	1.1b	1.0b	1.1b	0.0a
2	88.5b	44.9b	0.5b	2.7b	1.9b	0.0a
3	94.1a	61.8a	7.1a	24.2a	14.4a	0.0a

[a]Means within a column for each variable followed by the same letter are not significantly different (k = 100, P = 0.05) using Waller-Duncan k-ratio t-test (33).

[b]Percentage data transformed to arcsine $\sqrt{\%}$ for analysis.

[c]Aflatoxin concentration converted to Log 10 (aflatoxin + 1) for analysis.

[d]Planting dates: 1983 (1 = April 4, 2 = April 25, 3 = May 16, 4 = June 6); 1985 (1 = April 22, 2 = May 13, 3 = June 3).

[e]Harvest dates: 1983 (1 = Sept. 1, 2 = Sept. 8, 3 = Sept. 15); 1985 (1 = Sept. 5, 2 = Sept. 26, 3 = Oct. 21).

[f]Pod injury rated on a scale of 1 to 3, where 1 = no injury, 2 = external pod scarification, and 3 = pod penetration.

Table 4. Interaction of planting date, harvest date, and pod injury by the lesser cornstalk borer on the incidence of the *A. flavus* group fungi on peanut pods, Tifton, GA, 1985.

	Percent Pods with A. flavus group fungi when planted on:[a]											
	April 22				May 13				June 3			
Pod Injury Category	Harvest Date				Harvest Date				Harvest Date			
	Sept. 5	Sept. 26	Oct. 21	Mean	Sept. 5	Sept. 26	Oct. 21	Mean	Sept. 5	Sept. 26	Oct. 21	Mean
Uninjured	0.1b	0.1b	1.8b	0.7b	0.6b	0.6a	0.6b	0.6b	0.3c	4.8b	0.1b	1.7b
Externally Scarified	1.5b	0.4b	3.7b	1.9b	6.1b	4.9a	0.4b	3.8b	5.1b	0.0b	2.7b	2.6b
Pod penetrated	42.9a	19.0a	17.0a	26.3a	38.0a	7.7a	10.1a	18.6a	21.9a	40.3a	20.8a	27.7a

[a]Percentage data transformed to arcsine $\sqrt{\%}$ for analysis. Means within a column followed by the same letter are not significantly different (k = 100, P = 0.05) using Waller-Duncan k-ratio t-test (30).

for peanut contamination with fungi of the *A. flavus* group, even when over 50% of the LCB larvae that were collected in the field were naturally contaminated. Under hot, dry conditions that are favorable for both LCB injury to peanut pods and for pod contamination with fungi of the *A. flavus* group, a higher percentage of larvae might be expected to be contaminated with the fungus. Contaminated larvae serve as excellent carriers for the *A. flavus* group fungi to peanut pods. Peanut planting date and harvest date had little influence on the percentage of pods and seeds contaminated with species of the *A. flavus* group or aflatoxin content under the conditions of this research. However, the extent of pod injury by LCB larvae had a direct effect on the percentage of seeds contaminated with species of the *A. flavus* group. More important, peanut seeds from pods with only external scarification had a significantly higher percentage of *A. flavus* group infection than seeds from uninjured pods. Thus, pod penetration and seed injury are not necessary for enhanced infection of seeds with fungi of the *A. flavus* group. Therefore, the role of insects in *A. flavus* group infection and contamination of visibly uninjured seeds cannot be categorically denied, and may indeed be more important than previously noted in other reports (3, 4, 10). Theoretically, an increase in the percentage of infection of seeds with the *A. flavus* group in externally scarified pods should lead to an increased likelihood for aflatoxin development under the appropriate environmental conditions. Research is presently being conducted to verify this important point.

Acknowledgments

The authors express appreciation to Lloyd Copeland, Jamie Day, and Bonnie Evans for their technical assistance in this research. Appreciation is also expressed to Dr. Rodney Beaver for the aflatoxin analyses.

Literature Cited

1. Ashworth, L. J., Jr., and B. C. Langley. 1964. The relationship of pod damage to kernel damage by molds in spanish peanuts. Plant Disease Reporter 48:875-878.
2. Aucamp, J. L. 1969. The role of mite vectors in the development of aflatoxin in groundnuts. J. Stored Prod. Res. 5:245-249.
3. Blankenship, P. D., R. J. Cole, T. H. Sanders, and R. A. Hill. 1984. Effect of geocarposphere temperature on pre-harvest colonization of drought-stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin formation. Mycopathologia 85:69-74.
4. Cole, R. J., T. H. Sanders, R. A. Hill, and P. D. Blankenship. 1985. Mean geocarposphere temperatures that induce preharvest aflatoxin contamination of peanuts under drought stress. Mycopathologia 91:41-46.
5. Dickens, J. W., J. B. Satterwhite, and R. E. Sneed. 1973. Aflatoxin contaminated peanuts produced on North Carolina farms in 1968. J. Amer. Peanut Res. Educ. Assoc. 5:48-58.
6. Diener, U. L., R. E. Pettit, and R. J. Cole. 1982. Aflatoxins and other mycotoxins in peanuts, pp. 486-519. *in* H. E. Pattee and C. T. Young (eds.), Peanut Science and Technology. Amer. Peanut Res. Educ. Soc., Yoakum, TX.
7. Dorner, J. W., R. J. Cole, T. H. Sanders, and P. D. Blankenship. 1989. Interrelationship of kernel water activity, soil temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought-stressed peanuts. Mycopathologia 105:117-128.
8. Dowell, F. E., J. W. Dorner, R. J. Cole, and J. I. Davidson. 1990. Aflatoxin reduction by screening farmers stock peanuts. Peanut Sci. 17:6-8.
9. Graham, J. 1982. Aflatoxin in peanuts: occurrence and control. Queensland Agric. J. 108:119-122.
10. Hill, R. A., P. D. Blakenship, R. J. Cole, and T. H. Sanders. 1983. Effects of soil moisture and temperature on preharvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. Appl. Environ. Microbiol. 22:629-634.
11. Johnson, R. A., and M. H. Gumel. 1981. Termite damage and crop loss studies in Nigeria—the incidence of termite-scarified groundnut pods and resulting kernel contamination in field and market samples. Trop. Pest Mgt. 27:343-350.
12. Johnson, R. A., R. W. Lamb, and T. G. Wood. 1981. Termite damage and crop loss studies in Nigeria—a survey of damage to groundnuts. Trop. Pest Mgt. 27:325-342.
13. Leuck, D. B. 1967. Lesser cornstalk borer damage to peanut plants. J. Econ. Entomol. 60:1549-1551.
14. Lillehoj, E. B., W. W. McMillian, W. D. Guthrie, and D. Barry. 1980. Aflatoxin-producing fungi in preharvest corn: inoculum source in insects and soil. J. Environ. Qual. 9:691-694.
15. Lynch, R. E. 1984. Damage and preference of lesser cornstalk borer (Lepidoptera: Pyralidae) larvae for peanut pods in different stages of pod maturity. J. Econ. Entomol. 77:360-363.
16. Lynch, R. E., and T. Reed. 1985. Rearing the lesser cornstalk borer: Fungicides for the control of *Aspergillus niger*. J. Entomol. Sci. 20:26-33.
17. Mack, T. P., J. W. Smith, Jr., and R. B. Reed. 1987. A mathematical model of the population dynamics of the lesser cornstalk borer, *Elasmopalpus lignosellus* (Lepidoptera:Pyralidae). Ecol. Model.

39:269-286.
18. McDonald, D. 1969. *Aspergillus flavus* on groundnut (*Arachis hypogaea* L.) and its control in Nigeria. J. Stored Prod. Res. 5:275-280.
19. McDonald, D., and C. Harkness. 1963. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts. Part II. Trop. Sci. 5:143-154.
20. McDonald, D., and C. Harkness. 1964. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts. Part III. Trop. Sci. 6:12-27.
21. McDonald, D., C. Harkness, and W. C. Stonebridge. 1964. Growth of *Aspergillus flavus* and production of aflatoxin in groundnuts. Part IV. Trop. Sci. 6:131-154.
22. McMillian, W. W. 1983. Role of arthropods in field contamination, pp. 20-22. *in* U. L. Diener, R. L. Asquith, and J. W. Dickens (eds.), Aflatoxin and *Aspergillus flavus* in corn. Southern Coop. Series Bull. 279.
23. National Peanut Council. 1989. The peanut quality enhancement project. National Peanut Foundation, Alexandria, VA, 28 pp.
24. Pollet, A., and C. Decleret. 1987. Condition and extent of aflatoxin contamination of local groundnut stocks in Cote d'Ivoire. I. Preliminary data (1985-1986 seasons). Oleagineux 42:327-336.
25. Sanders, T. H., R. J. Cole, P. D. Blankenship, and R. A. Hill. 1985. Relationship of environmental stress duration to *Aspergillus flavus* invasion and aflatoxin production in preharvest peanuts. Peanut Sci. 12:90-93.
26. Sanders, T. H., R. A. Hill, R. J. Cole, and P. D. Blankenship. 1981. Effect of drought on occurrence of *Aspergillus flavus* in maturing peanuts. J. Am. Oil Chem. Soc. 58:966A-970A.
27. SAS Institute. 1985. SAS user's guide: Statistics. SAS Institute, Cary, NC.
28. Sellschop, J. P. 1965. Field observations on the conditions conducive to the contamination of groundnuts with the mould *Aspergillus flavus* Link ex Fries, pp. 47-52. Symposium on Mycotoxins in Foodstuffs, Agric. Aspects, Pretoria, S. Africa.
29. Sellschop, J. P., N. P. Kriek, and J. C. DuPreez. 1965. Distribution and degree of occurrence of aflatoxin in groundnuts, pp. 9-17. Symposium on Mycotoxins in Foodstuffs, Agric. Aspects, Pretoria, S. Africa.
30. Snedecor, G. A., and W. G. Cochran. 1980. Statistical methods, 7th edn. Iowa State Univ. Press, Ames, IA.
31. Subrehmanyam, P., and A. S. Rao. 1974. Occurrence of aflatoxin and citrinin in groundnut (*Arachis hypogaea* L). at harvest in relation to pod condition and moisture content. Curr. Sci. 43:707-710.
32. Thean, J. E., D. R. Lorenz, D. M. Wilson, K. Rodgers, and R. C. Gueldner. 1980. Extraction, cleanup, and quantitative determination of aflatoxins in corn. J. Assoc. Off. Anal. Chem. 63:631-633.
33. Waller, R. A., and D. B. Duncan. 1969. A Bayes rule for the symmetric multiple comparison problem. J. Amer. Stat. Assoc. 64:1484-1499.
34. Widstrom, N. W. 1979. The role of insects and other plant pests in aflatoxin contamination of corn, cotton, and peanuts—A review. J. Environ. Qual. 8:5-11.
35. Williams, E. J., and J. S. Drexler. 1981. A nondestructive method for determining peanut pod maturity. Peanut Sci. 8:134-141.
36. Wilson, D. M., W. W. McMillian, and N. W. Widstrom. 1986. Use of *Aspergillus flavus* and *A. parasiticus* color mutants to study aflatoxin contamination in corn, pp. 284-288. *in* G. Llewellyn, C. O'Rear, and S. Barry (eds.), Biodeterioration VI. Intern. Biodeterioration Soc., London.

Accepted August 10, 1991