# Effect of Chemical and Biological Agents on the Incidence of *Aspergillus flavus* and Aflatoxin Contamination of Peanut Seed

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## ABSTRACT

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Chemical and biological soil amendments were investigated for effects on seed colonization by fungi of the *Aspergillus flavus* group (*A. flavus* and *A. parasiticus*) and, in certain instances, on aflatoxin contamination of one or more genotypes of peanut (*Arachis hypogaea*). During 1976, 1977, 1981, and 1982, granular and/or liquid pesticide formulations and *Trichoderma harzianum* were applied either as soil amendments or as postharvest liquid applications to peanut pods. In 1981 and 1982, soil treatments also were applied to plots treated with surface applications of 0, 673, and 1,345 kg of gypsum per hectare. Peanut genotypes grown with chemicals applied to either the soil (both alone and in certain combinations) or to the pods of these genotypes varied in colonization of the seed by the *A. flavus* group of fungi. Gypsum applications reduced the percentage of seed colonized by these fungi. Applications of gypsum also enhanced the control of seed colonization in plots treated with *T. harzianum*, PCNB-fensulfothion, or CGA 64250, but treatment and genotype interactions were noted. No aflatoxin was detected in peanuts harvested from gypsum-treated plots, but it was occasionally found in peanuts from the non-gypsum treatments resulting in a highly significant treatment × genotype interaction.

*Additional key words*: biological toxins, pest resistance, seed contamination.

Aflatoxins, which are metabolites produced by many strains of the *Aspergillus flavus* group (*A. flavus* Link and *A. parasiticus* Speare) of fungi under certain environmental conditions, are highly toxic and are potent carcinogens (4,5,7,12,15). Aflatoxin may be produced in many food and feed products after plants are infected by species of the *A. flavus* group. Prevention of aflatoxin contamination in peanut seed is of vital concern to all segments of the peanut industry. Some production practices effectively reduce the incidence of aflatoxin in peanuts. These include: rotation with nonlegume crops (18) or planting on fallowed land (11), deep burial of surface litter (2), avoidance of drought stress (20), and proper harvesting and curing (19,21).

Results of previous studies (27) indicated that increased levels of calcium from gypsum applied over the row may reduce aflatoxin contamination of peanuts. Other workers (1,10,13,19,26) have applied chemical treatments during the curing and drying process in attempts to control invasion of peanut pods by members of the *A. flavus* group. Bell and Doupnik (1) applied 27 fungicides to peanut plots after digging the plants. Twenty of these fungicides decreased subsequent aflatoxin concentration in seed samples, but in many instances aflatoxin concentrations were not related to the prevalence of colonies of *A. flavus*. Attempts by Jackson (10) to reduce *A. flavus* on pod surfaces with fungicides had little success. Pettit et al (20) reported that six soil and five foliage fungicides were ineffective in controlling *A. flavus* and aflatoxin contamination of seed. Madaan and Chohan (13) treated peanut pods inverted in the windrow with 13 fungicides and acidic-type chemicals. They found that separate treatments with 5.0% proponic acid, 0.1% sorbic acid, and 0.15% chlorothalanil prevented the invasion of pods by *A. flavus* and aflatoxin development in the peanut seed during drying for 12 days in field windrows. Although no known practical chemical control of *A. flavus* and aflatoxin formation has been developed (4), further evaluation of chemical treatments in certain management systems appears to be desirable.

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Peanut germ plasm lines have been identified that develop 22–38% less seed colonization by *A. flavus* in laboratory evaluation (16). All of the cultivars grown commercially are susceptible to seed colonization by *A. flavus*.

The application of supplemental calcium in the form of highly water-soluble gypsum ($CaSO_4$) to the soil in the pegging zone of peanut plants is recommended to increase the seed quality of large-seeded Virginia-type peanuts (24). However, gypsum applications are not a standard recommendation for small-seeded runner-type peanuts grown extensively in the peanut-growing area of the United States. Gypsum applications have also been effective in reducing peanut pod-rot complex caused by species of *Fusarium*, *Pythium*, and *Rhizoctonia* (3).

## MATERIALS AND METHODS

A series of experiments were conducted in 1976, 1977, 1981, and 1982 on Tifton loamy sand soil near Tifton, GA, to study the influence of peanut (*Arachis hypogaea* L.) genotypes varying in resistance to seed invasion by *A. flavus* fungi, chemical applications, and soil management procedures on the infection of peanut seed by fungi of the *A. flavus* group. Peanut genotypes were Starr and peanut accessions PI 337409 and PI 331326 in 1976 and 1977, Florunner and Sunbelt Runner in 1981, and Florunner alone in 1982. Data were compared by analysis of variance and Duncan's multiple range test (22).

**Experiment 1.** In 1976, this experiment was conducted to evaluate the effects of applying chemicals to soil during the peanut-growing season on infection by *A. flavus* fungi and subsequent aflatoxin contamination of peanut seed. Previously, PI 337409 was found resistant and PI 331326 highly susceptible to invasion by aflatoxin-producing strains of the *A. flavus* group after inoculation (14,17). Cultivar Starr was included as a standard control cultivar. The test was planted 6 May in a split-plot, randomized block field experiment replicated five times. Whole plots were comprised of genotypes, and pesticides were applied as soil treatments in subplots. Chemicals were applied to the surface of the soil and seeds of each genotype were planted ~10 cm apart in the drill of two rows spaced 91.4 cm apart and 6.1 m long. Granular (in diatomaceous earth) pesticides were applied to the soil surface 53 days after planting to a 30-cm wide band over the row in their respective plots

and were replicated five times. Treatments were: PCNB [pentachloronitrobenzene] (11.2 kg/ha); ethoprop [*O*-ethyl *S,S*-dipropyl phosphorodithioate] (3.4 kg/ha); carbofuran [2,3-dihydro-2,2-dimethyl-7 benzofuranyl methylcarbonate] (3.4 kg/ha); PCNB and ethoprop (11.2 and 3.4 kg/ha); PCNB and carbofuran (11.2 and 3.4 kg/ha); PCNB, ethoprop plus carbofuran (11.2, 1.0, and 3.4 kg/ha), and no soil treatment. Treatment rates are expressed as active ingredients from commercial formulations of 10% in granules of diatomaceous earth, except where PCNB and ethoprop were applied together as a 10 and 3% combination. The test was mechanically dug 126 days (9 September) after planting. Green pod samples were hand picked, placed in open mesh bags, and dried for 7 days at 35 C in a forced-draft oven. Pods were stored at ambient temperature for ~30 days and hand shelled. Two 20-g samples of sound seed were processed according to the previously described laboratory procedure (without inoculation with *A. parasiticus*) (14), and placed in a humid chamber for 7 days at 28 C. This process is referred to as the *Aspergillus* "seed plate" method. The percentage of seed with visible conidia of the *A. flavus* group species was recorded.

**Experiment 2.** In 1977, this experiment was conducted to evaluate the effects of additional fungicides as soil applications during the growing season on the incidence of infection of peanut seed by *A. flavus*. The test was planted on 25 April in a split-split-plot randomized block with four replications. The same genotypes (PI 337409, PI 331326, and Starr) as in experiment 1 were used as whole plots. The subplots were half the length (91.4 cm × 3 m) of the 6.1 row and the sub-subplots were one fourth (91.4 cm × 1.5 m) of the row. At 102 days after planting, the sub-subplot treatments were applied with a sprinkler can as a drench in 4 L of water on a 30-cm-wide area of the row. The treatments were: benomyl [methyl 1-(butyl-carbamoyl)-2-benzimidazolecarbamate] (286 g/ha); carboxin [5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide] (3.4 kg/ha); captafol [*cis*-*N*(1,1,2,2-tetrachloroethyl thio) 4-cyclohexene-1,2-dicarboximide] 5 kg/ha; and none. Overhead irrigation water (2.5 cm) was applied 24 hr after the sub-subplot treatments. The subplots were infested with either cracked-corn medium containing inoculum of *A. parasiticus* (NRRL 2999) or cracked-corn medium with no inoculum 12 days after sub-subplot treatments were applied. The medium consisted of (by weight) milled-cracked corn (9.6%), air-dry Tifton loamy sand soil screened through a 2-mm-mesh sieve (70.4%), and tap water (20%). The mixture was autoclaved in 5-kg units in aluminum foil-covered greenhouse flats for 60 min at 1.1 kg/cm$^2$ of pressure on two successive days. After cooling, three colonies of *A. parasiticus* grown for 2 wk on Czapek's medium in 100 × 15-mm petri plates were thoroughly mixed into the soil in half of the flats each containing 5 kg of medium. The infested flats were incubated in a humid chamber (98% ± RH) for 4 days at 27 C. Just before application, each flat of the infested and noninfested media was screened through a 1-cm-mesh sieve, diluted with 1 kg of sterile cracked corn and 1 kg of sterile granules of diatomaceous earth (Celatom MP 78S, Eagle-Picher Industries, Cincinnati, OH). The field soil of respective subplots was treated 114 days after planting by manually distributing 500 g of the infested or uninfested media over a 30-cm-wide area of each plot row. The test was dug 126 days after planting with a mechanical peanut digger-shaker-inverter. Pods, air-dried in the field, were hand picked and stored at room temperature. On 12 October, pods were hand shelled and two 20-g samples of sound seed were assayed by using the seed plate method and incubated in a humid chamber for 7 days at 27 C. The percentage of seed colonized by *Aspergillus* spp. was recorded.

**Experiment 3.** This test was conducted in 1977 in a split-split-plot design with the same cultivars and fungicides used in the 1977 soil treatment study, but the chemical treatments were applied at harvest time to the peanut pods. After the plants were dug and inverted in each plot (126 days after planting), the pod portion of the plants of each sub-subplot was washed by immersing several times in a 112-L plastic container filled with water. Water was changed after washing all plants of respective plots in each of the four blocks. Then, the pod portion of plants from the sub-subplots was washed in treatment solutions of benomyl (6.3 mg/L), carboxin (62.5 mg/L), captafol (46.9 mg/L), and water. Plants were replaced in an inverted position on the plot area to air-dry in the field. After 24 hr, pods were inoculated with *A. parasiticus*. Inoculum was prepared by blending mycelial mats from three 15-day cultures grown on 100-mm-diameter plates in 4 L of distilled water, and it was applied with a sprinkling can.

The inoculated, field air-dried pods were hand-picked ~10 days after digging and stored at room temperature. After ~3 mo, representative samples of hand-shelled seed were assayed for seed colonization by *Aspergillus* spp. by using the seed plate method. From the remaining seed, 150 representative sound-mature seeds were plated on a microbial medium and incubated for 5–8 days. This medium contained (per liter): $KH_2PO_4$, 0.5 g; $K_2HPO_4$, 0.5 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; $NH_4NO_3$, 0.5 g; Bacto peptone, 1.0 g; yeast extract, 1.0 g; phytone, 1.0 g; malt extract, 1.0 g; sucrose, 10.0 g; glucose, 10.0 g; agar, 17.0 g; rose bengal, 25.0 mg, and demineralized water, 955 ml. The sugars were added to the hot medium after steam autoclaving.

The number of fungal colonies of the *A. flavus* group and other fungi were recorded. This method is referred to as the "microbial seed medium" method.

**Experiment 4.** In 1981, this experiment was conducted to evaluate the effects of soil amendments with supplemental gypsum and repeated soil applications of *Trichoderma harzianum* Rafai, a commercial pesticide formulation (PCNB plus fensulfothion), and an experimental pesticide (CGA 64250). The study was planted 15 April 1981. For each cultivar, two treatments of 673 or 1,345 kg/ha of gypsum were applied to subplots. A nontreated control was included. The gypsum was applied to a 30-cm-wide area over the rows 48 days after planting. Other treatments were applied to the soil in repeated applications 85 and 106 days after planting. Treatments were: a mixture containing *T. harzianum* (151 kg/ha); PCNB (10%) and fensulfothion (*O,O*-diethyl *O*-[4-(methylsulfinyl) phenyl] phosphorothioate) (3%) commercial granular formation (11.2 and 3.4 kg/ha, respectively); CGA 64250 (Ciba-Geigy, 2.5% granular formulation of the experimental material) 34 kg/ha of the commercial formulation; and a nontreated control. The inoculum mixture containing *T. harzianum* was prepared by mixing three mycelial mats of the fungus with a milled wheat middling-soil medium in the same manner as the medium for *A. parasiticus* was prepared for the 1977 soil treatments study (experiment 2). Dilution and application of the *Trichoderma* to the treated area were the same. One week following the four soil treatments, inoculum of *A. parasiticus* (NRRL 2999) was prepared in the same manner and applied to the entire test at 392 kg/ha in a 30-cm-wide area over the peanut rows. Plots were mechanically dug and the plants were inverted 3 and 14 September and air-dried in the field. Pod samples were hand-picked and stored at room temperature. In December, pod samples were hand-shelled and assayed by using the seed plate method. Pod samples (500 g) were taken from each plot for determining the percentage visible damage of sound mature seed according to the standard procedure of the Federal-State Inspection Service for peanut market grade (25). Aflatoxin analyses were run on 50-g subsamples by using the high-pressure liquid chromatography (HPLC) method developed by Thean et al (23).

**Experiment 5.** In 1982, experiment 4 was repeated but only cultivar Florunner was used in this field test. The test was planted 6 May and dug 15 September 1982. Gypsum treatments were applied 35 days after planting, and soil amendments were applied repeatedly at 90 and 110 days after planting. Pod samples were collected, stored at ambient room temperature until January 1983, and processed as in experiment 4.

## RESULTS

**Experiment 1.** The percentage of seed colonized by fungi of the *A. flavus* group was least with PI 337409, intermediate with PI 331326, and greatest on Starr when averaged over all treatments (Table 1). Two of the soil chemical treatments (PCNB plus ethoprop and PCNB plus carbofuran) significantly reduced the percentage of seed colonized in comparison with the nonchemical

treated control in all genotypes. For PI 331326, ethoprop alone also reduced the percentage of seed colonized.

**Experiment 2.** The results are given in Table 2 (soil treatment). Fewer PI 337409 seed were colonized by fungi of the *A. flavus* group than seed of Starr or PI 331326. Carboxin gave less colonized seed of PI 337409 than occurred with benomyl or in the nontreated control (Table 2). Seed colonization in PI 331326 was less with the carboxin treatment than with benomyl, captafol and in the nontreated controls. There were no significant differences between treatments with Starr. These treatment variations resulted in a significant ($P = 0.05$) genotype × treatment interaction.

**Experiment 3.** In 1977, seed samples from the three genotypes obtained from plots treated by dipping the pods into chemical solutions revealed that the genotype PI 337409 had fewer seed colonized by *A. flavus* fungi than Starr or PI 331326 (Table 2, pod treatment). The benomyl and carboxin treatments resulted in fewer seed of PI 337409 colonized, and the benomyl, carboxin, and captafol treatments resulted in fewer Starr seed colonized than the nontreated controls. There was a significant ($P = 0.01$) interaction between genotypes and pod treatments. No differences were evident for chemical treatments with PI 331326.

With the microbial seed medium assay, PI 337409 and Starr produced fewer colonies of the *A. flavus* group than PI 331326 (Table 2). Furthermore, the seed from treated pods of PI 337409 were colonized less by *A. flavus* fungi in each of the three chemical treatments than was the nontreated control.

*Fusarium* spp. were less frequent on seed from PI 337409 than on Starr or PI 331326 seed (Table 2). For the PI 337409 and Starr, captafol-treated pods had fewer colonies of *Fusarium* on the seed than those treated with benomyl or carboxin or the nontreated control. Starr treated with benomyl had more colonies of *Fusarium* on the seed than the nontreated control. A significant ($P = 0.01$) genotype × pod-treatment interaction was evident for the colonies of *Fusarium*.

Fewer colonies of other fungi occurred on seed of PI 337409 and Starr than on PI 331326 (Table 2). Fewer fungi were present on PI 337409 seed from pods treated with benomyl than with captafol or from the nontreated control. Starr seed contained fewer other fungi when pods were treated with benomyl than with carboxin, captafol, or in the control treatments. No treatment effect was evident for PI 331326.

Numbers of bacterial colonies did not differ for the chemical treatments on any genotype. However, PI 337409 and Starr had significantly ($P = 0.05$) fewer colonies than did PI 331326.

**Experiments 4 and 5.** The results of two gypsum and three soil treatments in 1981 and 1982 are given in Tables 3 and 4. In 1981, the 673 kg/ha application of gypsum resulted in less colonization of postharvest Florunner and Sunbelt Runner seed samples by *A. flavus* fungi than the nontreated control (Table 3). Also, the 1,345 kg/ha application gave a further reduction. In 1982, either of the two gypsum rates applied 35 days after planting to the cultivar Florunner reduced colonies of *A. flavus* when compared to the check (Table 4).

In 1981 and 1982, fewer *A. flavus*-type colonies developed on peanut seed when soil applications of *Trichoderma*, PCNB-fensulfothion, and the CGA 64250 were used with one or both levels of gypsum as compared to treatments of gypsum alone. In 1982, however, the pesticide treatments had no effect on soil amended with 1,345 kg/ha of gypsum. There was a highly

TABLE 1. Percentage of peanut seed colonized by fungi of the *Aspergillus flavus* group with and without soil-applied pesticides in 1976

| Treatment[y] | Genotype and percentage of seeds colonized[z] | | |
|---|---|---|---|
| | PI 337409 | Starr | PI 331326 |
| PCNB | 4.6 ab | 8.8 ab | 9.4 ab |
| Ethoprop (E) | 3.8 ab | 7.6 ab | 7.4 b |
| Carbofuran (C) | 3.6 ab | 8.8 ab | 9.4 ab |
| PCNB + E | 0.8 b | 5.4 b | 9.0 b |
| PCNB + C | 1.6 b | 5.6 b | 6.6 b |
| PCNB + E + C | 7.4 a | 8.0 ab | 9.8 ab |
| Pesticide (control) | 7.8 a | 10.6 a | 13.4 a |
| Mean | 4.2 | 9.3 | 7.8 |

[y] Actual ingredients of PCNB, ethoprop (E), carbofuran (C), PCNB + E, PCNB + C, PCNB + E + C were 11.2, 3.4, 3.4, 11.2 + 1.0, 11.2 + 3.4, and 11.2 + 1.0 + 3.4 kg/ha, respectively. Treatments were applied on a 30-cm-wide area over peanut rows 53 days after planting.

[z] Percentage based on average of two 20-g samples of seeds assayed for each genotype and treatment. Treatment means within a column with same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Genotype means over all treatments are also significantly different ($P = 0.05$).

TABLE 2. Microbial colonization of seed of three peanut genotypes following treatment of soil or pods with three fungicides in 1977[v]

| Genotype | Treatment[w] | Soil treatment | Pod treatment | | | |
|---|---|---|---|---|---|---|
| | | *A. flavus*[x] | *A. flavus*[x] | *A. flavus*[y] | *Fusarium* spp.[z] | Other fungi[z] |
| PI 337409 | Benomyl | 15.7 a[z] | 6.3 b | 0.0 b | 47.5 a | 20.0 c |
| | Carboxin | 10.0 b | 4.3 b | 0.3 b | 52.4 a | 24.0 bc |
| | Captafol | 11.1 ab | 6.5 ab | 0.3 b | 16.3 b | 32.0 a |
| | Control | 16.0 a | 9.0 a | 1.4 a | 37.3 a | 27.7 ab |
| | Mean | 13.2 | 6.5 | 0.5 | 37.9 | 25.9 |
| Starr | Benomyl | 21.0 a | 12.5 b | 4.1 a | 97.1 a | 23.4 c |
| | Carboxin | 18.7 a | 10.1 c | 1.7 a | 55.6 b | 37.3 b |
| | Captafol | 26.3 a | 4.7 d | 4.7 a | 37.3 b | 49.5 a |
| | Control | 21.1 a | 20.1 a | 1.7 a | 44.5 b | 26.0 b |
| | Mean | 21.8 | 12.6 | 3.1 | 58.7 | 36.5 |
| PI 331326 | Benomyl | 30.2 a | 10.1 a | 10.6 a | 70.3 a | 63.3 a |
| | Carboxin | 17.2 b | 12.3 a | 7.7 a | 59.4 ab | 74.3 a |
| | Captafol | 26.7 a | 10.7 a | 3.6 a | 34.4 c | 75.5 a |
| | Control | 30.6 a | 14.0 a | 5.1 a | 47.5 bc | 81.8 a |
| | Mean | 26.2 | 11.8 | 6.8 | 52.9 | 73.8 |

[v] Means with same letter within a column for each genotype are not significantly different at $P = 0.05$ according to Duncan's multiple range test (DMRT).

[w] Actual ingredients of benomyl, carboxin, and captafol were 286 g, 3.4 kg, and 5.0 kg, respectively, per hectare. Control was not treated.

[x] Percentage of seed colonized from four replications of duplicate 20-g sample averages as determined by seed-plate method revealed a significant ($P = 0.05$) genotypes × treatment interaction and significant differences between the mean for PI 337409 and those for Starr or PI 331326 means at $P = 0.05$ according to DMRT.

[y] Number of colonies from four replications of 150 seeds each as determined by the seed-medium method revealed significant differences between means for PI 337409 or Starr compared to PI 331326 mean at $P = 0.05$ according to DMRT.

[z] Number of colonies from four replications of 150 seeds each as determined by the seed-medium method revealed a significant ($P = 0.05$) genotype × treatment interaction and significant differences between the mean for PI 337409 and those for Starr or PI 331326 at $P = 0.05$ according to DMRT.

TABLE 3. Colonization by *Aspergillus* and alfatoxin contamination of seed of two peanut cultivars grown on soil with and without gypsum and chemical and biological treatments in 1981

| Treatment[w] | Florunner | | Sunbelt | |
|---|---|---|---|---|
| | *A. flavus*[x] | Aflatoxin[y] | *A. flavus*[x] | Aflatoxin[y] |
| No gypsum | | | | |
| *Trichoderma* | 8.2 a[z] | 0.0 | 6.2 a | 433 |
| PCNB-fensulfothion | 7.8 ab | 0.0 | 7.4 a | 0 |
| CGA 64250 | 4.2 c | 0.0 | 2.4 c | 0 |
| Control | 6.6 b | 0.0 | 4.2 b | 4,105 |
| Mean | 6.7 | 0.0 | 5.0 | 1.135 |
| Gypsum (673 kg/ha) | | | | |
| *Trichoderma* | 4.4 b | 0.0 | 3.8 b | 0 |
| PCNB-fensulfothion | 3.8 b | 0.0 | 3.8 b | 0 |
| CGA 64250 | 1.6 c | 0.0 | 2.8 b | 0 |
| Control | 7.6 a | 0.0 | 6.0 a | 0 |
| Mean | 4.3 | 0.0 | 4.1 | 0 |
| Gypsum (1,345 kg/ha) | | | | |
| *Trichoderma* | 3.2 b | 0.0 | 3.0 b | 0 |
| PCNB-fensulfothion | 3.0 b | 0.0 | 3.2 b | 0 |
| CGA 64250 | 0.8 c | 0.0 | 2.4 b | 0 |
| Control | 4.8 a | 0.0 | 4.6 a | 0 |
| Mean | 2.9 | 0.0 | 3.3 | 0 |

[w] *Trichoderma* medium applied at 151 kg/ha. PCNB (10%) and fensulfothion (3%) granules, and CGA 64250 (2.5%) Ciba Geigy experimental granules applied at rate of 123 and 34 kg/ha, respectively.
[x] Percentage of seed colonized from four replications of duplicate 20-g sample averages as determined by seed plate method revealed a significant interaction for cultivars × gypsum rate and gypsum rate × soil treatment at $P = 0.01$. Interactions for cultivars × soil treatment and differences between means of gypsum rates were significant at $P = 0.05$.
[y] Total aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$ (ng/g).
[z] Means within a column for each gypsum level with same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

TABLE 4. Colonization by *Aspergillus* and aflatoxin contamination of seed of Florunner peanuts grown on soil with and without gypsum and chemical and biological treatments in 1982

| Treatments[w] | *A. flavus*[x] | Aflatoxin[y] |
|---|---|---|
| No gypsum | | |
| *Trichoderma* | 5.8 a[z] | 0.0 |
| PCNB-fensulfothion | 7.8 a | 0.0 |
| CGA 64250 | 5.0 a | 0.0 |
| Control | 7.2 a | 5.0 |
| Mean | 6.5 | 1.3 |
| Gypsum (673 kg/ha) | | |
| *Trichoderma* | 2.8 b | 0.0 |
| PCNB-fensulfothion | 4.8 ab | 0.0 |
| CGA 64250 | 2.5 b | 0.0 |
| Control | 8.0 a | 0.0 |
| Mean | 4.5 | 0.0 |
| Gypsum (1,345 kg/ha) | | |
| *Trichoderma* | 2.7 a | 0.0 |
| PCNB-fensulfothion | 3.0 a | 0.0 |
| CGA 64250 | 2.7 a | 0.0 |
| Control | 4.0 a | 0.0 |
| Mean | 3.1 | 0.0 |

[w] Inoculum of *Trichoderma* was applied at 151 kg/ha. PCNB (10%) fensulfothion (3%) granules applied at 123 kg/ha commercial formulation, and CGA 64250 (2.5%) Ciba Geigy experimental granules applied at rate of 34 kg/ha commercial formulation.
[x] Percentage of seed colonized from four replications of duplicate 20-g sample averages as determined by the seed plate method. No-gypsum treatment mean is significantly different compared to gypsum treatments at $P = 0.05$ according to Duncan's multiple range test (DMRT).
[y] Total aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$ (ng/g).
[z] Means within a column for each gypsum level with same letter are not significantly different ($P = 0.05$) according to DMRT.

significant interaction ($P = 0.01$) of genotype × gypsum rate and of gypsum rate × soil treatment ($P = 0.01$) in 1981 for seed colonization by *A. flavus*. Genotype by soil treatment interaction was significant at $P = 0.05$. Total aflatoxin determinations revealed no aflatoxin on the seed from the gypsum-treated soil, but seed from plots without gypsum, *Trichoderma*, or pesticides contained detectable amounts of aflatoxin.

## DISCUSSION

These studies verified that the PI 337409 peanut genotype is partially resistant to seed infection by *A. flavus* as previously reported (14,17); it was colonized less than the Starr or PI 331326 genotypes (Tables 1 and 2). The incidence of seed colonization was greater in 1977 than in 1976. Apparently environmental conditions were more favorable for invasion of the peanut pods by the *A. flavus* group before digging the plants in 1977. Other workers have reported greater aflatoxin contamination of peanut seed when plants were exposed to drought stress and higher soil temperature (9), which was the case in 1977 as compared to 1976.

In 1976, the application of PCNB plus ethoprop or carbofuran, and in 1977, carboxin, reduced seed colonization by the *A. flavus* group of fungi. Colonization was the least when these chemicals were applied to plots with the resistant PI 337409 genotype. The results of chemical treatments of soil or pods, especially in 1977, were not considered effective enough in reducing the *A. flavus* group of fungi to be of economic importance to growers. Further investigation of treatments or treatment combinations is needed.

In 1981 and 1982, the chemical CGA 64250 and *T. harzianum* were more effective in reducing colonization by *A. flavus* in the gypsum-treated than in the non-gypsum-treated soil (Tables 3 and 4). Further, there was no aflatoxin contamination of seed from the gypsum-treated soil, but it was found in seed from the nontreated controls. Other studies have revealed that pod breakdown fungi (6,8) and aflatoxin (27) were reduced by supplemental gypsum applications.

From these experiments, it is of interest that gypsum applications alone may be effective in reducing aflatoxin produced by *A. flavus* during the infection of peanut seed. Results of this study demonstrate for the first time that soil applications of gypsum may reduce colonization of peanut seed by fungi of the *A. flavus* group and subsequent aflatoxin contamination. These results also indicate that gypsum may interact with soil-applied fungicides to reduce such colonization and seed contamination even further. These interactions have not been described previously.

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