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Effect of application of nontoxigenic strains of *Aspergillus flavus* and *A. parasiticus* on subsequent aflatoxin contamination of peanuts in storage

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Abstract

Experiments were conducted to determine the potential for biological control of aflatoxin contamination of peanuts during storage. Florunner peanuts were treated in field plots by applying competitive, nontoxigenic strains of *Aspergillus flavus* and *A. parasiticus*, at 76 and 67 days after planting in 1998 and 1999, respectively. After harvest, half the peanuts from both treated and control plots were sprayed with an aqueous conidial suspension containing the nontoxigenic strains; the other half of the peanuts from each group were not sprayed. The peanuts were then placed in separate compartments of a miniature warehouse. Therefore, storage treatments consisted of peanuts that were (1) not treated at all; (2) treated prior to storage only; (3) field-treated only; (4) treated both in the field and prior to storage. Peanuts were stored for 3–5 months under high temperature and relative humidity conditions designed to promote aflatoxin contamination. In 1998, peanuts were not contaminated with aflatoxins prior to storage. After storage, peanuts that were never treated with the competitive fungi contained an average of 78.0 ppb of aflatoxins. Peanuts not treated in the field but receiving the spray treatment before storage contained 48.8 ppb. Peanuts treated in the field only averaged 1.4 ppb, and peanuts treated both in the field and prior to storage contained 0.8 ppb. In 1999, peanuts suffered from late-season drought and were contaminated with aflatoxins at harvest, with controls averaging 516.8 ppb compared with 54.1 ppb in treated peanuts. After storage, non-field-treated peanuts averaged 9145.1 ppb compared with 374.2 ppb for peanuts that had been field-treated, a 95.9% reduction. Spraying of pods with the nontoxigenic strains postharvest but prior to storage provided no additional protection against aflatoxin contamination. Results demonstrated that field application of the nontoxigenic strains had a carry-over effect and reduced aflatoxin contamination that occurred in storage. Published by Elsevier Science Ltd.

Keywords: Groundnut; *Arachis hypogaea*; Warehouse; Biological control

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1. Introduction

Aflatoxins are potent hepatotoxic, carcinogenic metabolites produced by *Aspergillus flavus* Link, *A. parasiticus* Speare, and *A. nomius* Kurtzman, Horn and Hesseltine (CAST, 1989; Diener et al., 1987; Kurtzman et al., 1987). These fungi invade various agricultural commodities during maturation in the field or after harvest and contaminate them with aflatoxins. Maize, peanuts, cottonseed, and various tree nuts are particularly susceptible to preharvest aflatoxin contamination when environmental conditions during crop maturation are characterized by high temperatures and moisture stress and when insect injury is prevalent (Cole et al., 1989; Cotty and Lee, 1989; Hill et al., 1985; Payne, 1992). Postharvest contamination may also occur when stored products are not maintained at a safe moisture level. Owing to the toxicity and carcinogenicity of aflatoxins, contaminated commodities destined for human or animal consumption pose a serious health hazard and are, therefore, closely monitored and regulated (van Egmond, 1995). Apart from its effect on health, aflatoxin contamination also impacts the agricultural economy through the loss of produce and the time and costs involved in monitoring and decontamination efforts (Shane, 1994).

One strategy that has been developed for reducing preharvest aflatoxin contamination of crops is biological control, which is achieved by applying competitive, nontoxigenic strains of *A. flavus* and/or *A. parasiticus* to soil of developing crops. Application of non-aflatoxigenic strains of *A. parasiticus* to peanut (*Arachis hypogaea* L.) plots reduced aflatoxin contamination of peanuts in three successive years (Dorner et al., 1992). When different rates of a combined inoculum of nontoxigenic strains of *A. flavus* and *A. parasiticus* were applied to soil in a two-year study, reductions in aflatoxin contamination of peanuts ranged from 74.3% to 99.9% and demonstrated a treatment-rate effect (Dorner et al., 1998). Reduction in aflatoxin contamination of cottonseed was achieved by applying a non-aflatoxigenic strain of *A. flavus* to soil around developing cotton plants (Cotty, 1994). Inoculation of maize plots resulted in aflatoxin reductions in harvested maize of 66–87% (Dorner et al., 1999). A review of preharvest aflatoxin prevention strategies has recently been published (Brown et al., 1998).

Whereas the biological control strategy has shown great promise for reducing preharvest aflatoxin contamination of peanuts, there remains concern about contamination that occurs while peanuts are stored in large, bulk storage warehouses, which may last up to one year. Contamination of stored peanuts can result from several different scenarios, but all scenarios relate to the inability to maintain peanuts at a safe moisture content during the storage period. Improper kernel moisture during storage can result from leaky roofs, condensation due to inadequate ventilation in the warehouse, high-moisture foreign material associated with stored peanuts, and high-moisture peanuts initially going into storage (Davidson et al., 1982). This study was undertaken to determine if application of nontoxigenic strains of *A. flavus* and *A. parasiticus* might also have an effect on aflatoxin contamination of peanuts in storage.

2. Materials and methods

2.1. Fungal strains

Competitive fungi used in this study were a naturally occurring isolate of *A. flavus* (NRRL 21882) and an orange–brown color mutant of *A. parasiticus* (NRRL 21369), each lacking the

ability to produce aflatoxins, cyclopiazonic acid, and known biosynthetic precursors of aflatoxins (Dorner et al., 1998). Cultures were maintained on Czapek agar (Cz) slants at 5°C.

2.2. Field inoculum preparation

For application of fungi to soil, rice inoculum was prepared by culturing each strain on autoclaved, long-grain rice in 2800 ml Fernbach flasks (500 g of rice with 150 ml distilled water). Rice was inoculated with 1 ml of a conidial suspension (10^6 /ml) from Cz slants and incubated at 30°C for 4 days on a rotating platform (2 rev/min) tilted 70° from the horizontal to prevent clumping and inhibit fungal sporulation. Rice inoculum was then dried in shallow pans in a forced-air draft oven at 50°C for 6 h or until the moisture content was $\leq 7\%$. Rice inoculum was stored at 5°C until used.

2.3. Test plots

In both 1998 and 1999, Florunner peanuts were grown in 12 field plots of 0.1 ha each. Plots measured 45.7 m in length \times 21.9 m in width and contained 24 rows spaced 0.9 m apart. Peanuts were planted on April 24, 1998, and May 8, 1999. Rice inocula of *A. flavus* and *A. parasiticus* were thoroughly mixed and applied to six plots by spreading 24 kg/ha (20 lbs/acre) over the peanut rows from a Gandy box at 76 days after planting (DAP) in 1998 and 67 DAP in 1999. Six equivalent plots were not treated and served as controls. Treated and control plots were separated in the field by approximately 220 m to minimize cross-contamination. Individual plots within each treatment were separated by approximately 15 m, and soil between plots was periodically tilled to minimize possible substrate for biocontrol fungi outside treated plots. Peanuts were dug on October 13, 1998, and September 14, 1999, and harvested with a conventional peanut combine. In 1998, peanuts from all treated plots were placed together in a drying trailer and peanuts from control plots were combined in a separate trailer. Peanuts were dried and a sample of about 18 kg was obtained from each trailer with 15 random probes of a pneumatic sampler. In 1999, samples were obtained from each individual plot by collecting all peanuts from one complete windrow (2 rows) using a peanut combine with a bagging attachment. The remaining peanuts were combined on drying trailers as in 1998.

2.4. Pre-storage inoculation

Peanuts from each trailer were unloaded and divided in half in order to spray half the peanuts in each group with the competitive fungi before storage. Therefore, four treatment regimens were included in the storage phase of the study. These were (1) control–control, which were peanuts never treated with the biocontrol fungi; (2) control–treated, which were peanuts from untreated soil that were sprayed with the nontoxigenic strains before storage; (3) treated–control, which were peanuts from treated soil that were not sprayed before storage; and (4) treated–treated, which were peanuts from treated soil that were also sprayed with the nontoxigenic strains before storage (Fig. 1). A private fermentation company was contracted to supply a large quantity of conidia of nontoxigenic *A. flavus* and *A. parasiticus*. A conidial suspension was prepared by first adding 100 g of conidia of each strain to 20 l of water containing 0.1% Tween 20, which was

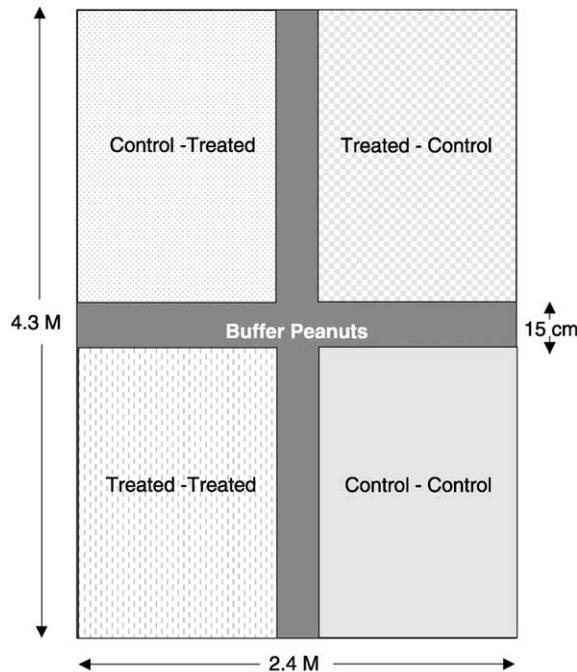


Fig. 1. Top view of miniature warehouse showing the location of each group of peanuts within the warehouse separated by a buffer zone of high-quality, irrigated peanuts. Treatments were: (1) control–treated, peanuts from untreated soil sprayed with nontoxigenic fungal conidia prior to storage; (2) treated–control, peanuts from treated soil not sprayed postharvest but prior to storage; (3) treated–treated, peanuts from treated soil and sprayed with nontoxigenic fungal conidia postharvest but prior to storage; (4) control–control, peanuts from untreated soil not sprayed prior to storage.

vigorously shaken and then stirred overnight. The suspension was then diluted to 1201 with water containing 0.01% Tween 20 in an agricultural spray tank attached to a tractor (typical equipment used for spraying pesticides). The conidial suspension contained 2.1×10^7 and 1.1×10^7 colony forming units (CFU)/ml of nontoxigenic *A. flavus* and *A. parasiticus*, respectively, in 1998, and 9.0×10^7 and 3.2×10^7 CFU/ml, respectively, in 1999. Peanuts were fed onto a vibrating trough from which they were discharged at a flow rate of 38 kg/min onto a belt conveyer. Two spray tips were mounted opposite each other between the trough and the conveyer so that two sides of the falling stream of peanuts were sprayed with the conidial suspension as they dropped from the trough to the conveyer. Peanuts were sprayed at a rate of approximately 60 ml of suspension per kg of peanuts. Peanuts were conveyed into pallet bins for transport to the warehouse.

2.5. Storage

A miniature warehouse at the National Peanut Research Laboratory was used to store the peanuts. The warehouse had a capacity of 3850 kg of farmers' stock peanuts and was a 1/10 scale model of a conventional warehouse (Smith et al., 1989). To separate the treatments within the warehouse, longitudinal and cross section wire-mesh partitions were built, producing four

separate sections with a 15 cm buffer zone between sections. The buffer zone was filled with high-quality, irrigated peanuts not associated with the experiment to separate the various treatments while allowing unrestricted air flow throughout the warehouse (Fig. 1). The warehouse was loaded on November 2, 1998, and September 16, 1999. It was unloaded on February 11, 1999, and February 22, 2000.

The moisture content of peanuts going into the warehouse in 1998 was about 11%. Due to the concern that there was not enough moisture to support mold growth and aflatoxin contamination during the storage period, a heating system with waffle moisture columns was attached to the warehouse so that heated, moist air could be pumped into the warehouse. This system was run for 25 days from November 20 to December 15, 1998, in order to ensure conditions conducive for aflatoxin contamination. Moisture content of peanuts in 1999 averaged about 13.5%, which was considered sufficient to promote aflatoxin development without modification of the warehouse environment.

After the storage period each year, peanuts were unloaded into pallet bins. Random samples were pulled by pneumatic probe as needed for various analyses.

2.6. Sampling and processing of field plot samples

The 18 kg samples pulled from trailers in 1998 were divided to yield a subsample of about 1 kg for fungal analysis; the remaining peanuts were riffle divided to produce eight 2 kg subsamples for aflatoxin analysis. Subsamples were shelled with a Federal State Inspection Service (FSIS) sample sheller. Peanuts from each shelled subsample were screened over a 0.635 cm slotted screen to separate edible categories (peanuts riding the screen) from oil stock (peanuts falling through the screen). Visually damaged kernels were picked out of the edible fraction and combined with the oil stock fraction. For fungal analysis, 500 kernels were randomly selected from the edible fraction. For aflatoxin analysis, all peanuts in the edible and oil stock (containing damaged kernels) fractions were separately analyzed.

The samples gathered at harvest in 1999 varied in size from 7.4 to 18.8 kg (depending on plot yield). These samples were shelled with a “model 4 sample sheller” designed to produce the same shelling characteristics as equipment used in a typical shelling plant (Davidson et al., 1981). Peanuts were separated into commercial size categories including jumbo, medium, number 1, sound splits, and oil stock; damaged kernels were picked out of each category except oil stock and combined. For fungal analysis, jumbo, medium, and number 1 size peanuts were combined and riffle divided to yield ≥ 100 kernels for plating. The remaining peanuts were re-sized into jumbo, medium, and number 1 categories. For aflatoxin analysis, subsamples containing ≥ 500 g were homogenized to a paste in a vertical cutter mixer (VCM), and a 200 g subsample was taken for analysis (Dorner and Cole, 1993). Samples of less than 500 g were extracted directly in Waring blenders of appropriate size.

2.7. Sampling and processing of storage samples

For post-storage fungal analysis in 1998, about 1 kg subsamples were obtained from each treatment and processed as described for field test samples to give a 500-kernel subsample for plating. Post-storage fungal analysis of 1999 peanuts was carried out by collecting ten 1.5 kg

samples from each treatment. Peanuts were shelled with the FSIS sample sheller and sized. Jumbo, medium, and number 1 sizes (edible category) were combined, and 1 kg of kernels was homogenized with an equal weight of water in a Robot Coupe RSI6Y VCM to produce a peanut/water slurry (Dorner, 2000). The slurry (100 g) was diluted with 200 g of water and blended for 1 min. The mixture was dilution plated on modified dichloran-rose bengal medium (Horn and Dorner, 1998) to determine CFU/g of all *A. flavus* and *A. parasiticus* associated with the peanuts.

For post-storage aflatoxin analysis, four 30 kg samples were drawn from each treatment and shelled with the Penco Pearman moisture sheller. All kernels in each sample were homogenized in a large VCM to produce a homogeneous paste, and a 200 g subsample was taken for analysis.

2.8. Fungal analyses

All kernels for plating were surface sterilized for 2 min in a 2% sodium hypochlorite solution followed by two sterile water rinses and placed in Petri dishes containing Cz agar (five kernels per plate). Plates were incubated for 7 days at 30°C. To determine the incidence of the applied, competitive strains in peanuts, *A. parasiticus* NRRL 21369 was identified by its orange–brown color and gross morphology. However, colonies of *A. flavus* NRRL 21882 could not be conclusively differentiated from wild-type strains. Therefore, all *A. flavus* colonies from infected peanuts and 10 randomly selected colonies from each slurry sample (400 total colonies) were transferred to both Cz slants and 4 ml vials containing 1 ml of an enriched liquid growth medium (Horn et al., 1996) consisting of sucrose (150 g), glucose (40 g), soytone (10 g), yeast extract (20 g), and distilled water (1 l) adjusted to pH 5.9 with HCl. Slants were incubated at 30°C for 7 days and examined for partial identification of NRRL 21882 by its gross morphology, including production of abundant exudate and sclerotia. Many wild-type strains were identified on the basis of lacking these morphological characteristics. Inoculated vials were incubated at 30°C for 7 days and were extracted and analyzed for aflatoxins and cyclopiazonic acid according to Horn et al. (1996). Strains that exhibited morphological characteristics consistent with NRRL 21882 and also were negative for production of aflatoxins and cyclopiazonic acid were presumed to be the applied *A. flavus*. Strains with these criteria are uncommon in southwestern Georgia according to Horn et al. (1996), who found that among 44 vegetative compatibility groups of *A. flavus*, only one group contained isolates that produced neither aflatoxins nor cyclopiazonic acid.

2.9. Aflatoxin analyses

Subsamples from the homogenized samples or whole kernel samples were extracted with methanol–water and analyzed by a slightly modified high-performance liquid chromatography (HPLC) method of Dorner and Cole (1988). The HPLC system consisted of a Waters 3.9 × 150 mm² Nova-PAK C₁₈ column with a mobile phase of water–methanol–butanol (700 + 355 + 12; v/v/v). Instead of using postcolumn iodination to enhance fluorescence of aflatoxins B₁ and G₁ (Dorner and Cole, 1988), postcolumn derivatization was achieved with a photochemical reactor (Joshua, 1993) placed between the column and a Shimadzu Model RF551 fluorescence detector with excitation and emission wavelengths of 365 and 440 nm, respectively. Injection solvent consisted of methanol–water (62 + 38) with 0.1% acetic acid. Aflatoxin standards were prepared from crystals according to AOAC method 971.22 (Scott, 1995), and

aflatoxin determinations were not corrected for recovery. For reporting purposes, aflatoxin data from individual analyses were combined on a weighted basis to produce a single total aflatoxin value for each composite sample. For example, when samples were divided into jumbo, medium, number 1, etc. categories and analyzed separately, a composite sample aflatoxin value was determined by multiplying the concentration of total aflatoxins (ng/g) in each category by the weight (g) of peanuts in the category, summing aflatoxin weights (ng) thus determined, and dividing by the total peanut weight (g) for all categories.

2.10. Statistics

Aflatoxin concentrations and quantitative fungal data were log transformed, and fungal infection percentages were transformed to the arcsine of the square root to normalize distributions. Data were analyzed by *t*-tests or ANOVA as appropriate, and multiple comparisons were made with the Student–Newman–Keuls method using SigmaStat for Windows Version 1.0 (Jandel Corporation, San Rafael, CA).

3. Results and discussion

3.1. 1998 studies

The 1998 growing season was characterized by periods of extensive drought early in the season that delayed setting of the crop. However, rains late in the growing season allowed for reasonable peanut production but delayed harvest until October 13. Total yield from treated and control plots was 3600 and 2960 kg/ha, respectively. Aflatoxin analyses of harvested peanuts showed no preharvest contamination. Thus, peanuts entering the warehouse were not contaminated (Table 1).

After storage, peanuts that were never treated with the competitive strains (control–control) averaged 78.0 ppb of aflatoxins (Table 1). Although peanuts that were treated with the competitive strains prior to storage only (control–treated) contained significantly less aflatoxin than those not treated (control–control), they still had significantly higher aflatoxin concentrations than peanuts that had been treated in the field, irrespective of pre-storage treatment. Peanuts that came from soil treated with the competitive strains contained very little aflatoxin, even after being stored under conditions that were conducive for contamination (Table 1). Peanuts that had

Table 1
Mean aflatoxin concentrations (ppb) in peanuts from the 1998 crop year

| Treatment (field only) | Field | Treatment (field-storage) | Storage ^a |
|------------------------|-------|---------------------------|----------------------|
| Control | 0.0 | Control–control | 78.0 ± 12.2a |
| | | Control–treated | 48.8 ± 11.2b |
| Treated | 0.0 | Treated–control | 1.4 ± 1.2c |
| | | Treated–treated | 0.8 ± 0.9c |

^a Means in a column followed by the same letter are not significantly different (*P* > 0.05).

been treated with competitive fungi in the field only (treated–control) were not significantly different in aflatoxin content compared to peanuts treated both in the field and prior to storage (treated–treated). These data demonstrate that the best control of aflatoxin in storage occurred when peanuts were treated with the competitive fungi in the field. Additional treatment prior to storage had no increased beneficial effect.

Field infection of peanuts by *A. flavus* and *A. parasiticus* in 1998 (Table 2) indicated a slightly higher infection rate in control peanuts compared with peanuts from soil treated with competitive strains. However, because these were results from the plating of 500 kernels grouped from all plots, it could not be determined whether or not that difference was significant. The much higher incidence of applied strains (88.2%) in peanuts from treated plots than in control peanuts (13.1%) indicated preferential invasion by the applied strains. Similarly, for storage peanuts, fungal infection was numerically higher in peanuts that were sprayed prior to storage, and the highest incidence of applied strains was associated with those same peanuts.

3.2. 1999 studies

The 1999 growing season was characterized by adequate rainfall early in the growing season, but peanuts suffered from extensive late-season drought stress, which negatively impacted yield. Total yield was 2576 kg/ha from control plots and 1673 kg/ha from treated plots. The extensive late-season drought also resulted in considerable preharvest aflatoxin contamination (Table 3). However, peanuts from plots treated with the competitive, nontoxigenic strains of *A. flavus* and *A. parasiticus* showed a statistically significant 89.5% reduction in aflatoxin contamination compared with peanuts from control plots.

Subjecting these peanuts to poor storage conditions produced increased levels of aflatoxin in both control and treated groups (Table 3). Peanuts that were spray-treated with the competitive strains prior to storage only (control–treated) contained significantly less aflatoxin than the control–control group, but levels were still extremely high. Peanuts from soil that had been treated with the competitive strains averaged about 374 ppb of aflatoxins regardless of whether or not they were sprayed with the strains prior to storage. This represented a 95.9% reduction in aflatoxin compared with the average aflatoxin concentration of 9145.1 ppb for the control–control and control–treated groups. Even though high concentrations of conidia of nontoxigenic strains

Table 2

Percentage of kernels infected by *Aspergillus flavus* and *A. parasiticus*^a and incidence of applied, nontoxigenic strains (as a percentage of total *A. flavus* and *A. parasiticus*) in peanuts from the 1998 crop year

| Field | | | Storage | | |
|-----------|------------|-----------|-----------------|------------|-----------|
| Treatment | % Infected | Incidence | Treatment | % Infected | Incidence |
| Control | 16.8 | 13.1 | Control–control | 5.6 | 55.6 |
| | | | Control–treated | 15.0 | 80.0 |
| Treated | 6.4 | 88.2 | Treated–control | 3.8 | 42.1 |
| | | | Treated–treated | 25.4 | 93.7 |

^a Includes both toxigenic and nontoxigenic strains of *A. flavus* and *A. parasiticus*.

Table 3
Mean aflatoxin concentrations (ppb) in peanuts from the 1999 crop year

| Treatment (field only) | Field ^a | Treatment (field-storage) | Storage ^a |
|------------------------|--------------------|---------------------------|----------------------|
| Control | 516.8 ± 320.0a | Control–control | 11,579.3 ± 1755.1a |
| Treated | 54.1 ± 46.4b | Control–treated | 6,711.0 ± 645.8b |
| | | Treated–control | 380.0 ± 64.4c |
| | | Treated–treated | 368.4 ± 85.4c |

^a Means in a column followed by the same letter are not significantly different ($P > 0.05$).

Table 4
Quantity (CFU/g) of *Aspergillus flavus* and *A. parasiticus*^a and incidence of applied, nontoxigenic strains (as a percentage of total *A. flavus* and *A. parasiticus*) in 1999 crop year peanuts after storage

| Treatment | CFU/g ^{b,c} | Incidence (%) ^c |
|-----------------|----------------------|----------------------------|
| Control–control | 429,600 ± 181,119a | 3.9 ± 5.0a |
| Control–treated | 780,800 ± 307,517b | 17.7 ± 11.4b |
| Treated–control | 156,800 ± 70,875c | 96.4 ± 6.2c |
| Treated–treated | 900,400 ± 225,451b | 89.7 ± 15.7c |

^a Includes all toxigenic and nontoxigenic strains of *A. flavus* and *A. parasiticus*.

^b Values are the means of ten determinations.

^c Means in a column followed by the same letter are not significantly different ($P > 0.05$).

were sprayed onto pods prior to storage, that did not have nearly the same protective effect as applying the fungi in the field. These data support data from 1998 indicating that no benefit is gained by spray-inoculating peanuts prior to storage if they have been treated in the field.

No significant difference in preharvest infection of peanuts by *A. flavus* and *A. parasiticus* was found between field-treated and control peanuts in 1999. Infection percentages averaged 51.3% in peanuts from control plots and 54.7% in peanuts from treated plots. However, the incidence of applied, nontoxigenic strains was significantly higher ($P < 0.0001$) in peanuts from soil treated with those strains (89.2%) compared with peanuts from control plots (8.2%). These data suggest that treatment of soil with nontoxigenic strains did not increase total infection of peanuts by *A. flavus* and *A. parasiticus*, but rather, it resulted in the preferential invasion of peanuts by nontoxigenic strains compared with native toxigenic strains.

The two-year increase in aflatoxins during storage to much higher levels in peanuts from control plots irrespective of spray treatment suggests that the fungi that proliferated in those peanuts during storage were the aflatoxigenic strains that infected the kernels in the field. To obtain a better understanding of which fungi were proliferating in peanuts during storage, dilution plating of peanut slurries was used to quantify the fungi present in peanuts after storage in 1999. The highest quantity of *A. flavus* and *A. parasiticus* was present in peanuts that were spray-treated prior to storage, with CFU/g of 780,000 in the control–treated group and 900,400 in the treated–treated group (Table 4). The lowest quantity was in peanuts treated in the field but not sprayed prior to storage (treated–control). The incidence of the applied, competitive strains was not significantly different between treated–control and treated–treated groups even though the

quantity was significantly higher in the treated–treated group. Although peanuts that were spray-treated prior to storage only (control–treated) had an equally high level of total *A. flavus* and *A. parasiticus* as the treated–treated peanuts, the incidence of applied strains was significantly lower in the spray-treated peanuts. That incidence was also significantly lower in the spray-treated group compared with peanuts only treated in the field (treated–control). These data affirm the suggestion made on the basis of aflatoxin contamination that the proliferation of fungi that occurs in peanut kernels during storage is predominately by those strains that infect the kernels in the field.

4. Conclusion

This study has confirmed earlier work showing that treatment of soil with specific, nontoxigenic strains of *A. flavus* and *A. parasiticus* significantly reduces preharvest aflatoxin contamination. In addition, soil treatment with nontoxigenic strains had the beneficial carry-over effect of reducing aflatoxin contamination that occurred during storage. Reduced aflatoxin contamination was accomplished without increasing either the percentage of kernels infected by *A. flavus* and *A. parasiticus* in the field or the overall quantity of those fungi present in peanuts after storage. Applying conidia of the nontoxigenic strains directly to peanut pods prior to storage was not nearly as effective in reducing aflatoxin contamination in storage as was treating soil with the nontoxigenic strains. *Aspergillus flavus* and *A. parasiticus* proliferation in peanut kernels during storage appeared primarily to be associated with infection of kernels that occurred in the field rather than with infection during storage. Field inoculation with nontoxigenic strains shows great potential for reducing not only preharvest aflatoxin contamination, but also contamination that occurs in storage.

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