Relationship of Damage from the Lesser Cornstalk Borer to *Aspergillus flavus* Contamination in Peanuts[1,2]

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ABSTRACT The ability of larvae of the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller), to augment contamination of peanut pods with aflatoxigenic fungi, *Aspergillus flavus* Link and *A. parasiticus* Speare (*A. flavus*-type fungi), was investigated in laboratory and field studies. Aflatoxigenic fungi were found in or on frass from 28.6% of field-collected larvae and in 8.9% of sterilized and macerated larvae. More aflatoxigenic fungi tended to be found in pods from untreated plots than in plots treated with chlorpyrifos in field trials. Contamination of pods or seeds with *A. flavus*-type fungi was positively correlated in all four trials with scarification of pods, and this relationship has been quantified. Since appropriate insecticide treatments can decrease populations of lesser cornstalk borers, which would decrease pod scarification, these same treatments may decrease contamination with aflatoxigenic fungi. Treatment thresholds for the lesser cornstalk borer need to be reconsidered based upon this information.

KEY WORDS *Aspergillus flavus,* aflatoxins, *Elasmopalpus lignosellus,* peanut quality, lesser cornstalk borer.

Aflatoxins are highly carcinogenic compounds that are metabolic products of the fungi, *Aspergillus flavus* Link and *A. parasiticus* Speare [Fungi Imperfecti: Hyphales], two closely related species, herein referred to as *A. flavus* (Diener et al. 1982). The presence of these potent carcinogens in food products has become a worldwide concern, and many governments have reduced allowable tolerances for aflatoxins in peanuts below the U. S. standard of 20 ppb to as low as 5 ppb (K. Cutchins, National Peanut Foundation, personal communication). Aflatoxin contamination in peanuts used for food is suspected at the buying point when grade samples are inspected and found to have visible *A. flavus* on seed (Diener et al. 1982). Peanuts with these fungi are not used as food and feed, even though fungal presence is not always indicative of a toxin problem (Dorner et al. 1989).

The lesser cornstalk borer (LCB), *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), is one of the most devastating insect pests of peanuts in the Southeast (Smith and Barfield 1982), feeding on the root hypocotyl (Mack et al. 1990) and developing pegs and pods (Leuck 1966). Damage to peanut pods

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prior to harvest is associated with this pest (Diener et al. 1982). Lesser cornstalk borers are typically pests during hot and dry weather in peanuts grown in sandy soils (Luginbill and Ainslie 1917, Smith and Barfield 1982). The LCB is well adapted to xeric conditions, experiencing little to no mortality from 24-h exposure to 30°C and 0% RH (Mack and Appel 1986).

Sanders et al. (1985) noted that water deficits during the last 45-50 days before harvest and soil temperatures of 28 to 30.5°C are optimum for the development of *A. flavus* in peanut fields. Invasion of peanut seed by these fungi, and subsequent aflatoxin contamination, is facilitated by hot, dry weather and is greater when pods and seeds are damaged (Diener et al. 1982, Cole et al. 1989). Thus, hot/dry conditions are conducive to the development of both *A. flavus* and LCB. Climatic conditions in the Southeastern U.S., where about 65% of U. S. peanuts are grown, are such that isolated outbreaks of LCB's and limited aflatoxin problems occur yearly. However, region-wide drought occurs about one year in five, resulting in a severe outbreak of the LCB and high aflatoxin contamination in peanuts. Over the past 15 years, outbreaks have occurred in 1980, 1986 and 1990 (Mack et al. 1992). Lesser cornstalk borer has been implicated as a factor in the development of aflatoxigenic fungi in peanuts (Dickens et al. 1973, Widstrom 1979, Sanders et al. 1985, Lynch et al. 1989, Lynch et al. 1990). Lynch and Wilson (1991) recently showed that LCB vectored an *A. parasiticus* color mutant to peanut pods in the laboratory. However, no studies have shown that the LCB transmits any regenerative portion of *A. flavus* (propagules) to peanut pods in the field.

In 1990, an outbreak of LCB occurred, and studies were initiated to determine if LCB larvae could transmit *A. flavus* propagules to peanut pods, and to determine if a relationship exists among larval damage, incidence of *A. flavus* in peanut seed, and aflatoxin contamination.

Materials and Methods

LCB as a Vector for Aflatoxigenic Fungi. On 28 August 1990, 70 larvae were randomly collected from an untreated peanut field at the Wiregrass Experiment Substation in Headland, AL that had an outbreak infestation of LCB. Peanut plants were in the R7 plant growth stage (Boote 1982). Larvae were collected from randomly selected, uprooted plants, placed individually into 59 cc (2 oz) plastic cups filled with artificial diet (Chalfant 1975), and maintained in a laboratory of 23°C and 80% RH. Medium to large larvae from this collection (≥ 6 mm long) were individually placed in 90-mm sterile, empty petri plates for 2 hr, to determine superficial presence of aflatoxigenic fungi. Larvae were then individually moved to rearing cups for 24 hr. The first fecal pellet (frass) produced by each larva, after placement in the sterile petri plate, was collected aseptically using flame-sterilized forceps while working under a laminar flow hood, and placed in a petri plate containing 25 ml of potato dextrose agar to determine if the frass contained *A. flavus* propagules.

Presence of *A. flavus* inside LCB larvae was investigated by collecting 56 larvae on 4 September from the same field, as before. Larvae were surface-sterilized by dipping in ethanol and flaming; larval remains were then macerated and spread onto potato dextrose agar in sterile petri plates. All plates were

incubated for 3 days at 30°C before visual inspection for fungi. *A. flavus* was identified by the distinctive yellow-green color of conidia (G. Morgan-Jones, personal communication). Incubation temperature allowed *Aspergillus* species to out-compete most fungi during the three days of incubation (Semeniuk 1954).

Field Trials. Plant and insect samples were collected in 1990 from unirrigated insecticide trials. All fields had soil characterized as a Dothan sandy loam with pH 6.5 and <1% organic matter. Fields were conventionally-tilled and planted to Florunner peanuts with 0.9-m row spacing at 50 kg seed/ha. Standard weed and disease management recommendations were followed (French et al. 1991).

Field trial I, located at the Wiregrass Substation, was planted 7 May 1990. Treatments, applied at the R2 plant growth stage (52 DAP) were: 1) an untreated control, 2) chlorpyrifos 15G granules at 2.24 kg (AI)/ha, 3) ethoprop 15G at 3.36 kg (AI)/ha, 4) fonophos 10G at 2.24 kg (AI)/ha, 5) Fortress^{R} 10G at 0.6 kg (AI)/ha, and 6) terbufos 15G at 2.24 kg (AI)/ha. All insecticides were applied with a single-row, small-plot granular applicator. Treatments were arranged in a randomized complete block design with four replications. Plots were eight rows wide and 15.2 m long.

Insect populations were sampled weekly beginning ca. 1 wk after planting and ending in early September. Relative abundance of LCB was determined with pitfall traps (Mack and Backman 1990). Two pitfall traps per plot were placed in a central row, shortly after emergence of seedlings, and were monitored weekly. In addition, population densities of larvae and pupae were determined on 15 August and 5 September 1990 by soil sieving (Mack and Backman 1987). One soil sample, 0.9-m-long by 0.3-m-wide by 3-cm-deep, was sieved from each plot. The longitudinal axis of each sample was centered over the row; the sample was passed through a 10-mesh sieve. Plants were removed from the soil at each sieve site and examined for larvae. Larvae found by sieving and plant examination were identified and counted.

Five whole plants were randomly selected and pulled from inner rows of each plot on 15 and 30 August and 13 September 1990 to determine incidence of *A. flavus* on developing pegs and pods (see below, "Fungal Incidence"). Incidence of *Aspergillus niger* Van Theighem was also determined on developing pegs and pods, since it can interfere with aflatoxin production by *A. flavus* (Horn and Wicklow 1983). Plots were dug on 20 September 1990; yield was determined in each plot by harvesting two adjacent central rows that had not been sampled for fungal incidence. Samples of ca. two kg were taken at harvest from each plot for evaluation of seed quality and grade determination.

Field trial II was planted on 23 May 1990 and also located at the Wiregrass Substation. Four treatments were used: an untreated control and chlorpyrifos 15G at 2.24 kg (AI)/ha applied either 30, 45, or 71 days after planting (DAP). Treatments were arranged in a completely randomized design and replicated eight times. A randomly selected 0.9-m section of row was chosen weekly throughout the season and the soil (0.3-m-wide and 3-cm-deep) was sieved for larvae as in Field Trial I. Whole plants were sampled, as before, on 21 August and 4 September 1990 to determine the incidence of *A. flavus* and *A. niger* on pegs and developing pods. Plots were dug on 5 October 1990, yields were determined and 2 kg samples collected from the center two rows of each plot.

Field trial III was planted on 25 April 1990 and located in a production field near Midland City, AL. Seven treatments were used: an untreated control; two treatments with chlorpyrifos 15G at 2.24 kg (AI)/ha, one applied in a 10-cm band over the row (narrow band) and one applied in a 23-cm band over the row (wide band); two treatments of fonophos 10G at 2.24 kg (AI)/ha, narrow and wide band; and two treatments of fonophos at 4.48 kg (AI)/ha, narrow and wide band. Treatments, arranged in a randomized complete block design with four replications, were applied 49 DAP. Density of larvae was not monitored during the growing season. Yields and 2 kg samples were collected, as before.

Field trial IV was planted on 30 April 1990 and located in a production field in Houston Co., AL. Seven treatments were used: an untreated control; three treatments with chlorphyrifos 15G at 2.24 kg (AI)/ha, one applied at flowering (13 June, 44 DAP), another applied at early-pegging (26 June, 57 DAP), and the third applied at late-pegging (23 July, 84 DAP); and three treatments of fonophos 10G at 2.24 kg (AI)/ha, applied at the same dates as the chlorpyrifos. Treatments were arranged in a randomized complete block design with four replications. Density of larvae was not monitored during the growing season. Yields and 2 kg samples were collected, as before.

Fungal Incidence. Incidence of aflatoxigenic fungi during plant development was determined by selecting 10 developing pegs and 10 pods from each field-collected plant from Trials I and II. All pegs and pods found were sampled when fewer than 10 were present. Pegs and pods were removed from plants, surface-sterilized in a 0.525% sodium hypochlorite solution for 1 min, then placed on cotton padding moistened with 20% NaCl solution. *Aspergillus flavus, A. niger* and other fungi that developed from plant parts were identified based on conidial color; incidence of occurrence was recorded after three days incubation in the dark at 30°C, 99% RH.

Yield and Quality. Plants from specific yield rows in each plot were inverted at optimal maturity, combined, bulked by plot and trailer-dried. Yields were recorded for each plot in the field trials. Samples of ca. 2 kg were taken, after drying, from each bulked plot yield for damage ratings and grading (quality evaluation). One hundred whole-pod subsamples were randomly selected from samples from each plot. Subsamples were evaluated for discoloration, visible *A. flavus* and *A. niger* and incidence of: scarification (likely from larvae of the LCB), other hull damage, damage due to LCB larval feeding (silken tube or larvae present on/in pod) and immaturity. A total of 2,400 pods were evaluated in this manner in Trial I; 3,200 pods in Trial II; 2,800 pods in Trial III, and 2,800 pods in Trial IV. A separate 500-g subsample was shelled and graded according to Federal standards (USDA 1990). Percentage sound mature kernels (SMK's) and damaged and discolored kernels (DDK's) were recorded for each sample, along with value per ton. Ten SMK and DDK subsamples were placed on cotton pads moistened with 20% NaCl solution and incubated at 30°C for determination of "hidden" fungi (i.e., fungi that colonized interior tissue). After 3 days, incidence of *A. flavus, A. niger, Penicillium* spp., *Rhizopus* spp., other fungi, and all bacterial colonies growing on seeds were recorded. Aflatoxins B_1,B_2,G_1, and G_2 were extracted from 50-g subsamples of SMK's (from Trial I and four replications in Trial II) with 90 ml acetonitrile and 10 ml of 4% KCl buffer. Aflatoxin levels were quantitated with HPLC by the modified method of Hutchins et al. (1989).

Data Analyses. Levels of individual aflatoxins were summed and transformed to the natural logarithm, ln(ng/g + 1), before analysis. Analyses of variance were performed to determine possible treatment effects on all variables – insect count, incidence of aflatoxigenic fungi, damage ratings, yields and grades. Waller-Duncan k-ratio t tests on treatment means (at $P = 0.10$ and $P = 0.05$) were performed on each of the following variables: mean number of larvae from pitfall traps and soil sieves, percentage of visible damage by LCB larvae, scarification, pods with visible *A. flavus*-type fungi, SMK's, hidden *A. flavus,* yield, value and aflatoxin levels. Due to the critical importance of aflatoxin occurrence, a probability of 0.10 was used. Another reason for the use of 0.10 probability instead of 0.05 was because the consequences of a type I error were not considered critical, as outlined in Little and Hills (1972).

Relationships between pod quality variables, fungal incidence, larval counts and aflatoxin levels were determined through multivariate techniques (SAS Institute, 1987). Principal component analysis was initially performed on data from each field trial for determining relationships among pod quality variables and for reducing the number of variables in regression. Pearson's correlation coefficients were also calculated among all variables (SAS Institute, 1987). Pod quality variables and larval counts were regressed on incidence of *A. flavus* and aflatoxin levels using the GLM procedure in SAS. Toxin levels from Trials I and II were regressed on all pod quality variables and fungal incidence. Type III sums of squares (which represent a variable's contribution to a model after all other variables have been added) were compared in each regression and used to assess the relative contribution to toxin levels of each independent variable.

Results

LCB as a Vector for Aflatoxigenic Fungi. *Aspergillus flavus* was found in or on frass from 28.6% of larvae and on 64.3% of plates on which larvae had been held for 2 hr. In the second collection of larvae, 8.9% of surface-sterilized, macerated larvae yielded *A. flavus*-type fungi.

Field Trials. Ambient temperatures for August 1990 were 0.4°C above normal, and rainfall was 9.6 cm below normal. Lesser cornstalk borer larvae were the dominant soil insects found in pitfall traps and by soil sieving in all field trials. Throughout the 1990 growing season, fewer than 5% of insects trapped in any of the authors' field studies belonged to the wireworm complex or were southern corn rootworms (*Diabrotica undecimpunctata howardi*). The number of LCB larvae increased during the latter part of the growing season in all field trials. For example, the average number of LCB larvae found per soil sieve in Field Trial I were 0.75, 2.00 and 3.00 on 15, 30 August and 13 September, respectively.

In Field Trial I, incidence of *A. flavus*-type fungi on pegs and pods increased ($P < 0.01$) from 30 August to 13 September (Fig. 1A). Aflatoxigenic fungal incidence on pods apparently decreased from 15 August to 30 August, but this may have been a period of time when pods were rapidly forming (Fig. 1A). Prior to harvest, many peanut plants died in this trial, probably due to the severe drought, so that yields were not recorded. However, samples were available for assessment of insect damage, fungal invasion, grade assessment and aflatoxin determination. No differences in number of LCB larvae from pitfall traps, scarification, fungal incidence

or grade (%SMK) were observed among the various insecticide treatments in Field Trial I.

In Field Trial II, pegs and pods had increasing fungal incidence ($P = 0.0001$) from 21 August to 4 September 1990 (Fig. 1B). More *A. flavus* was observed ($P < 0.10$) in pods from untreated plots than from any of the chlorpyrifos-treated plots (Table 1) of this trial. In addition, fewer pods were scarified, grade (% SMK) was better, and yield and value were greater ($P < 0.10$) in plots treated with chlorpyrifos at 45 DAP than other treatment plots (Table 1). Yields and values were better from plots treated with chlorpyrifos at 30, 45 or 71 DAP than from untreated plots.

In Field Trial III, there were no significant differences between treatments in incidence of *A. flavus* (Table 2). Pods from plots treated with the lower rate of fonophos or chlorpyrifos, in either band width, had less ($P < 0.10$) pod scarification than untreated plots. Plots treated with chlorpyrifos, applied in a narrow band (standard grower practice), had higher ($P < 0.10$) yields than untreated plots (Table 2).

In Field Trial IV, seed from plots treated with chlorpyrifos at flowering (44 DAP) or early pegging (57 DAP) had lower ($P < 0.10$) incidence of *A. flavus* than other treatments; pods from the latter treatment also had less scarification than the 57 DAP fonophos treatment (Table 3). Percent SMK, yield and value/ton also tended to be better from the chlorpyrifos treatments than from fonophos treatments or the untreated control (Table 3).

Determination of a Relationship. Principal component analysis indicated that occurrence of discoloration, scarification, holes, and LCB larval feeding damage (silken tube or larvae present) were interrelated in all four data sets. These variables had similar positive loadings in the first principal component, and thus, are considered equal in their contribution to pod quality. This first principal component, representing pod quality, described 35%, 39%, 40% and 37% of the variance of data from Field Trials, I, II, III and IV, respectively; at least twice as much as the second variable created.

In data sets from each of the field trials, the presence of a silken tube or LCB larva was positively correlated to the percentage of pods that were penetrated and scarified (Table 4). Also, incidence of *A. flavus* in pods was positively correlated with percentage of scarified pods (Table 4) in each data set. In Trial I, abundance of LCB larvae from soil sieves was positively correlated to total aflatoxins in samples (Table 4).

Scarification as an independent variable and fungal incidence as a dependent variable were used for developing regression models for all field trials. Individual linear models for each trial were significant ($P \leq 0.04$) and the equation developed from data from all trials indicated a quadratic effect of scarification on incidence of *A. flavus* (Fig. 2).

Type III sums of squares from the multiple regression models describing aflatoxin levels in samples from Trials I and II were used to determine those variables that contributed most to the model. In Trial I, number of LCB larvae from soil sieves, scarification and discoloration contributed 36.5%, 25.1% and 13.5%, respectively, to variation in aflatoxin levels. In Trial II, scarification contributed 72.1%, while discoloration, LCB damage and incidence of *A. flavus* contributed 17.9%, 16.3% and 13.1%, respectively, to aflatoxin levels according to the Type III sums of squares.

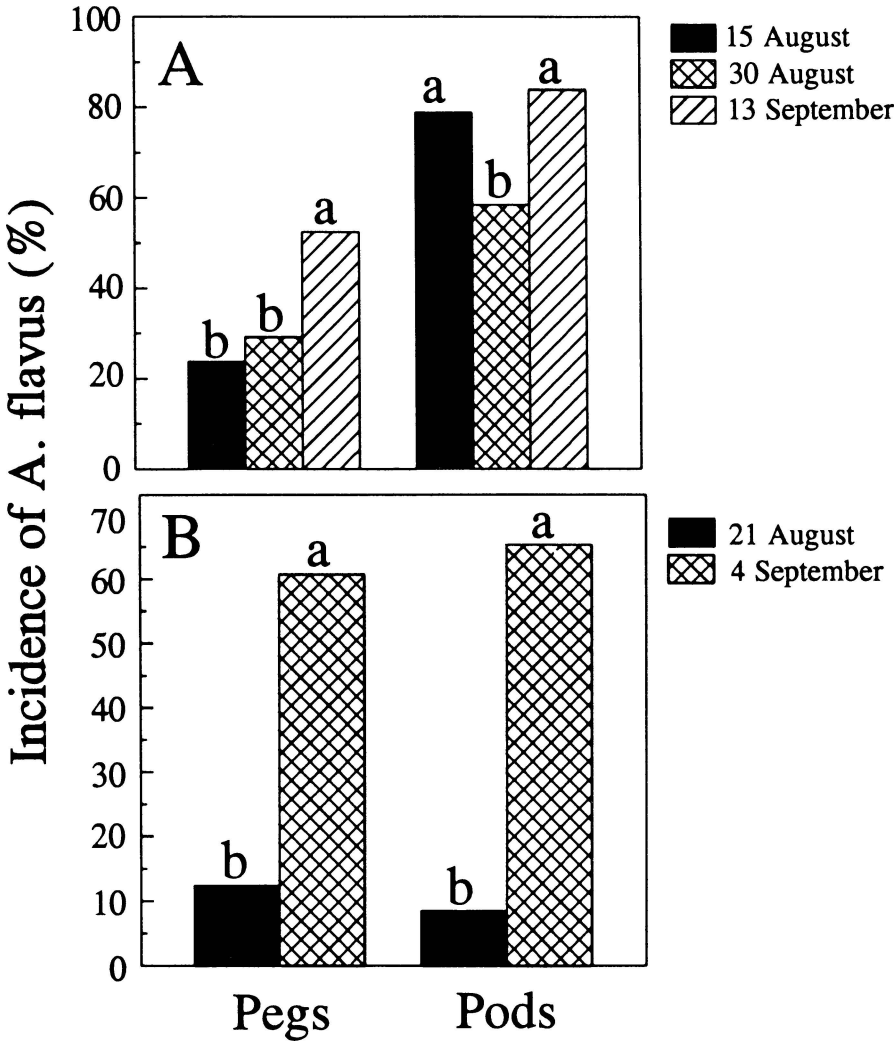

Fig. 1. Incidence of *Aspergillus flavus*-type fungi on developing pegs and pods collected from field trials: A) insecticide Trial I; and B) chlorpyrifos timing Trial II, 1990.

Table 1. Incidence of pod damage and fungal occurrence, yield value per metric ton and total aflatoxin content in peanuts from Trial II, 1990.*

Treatment	Scarification (%)	Observed *A. flavus* (%)	SMK† (%)	Yield (kg/ha)	Value/ Metric Ton
Untreated	43.50 a	11.00 a	68.31 c	795 d	675.26 c
Chlorpyrifos, 30 DAP‡	35.50 ab	6.25 b	70.29 b	1394 b	704.76 b
Chlorpyrifos, 45 DAP	22.50 b	3.63 b	71.50 a	1630 a	724.79 a
Chlorpyrifos, 71 DAP	37.13 a	4.88 b	69.10 bc	1192 c	692.48 b

* Means within each column are not significantly different if followed by the same letters, according to Waller-Duncan k-ratio t test at $P = 0.10$.
† SMK = Sound Mature Kernels.
‡ DAP = Days After Planting.

Discussion

LCB as a Vector for Aflatoxigenic Fungi. Most of the larvae collected on 28 August carried propagules of *A. flavus*-type fungi. This result was expected, since *Aspergillus* species are ubiquitous in soil environments, and it is impossible to prevent infestation of larvae when they are field-collected. More interestingly, *A. flavus*-type fungi were found in or on frass from almost 30% of LCB larvae inspected, and 8.9% of surface-sterilized, macerated larvae contained propagules of *A. flavus*. This evidence supports observations made by Lynch and Wilson (1991), and further substantiates that propagules of *A. flavus* are carried internally by LCB's in the field. Larvae defecate near their food source when they eat, in effect carrying *A. flavus* propagules to feeding sites on pods. Thus, LCB larvae may be passive vectors for aflatoxigenic fungi in the field, which in part explains why insect-damaged peanuts generally have higher aflatoxin levels. Additionally, aflatoxin contamination of peanuts is augmented by insects' injury (Lynch and Wilson 1991) which allow entry of fungal pathogens and soil into pods and seed.

Field Trials. Field Trials II and IV compared the timing of chlorpyrifos applications and had somewhat different results. In Trial II, chlorpyrifos applications were made at vegetative (30 DAP), flowering (45 DAP) and mid-pegging (71 DAP) stages. The application at flowering was best for minimizing scarification and maximizing grade, yield and value. These results, similar to previous observations (Mack et al. 1989), indicate that damaging populations of LCB were most effectively controlled by chlorpyrifos application at flowering. The later application may have allowed higher LCB populations and more damage, and the earlier application did not have long enough residual activity.

In Field Trial IV, chlorpyrifos applications at flowering (44 DAP) and at early-pegging (57 DAP) resulted in lower incidence of *A. flavus* in seed; the 57 DAP application had lower incidence of scarification than other treatments. Applications at late-pegging (84 DAP) had higher grades (% SMK) and values than other treatment plots. Thus, at this location, the latest application of chlorpyrifos might be considered most effective. Differences in results between Field Trials II and IV may have been due to variation in rainfall at the two locations.

Table 2. Incidence of pod damage and fungal occurrence, yield and value per metric ton of peanuts from Trial III, 1990.*

Insecticide Treatment (rate AI)	Scarification (%)	Observed *A. flavus* (%)	SMK† (%)	Yield (kg/ha)	Value/ Metric Ton
Untreated	17.00 a	6.75 a	67.12 a	3260 b	689.99 a
Chlorpyrifos (2.24 kgha) Narrow band	5.00 b	4.00 a	68.15 a	3889 a	697.94 a
Chlorpyrifos (2.24 kgha) Wide band	6.50 b	3.50 a	69.50 a	3841 ab	712.32 a
Fonophos (2.24 kg/ha) Narrow band	7.50 b	4.75 a	68.80 a	3783 ab	702.83 a
Fonophos (2.24 kg/ha) Wide band	8.25 b	4.00 a	68.50 a	3617 ab	699.31 a
Fonophos (4.48 kg/ha) Narrow band	13.00 ab	4.25 a	70.30 a	3525 ab	720.84 a
Fonophos (4.48 kg/ha) Wide band	10.50 ab	3.25 a	68.25 a	3547 ab	699.52 a

* Means within each column are not significantly different if followed by the same letters, according to Waller-Duncan k-ratio t test at $P = 0.10$.
† SMK = Sound Mature Kernels.

Weather conditions influence the development of LCB populations as well as the residual effectiveness of soil insecticides. Activity of granular insecticides degrades in hot dry conditions, so it is critical that applications are properly timed. Growers need to consider residual effectiveness of their insecticide along with weather influences, and not presume that an early application of a soil insecticide will remain effective throughout the growing system. Reduced length of insecticide effectiveness in seasons that are conducive to LCB outbreaks is a primary reason for recommending scouting for LCB damage prior to insecticide application (French et al. 1991).

In Field Trials III and IV, the insecticide chlorpyrifos was better than fonophos in reducing pod damage and fungal incidence, and increasing yields. Chlorpyrifos has longer residual effectiveness (Mack et al. 1991) for controlling soil insects such as LCB, and minimizes cumulative LCB damage that would increase aflatoxigenic fungal infection. These results, similar at two locations, demonstrate the possibility of treating for insects and reducing aflatoxin problems while optimizing yields. This is extremely important because of the reduction in allowable tolerances of aflatoxins in peanuts.

Table 3. Incidence of pod damage and fungal occurrence, yield and value per ton of peanuts from Trial IV, 1990.

Treatment Insecticide*	Timing†	Scarification (%)	Observed *A. flavus* (%)	SMK‡ (%)	Yield (kg/ha)	Value/ Metric Ton
Untreated		35.25 a£	7.00 b	70.90 ab	1812 a	718.73 abc
Chlorpyrifos	44 DAP	23.25 a	3.50 a	71.92 a	2245 a	730.40 ab
	57 DAP	18.75 a	3.50 a	71.85 ab	2097 a	730.79 ab
	84 DAP	26.00 a	6.20 ab	72.46 a	2132 a	737.24 a
Fonophos	44 DAP	31.00 a	9.75 c	70.65 ab	2089 a	717.72 abc
	57 DAP	38.75 a	8.50 bc	69.47 b	2303 a	694.51 c
	84 DAP	28.67 a	6.67 b	70.13 ab	1631 a	701.49 bc

* Chlorpyrifos 15G applied at 2.24 kg (AI)/A; Fonophos 10G applied at 2.24 kg (AI)/A.
† DAP = days after planting.
‡ SMK = Sound Mature Kernels.
£ Means within each column are not significantly different if followed by the same letters, according to Waller-Duncan k-ratio t test at $P = 0.05$.

Decreased incidence of aflatoxigenic fungi in peanut seed may also be due to fungistatic activity of the insecticides used in this study. Chlorpyrifos, ethoprop and fonophos have all shown fungistatic activity (Backman and Hammond 1981, Csinos 1985) and are recommended for control of the fungus, *Sclerotium rolfsii* (Basidiomycetes: Hymenomycetes) (French et al. 1991). Chlorpyrifos, at least in an emulsifiable formulation, inhibits *A. flavus* at 1µg (AI)/ml agar (KLB, unpublished data), but the granular formulation used in our studies may not provide the same level of fungistasis (Backman and Hammond 1981). Regardless of the fungistatic activity of these insecticides, pod damage by LCB would eventually allow entry of aflatoxigenic fungi into seeds after the insecticides degraded.

Relationships Between LCB Damage and Aflatoxigenic Fungal Infection. Scarification of pods in all four trials was positively correlated to contamination of pods or seeds with *A. flavus* and to LCB damage. No evidence of nematode damage was observed on pods, and no other subterranean insect pests of peanuts were found in these fields. Soil moisture in August and September 1990 was unfavorable for many soil pests, so scarification observed in pods was caused by LCB larvae. These results, similar to observations made by Lynch and Wilson (1991), correlate injury from LCB larvae to contamination by *A. flavus* which is a primary determinant of aflatoxin contamination. In addition, scarification was found to contribute to aflatoxin levels in two trials. We used scarification for exploring relationships among variables describing pod damage, and since these variables were interrelated, regression models developed for scarification should reflect relationships with other variables.

Regression of data from all trials indicated a quadratic relationship between scarification and *A. flavus* contamination. According to this equation, fewer

Table 4. Correlation coefficients among selected variables describing incidence of peanut pod damage due to lesser cornstalk borer, aflatoxigenic fungal incidence, aflatoxin levels and numbers of lesser cornstalk borer larvae from four field trials, 1990.*

Variable	with	Trial I	Trial II	Trial III	Trial IV
LCB Larva/ Silken Tube	Pod Penetration	0.92***	0.64***	0.77***	0.88***
	Scarification	0.56**	0.34*	0.33**	0.67***
A. flavus	Pod Penetration	0.19	0.64***	0.17	0.53***
	Scarification	0.43**	0.37**	0.66***	0.56***
	LCB Larva/Silken Tube	0.16	0.13	0.14	0.52***
	No. Larvae from Soil Sieves	0.33	-0.51	n.d.†	n.d.
Toxin Levels	Scarification	0.33	-0.34	n.d.	n.d.
	Pod Penetration	-0.00	-0.02	n.d.	n.d.
	No. Larvae from Soil Sieves	0.50***	-0.21	n.d.	n.d.

* Astericks indicate level of signficance of correlation coefficients: *, $P < 0.10$; **, $P < 0.05$; and ***, $P < 0.01$.
† n.d. indicates data insufficient to calculate correlation coefficients.

than 8% of pods will be contaminated with *A. flavus*-type fungi when less than 30% are scarified. Incidence of *A. flavus* in peanut samples indicates a potential for aflatoxin contamination (Blankenship et al. 1984, Lynch and Wilson 1991); and we now have a relationship between scarification (thus, LCB damage) and incidence of *A. flavus* in peanut seed. Shellers can use these relationships to help determine which peanut lots need to be sampled more closely for determination of aflatoxin contamination.

Implications for Peanut Pest Management. Both *A. flavus* and the LCB are economic pests of peanuts, so linking damage from one of these pests to increases in infection from the other is important. An economic injury level of 3.63 to 5.44 larvae per row-meter was recently developed for the LCB (Mack et al. 1988), but no synergism with other pests was assumed. Several Cooperative Extension Services in the Southeast recommend insecticide treatment of peanuts based on a predetermined percentage of insect-infested sampling sites, and may or may not include a component for disease augmentation. LCB treatment thresholds should be reconsidered because of the link between *A. flavus* and the LCB. Further, *A. flavus* may not be the only disease that the LCB augments. Experiments characterizing LCB injury to the root-hypocotyl region of peanut plants suggest that damage to the periderm in this region may augment diseases such as white mold, *S. rolfsii* (Mack et al. 1990). Disease-augmentation by LCB larvae may explain why yield increases have been reported from the application of insecticides when low densities of the LCB were present (e.g., Gilreath et al. 1989).

Growers now use one application of an insecticide during the growing season for the LCB in population outbreak years. In outbreak years, two applications of an insecticide might be required, since none of the current insecticides can protect pods from the time of blossom until harvest (Mack et al. 1989). One of these applications might be made at flowering, which yielded best and produced

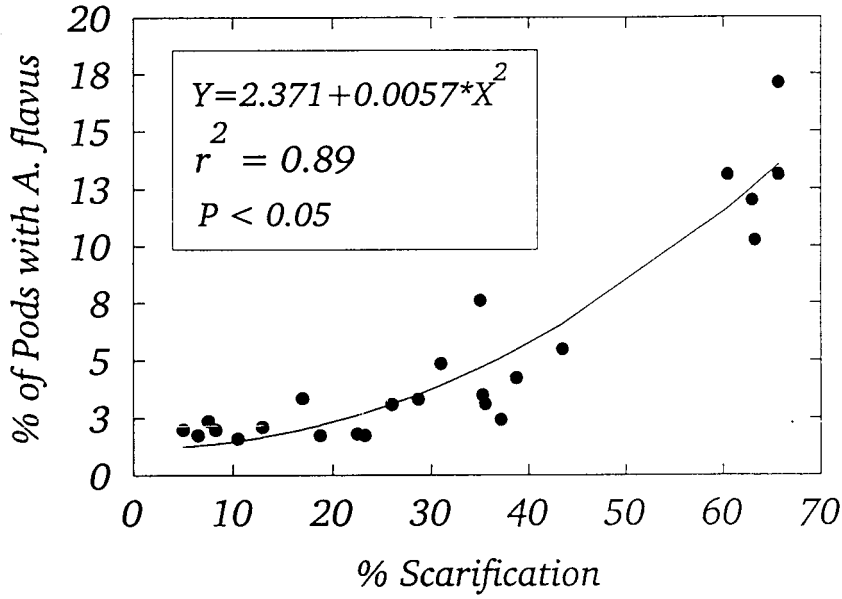

Fig. 2. Relationship between percentage of scarification and incidence of aflatoxigenic fungi in pods from harvest samples from four field trials in 1990. Lines represents predicted values from the regression model: *Afl* = 2.37 + 0.0057 (% Scar^2), r^2 = 0.89.

the least contamination from *A. flavus* in one trial in this study; a second application could be made later in the season to reduce the abundance of the LCB. Further studies are needed to verify this.

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