

## Evaluation of biological control formulations to reduce aflatoxin contamination in peanuts

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### Abstract

A two-year study was conducted to evaluate the efficacy of three formulations of nontoxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* to reduce preharvest aflatoxin contamination of peanuts. Formulations included: (1) solid-state fermented rice; (2) fungal conidia encapsulated in an extrusion product termed Pesta; (3) conidia encapsulated in pregelatinized corn flour granules. Formulations were applied to peanut plots in 1996 and reapplied to the same plots in 1997 in a randomized design with four replications, including untreated controls. Analysis of soils for *A. flavus* and *A. parasiticus* showed that a large soil population of the nontoxigenic strains resulted from all formulations. In the first year, the percentage of kernels infected by wild-type *A. flavus* and *A. parasiticus* was significantly reduced in plots treated with rice and corn flour granules, but it was reduced only in the rice-treated plots in year two. There were no significant differences in total infection of kernels by all strains of *A. flavus* and *A. parasiticus* in either year. Aflatoxin concentrations in peanuts were significantly reduced in year two by all formulation treatments with an average reduction of 92%. Reductions were also noted for all formulation treatments in year one (average 86%), but they were not statistically significant because of wide variation in the aflatoxin concentrations in the untreated controls. Each of the formulations tested, therefore, was effective in delivering competitive levels of nontoxigenic strains of *A. flavus* and *A. parasiticus* to soil and in reducing subsequent aflatoxin contamination of peanuts.

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### 1. Introduction

Aflatoxin contamination of peanuts (*Arachis hypogaea* L.) results from growth in peanut kernels by toxigenic strains of the fungi, *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare (Diener et al., 1987). These fungi are commonly found in soil where peanuts are grown, and many strains of *A. flavus* and most strains of *A. parasiticus* produce aflatoxins (Horn et al., 1996). Aflatoxins are

potent hepatotoxic and carcinogenic metabolites, and their presence in peanuts is heavily monitored and regulated to ensure a safe food supply (van Egmond, 1995; Wood and Trucksess, 1998). Contaminated lots of peanuts cannot be used for human consumption and therefore represent great economic losses for the peanut industry (Lamb and Sternitzke, 2001). Aflatoxin contamination of peanuts can occur in the field (preharvest) when severe late-season drought stress occurs and during storage (postharvest) when improper conditions of moisture and temperature exist (Cole et al., 1995).

Biological control is a promising approach for reducing both preharvest and postharvest aflatoxin contamination in peanuts (Dorner et al., 1992, 1998; Dorner

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and Cole, 2002). Reductions in aflatoxin are achieved by applying nontoxigenic strains of *A. flavus* and *A. parasiticus* to soil around developing peanut plants. When late-season drought conditions make peanuts susceptible to invasion and growth by these fungi, the applied nontoxigenic strains competitively exclude toxigenic strains present in the soil and thereby reduce subsequent aflatoxin concentrations. Reductions in aflatoxin contamination with the use of nontoxigenic strains has also been demonstrated in corn and cottonseed (Brown et al., 1991; Cotty, 1994; Dorner et al., 1999).

In previous studies, we have applied the nontoxigenic strains as a conidial suspension (Dorner et al., 1992) and by using colonized rice as a carrier (Dorner et al., 1998, 1999). The rice inoculum was produced through solid-state fermentation (SSF) of the nontoxigenic strains on sterile rice. This basic technique has also been used to produce a wheat-based inoculum for application of a nonaflatoxigenic strain of *A. flavus* to cotton fields (Bock and Cotty, 1999; Cotty, 1994).

Whereas inoculum produced by SSF is effective for delivering the desired competitive strains to the field, the process is laborious, time-consuming, and not well suited to potential large-scale commercial production. Other methods that have been used for formulating microbial biological control agents have been reviewed (Connick et al., 1990; McGuire et al., 1999). Connick et al. (1991) adapted the extrusion process for making pasta to encapsulate mycoherbicide fungal propagules in a wheat gluten matrix and called the product 'Pesta.' Extrusion technology has been employed in the production of Pesta containing a variety of fungal biocontrol agents, including nontoxigenic strains of *A. flavus* and *A. parasiticus* (Daigle et al., 1998). Microbial agents have also been encapsulated in a variety of other matrices, including starch, alginate, and pregelatinized corn flour (Connick et al., 1990; Dunkle and Shasha, 1988; McGuire and Shasha, 1992). Although most work involving starch and flour matrices was done with bacteria and viruses active against insects, Pereira and Roberts (1991) adapted the formulations for use with the insect pathogenic fungus *Beauveria bassiana*.

The objective of this two-year study was to compare formulations of SSF rice, Pesta, and corn flour-based granules for establishing populations of nontoxigenic strains of *A. flavus* and *A. parasiticus* in peanut plots and for reducing preharvest aflatoxin contamination of peanut.

## 2. Materials and methods

### 2.1. Fungi

Nontoxigenic color mutants of *A. flavus* (NRRL 21368) and *A. parasiticus* (NRRL 21369), which have

been shown to be effective in reducing preharvest aflatoxin contamination (Dorner et al., 1998), were used as biological control agents. Strains were maintained on Czapek agar slants at 5 °C.

### 2.2. Formulations

A rice formulation was produced by SSF of each fungal 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) and incubated at 30 °C for 4 days on a rotating platform (2 rev/min) tilted 70° from horizontal to gently agitate the rice and prevent fungal sporulation. The rice was then dried in shallow pans in a forced air draft oven at 50 °C for 6 h or until the moisture content was  $\approx 7\%$ .

For production of Pesta (at SRRC, New Orleans, LA), dry conidia of *A. flavus* ( $1.1 \times 10^{10}$  CFU/g) and *A. parasiticus* ( $5.7 \times 10^9$  CFU/g) color mutants were obtained from Sylvan Bioproducts (Kittanning, PA); semolina (King Midas #1) from Con-Agra (Omaha, NE); kaolin (RC-32AF) from Thiele Kaolin (Wrens, GA); Kelzan, a xanthan gum, from Kelco Industrial Biopolymers (Chicago, IL); and Tween 20 from Sigma (St. Louis, MO). Separate suspensions were made by adding 0.165 g of *A. flavus* and 0.32 g of *A. parasiticus* conidia to 324 ml of water containing 0.02% Tween 20. Then, with stirring, 0.49 g of Kelzan was added to aid in suspending the conidia. A mixture of 0.726 kg of semolina, 0.182 kg kaolin, and 325 ml of conidial suspension was blended in a model AS200-DT Hobart mixer (Hobart, Youngstown, OH) with a wire whip attachment. The resulting dough was extruded through a twin-screw extruder by the procedure of Daigle et al. (1997) and granules were dried at 50 °C for 1 h.

For production of corn flour-based granules, conidial suspensions were prepared by separately growing color mutant strains in nine Fernbach flasks containing 200 ml of YES broth (Davis et al., 1966) for 2 weeks at 30 °C. Cultures were shaken with water containing 0.01% Tween 20 to dislodge conidia and filtered through cheesecloth. Filtrate was dilution-plated to determine the concentration of conidia, which was  $2.2 \times 10^7$  CFU/ml for *A. flavus* and  $2.9 \times 10^7$  CFU/ml for *A. parasiticus*. Suspensions were shipped overnight to NCAUR, Peoria, IL, where they were stored at 4 °C until use. The formulation was prepared by mixing 1000 g pregelatinized corn flour 961 (Illinois Cereal Mills, Paris, IL), 400 g molasses (Nugget Brand, Stockton, CA) and 200 ml of conidial suspension in a 7.75 L double planetary mixer (Charles Ross and Son, Hauppauge, NY). After mixing, the product was sieved through a 4-mesh screen and collected on a 10-mesh screen. Granules were allowed to air-dry in a fume hood then shipped overnight to Georgia.

All formulations were stored at 4 °C and were assayed periodically to determine their stability during storage. The quantity (CFU/g) of fungi associated with the various formulations was determined by homogenizing five 1-g samples of each formulation in 10 ml of 0.2% water agar. The homogenate was dilution-plated on Czapek agar plates and incubated for 4 days at 30 °C.

### 2.3. Test plots

Experiments were carried out during two crop years in the environmental control plot facility at the National Peanut Research Laboratory, Dawson, GA. To produce an optimum environment for aflatoxin development in peanuts, the plots were equipped with a mechanized roof system to impose drought stress late in the growing season, and heating cables were present in the soil to elevate soil temperature (Blankenship et al., 1984, 1989; Cole et al., 1985, 1989). Four environmental control plots of 12.0 m × 5.5 m were each divided into four 3.0 m × 5.5 m subplots by placing three partitions across the plots. Partitions were made of plywood and were placed into the soil at a depth of 15 cm and extended to a height of 51 cm. Florunner peanuts were planted on April 29, 1996 and May 6, 1997 with six rows per plot.

Equal amounts of the color mutant *A. flavus* and *A. parasiticus* preparations of each formulation were thoroughly mixed prior to application to the soil. Treatments included the three biocontrol formulations and an untreated control with four replications each. Replicate subplots were randomized after ensuring that each environmental control plot contained one of each of the three treatments and a control. Each formulation was applied at a rate of 10 g/m of row (107 kg/ha) 58 days after planting (DAP) in 1996, and reapplied to the same subplots at 66 DAP in 1997.

Florunner peanuts were grown according to practices recommended by the Georgia Cooperative Extension Service until the time drought stress was imposed. In 1996, peanuts received a final irrigation at 86 DAP; soil temperature modification began 98 DAP; and plants were dug by hand 142 DAP. In 1997, final irrigation occurred 79 DAP; soil temperature modification began 112 DAP; and plants were dug 139 DAP. Mean soil temperatures at the 5 cm depth during the period of temperature control averaged 30.0 °C in 1996 and 30.5 °C in 1997.

All peanuts were picked with a mechanical picker, shelled with a Federal State Inspection Service sample sheller, and sized into commercial-size categories (jumbo, medium, number 1, sound splits, and oil stock). Damaged and visibly molded kernels were hand-picked from each category except oil stock and combined as a single category for each subplot.

### 2.4. Microflora analyses

Six soil samples (approximately 100 cm<sup>3</sup> each) were collected from each subplot immediately after planting and prior to harvest to determine populations of *A. flavus* and *A. parasiticus* in the pod zone. Soil was removed with a sterile trowel from the top 5 cm in the area where peanuts would be or were forming. Samples from each subplot (averaging 500 g) were thoroughly mixed, air dried in paper bags, and stored at 4 °C. Final moisture contents were not determined. Soil was screened through a No. 4 sieve, and 30 g was blended for 1 min at low speed with 300 ml of a 0.2% water agar solution that was cooled to 5 °C (Horn et al., 1994). Dilutions were plated on a dichloran-rose bengal medium (King et al., 1979) modified with 3% NaCl. Color mutants were readily distinguishable from wild-type strains of *A. flavus* and *A. parasiticus*, but the wild-type strains of *A. flavus* and *A. parasiticus* were not enumerated separately.

After peanuts were shelled and sized, jumbo, medium, and number 1 size categories were randomly divided to yield a subsample of approximately 6.25% of the original amount for plating. The number of kernels plated varied depending on yield and the quantity of peanuts in each category. The average numbers plated of jumbo, medium, and number 1 categories were: 122 ± 15, 288 ± 25, and 100 ± 10, respectively, in 1996 and 64 ± 13, 274 ± 39, and 118 ± 29, respectively, in 1997. Plated kernels totaled 8170 in 1996 and 7287 in 1997. Kernels were surface disinfected for 2 min in a 2% sodium hypochlorite solution and placed in Petri dishes containing Czapek agar (five kernels per plate). Plates were incubated for seven days at 30 °C.

### 2.5. Aflatoxin analyses

All remaining peanuts from each subplot were analyzed for aflatoxin by the high performance liquid chromatography (HPLC) method of Dorner and Cole (1988) with certain modifications. Peanuts were not subsampled prior to analysis; rather, all peanuts were extracted with methanol–water in a blender of the appropriate size so that no sampling error was introduced. The HPLC system consisted of a Waters 3.9 × 150 mm Nova-PAK C<sub>18</sub> 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 B<sub>1</sub> and G<sub>1</sub>, 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. Individual aflatoxin standards (B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>) were prepared from crystals

(Sigma) according to AOAC Official Method, 970.44 (Official Methods of Analysis, 1995).

### 2.6. Statistics

To determine aflatoxin concentrations for combined categories (total = jumbo + medium + number 1 + splits + oil stock + damage), the concentration of aflatoxin (ng/g = ppb) in each category was multiplied by the weight of kernels in the category, and the sum of the aflatoxin weight (ng) was divided by the total kernel weight (g). Aflatoxin concentrations were transformed to  $\log(\text{ng/g} + 1)$ , fungal densities were log transformed, and kernel infection percentages were transformed to the arcsine of the square root if necessary to normalize distributions. Data were analyzed by ANOVA, and multiple comparisons were made with the Student–Newman–Keuls method using SigmaStat for Windows Version 1.0 (Jandel Corporation, San Rafael, CA). Correlations between aflatoxin contamination in the jumbo, medium, and number 1 size categories and percent kernels infected by wild-type *A. flavus*/*A. parasiticus* and color mutant strains were determined using the Pearson product moment correlation method.

## 3. Results

### 3.1. Formulations

Analysis of each formulation revealed the following respective CFU/g of *A. flavus* and *A. parasiticus* color mutants: rice,  $1 \times 10^6$  and  $3 \times 10^6$ ; Pesta,  $3 \times 10^5$  and  $8 \times 10^5$ ; and corn flour granules,  $3 \times 10^6$  and  $4 \times 10^6$ . During storage at 4 °C for 16 months those quantities did not change significantly.

### 3.2. Soil populations

Analysis of soils at planting and harvest showed that applications of all formulations altered the quantity and composition of *A. flavus* and *A. parasiticus* (Table 1). By harvest time in the first year that color mutants were applied, total populations of *A. flavus* and *A. parasiticus* were very high but not significantly different among treatments. A large percentage of the population in

control plots was represented by the color mutants (61%). That percentage remained fairly steady throughout the study, although total *A. flavus* and *A. parasiticus* populations decreased. At planting time in 1997, subplots that had been treated with the rice formulation contained a significantly higher population than other treatments, but the percentage of color mutants comprising that population remained very high in all treatments. At harvest time in 1997 total populations were generally lower than they had been in 1996, particularly in the control subplots. Subplots treated with the rice formulation continued to have significantly higher total populations, and subplots treated with Pesta and corn flour granules were still significantly higher than control subplots. The composition of the *A. flavus* and *A. parasiticus* populations in treated subplots continued to heavily favor the applied color mutant strains.

### 3.3. Kernel infection

The percentages of peanut kernels infected by various strains of *A. flavus* and *A. parasiticus* in 1996 and 1997 are shown in Figs. 1 and 2, respectively (results from size categories totaled). In both years there were no differences in the percentage of kernels infected with all strains of *A. flavus* and *A. parasiticus* (total). In 1996,

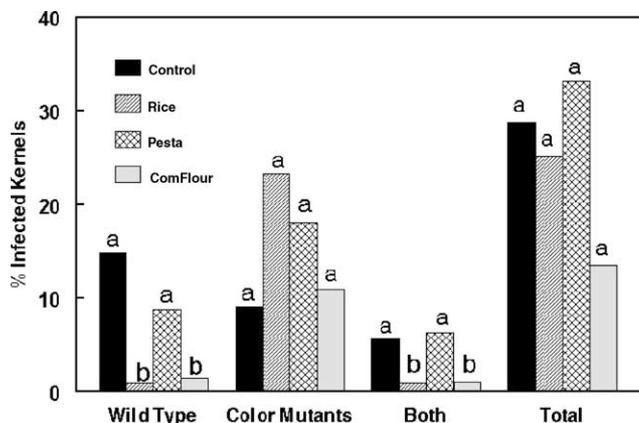


Fig. 1. Percentage of peanut kernels infected by wild-type *A. flavus* and *A. parasiticus*, applied color mutant strains, both wild-type and color mutant strains, and total kernels infected by any strain of *A. flavus* or *A. parasiticus* in 1996.

Table 1

Mean colony forming units (CFU)/g of all strains of *A. flavus* and *A. parasiticus* in soil at various sampling times and the percentage of those strains represented by applied color mutants (CM)<sup>a,b</sup>

Treatment	Plant 1996	CM (%)	Harvest 1996	CM (%)	Plant 1997	CM (%)	Harvest 1997	CM (%)
Control	43.3 a	0.0	20,218 a	61.0	3345 a	57.6	1433 a	52.2
Rice	20.0 a	0.0	80,988 a	99.9	41,467 b	99.8	51,193 b	99.9
Pesta	20.0 a	0.0	57,848 a	98.0	26,135 c	98.1	19,938 c	90.8
Corn flour	46.7 a	0.0	77,945 a	99.2	18,680 c	99.3	21,805 c	86.0

<sup>a</sup> Means in a column followed by a different letter are significantly different ( $P < 0.05$ ).

<sup>b</sup> Inoculum reapplied in 1997.

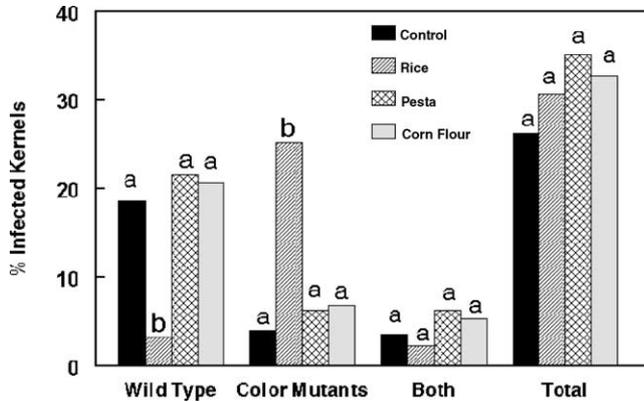


Fig. 2. Percentage of peanut kernels infected by wild-type *A. flavus* and *A. parasiticus*, applied color mutant strains, and total kernels infected by any strain of *A. flavus* or *A. parasiticus* in 1997.

significantly fewer kernels were infected by wild types of *A. flavus* and *A. parasiticus* in subplots treated with rice and corn flour formulations. However, there were no differences in the percentage of kernels infected by applied color mutant strains. In all treatments some individual kernels were infected by both wild-type and color mutant strains (both), but there were significantly less of these in subplots treated with the rice and corn flour formulations. Very few significant differences in kernel infection were seen in 1997. Subplots treated with the rice formulation again had significantly fewer kernels infected by wild-type strains, but they also had significantly more kernels infected by the applied color mutant strains.

Table 2  
Mean total aflatoxin (sum of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>) concentrations (ng/g) in peanuts from 1996<sup>a</sup>

Treatment	Commercial size category						
	Jumbo	Medium	Number 1	Splits	Oil stock	Damage	Total <sup>b</sup>
Control	3.1 a	5.6 a	30.7 a	0.6 a	252.6 a	21054.5 a	119.8 a
Rice	0.5 a	1.3 a	8.2 a	6.9 a	14.3 a	508.5 a	5.0 a
Pesta	0.0 a	3.8 a	12.2 a	2.4 a	150.2 a	5489.0 a	30.6 a
Corn flour	1.4 a	2.8 a	3.5 a	2.8 a	17.9 a	2718.2 a	13.8 a

<sup>a</sup> Means in a column followed by the same letter are not significantly different (Student–Newman–Keuls method,  $P > 0.05$ ).

<sup>b</sup> The weighted aflatoxin concentration for all size categories combined.

Table 3  
Mean total aflatoxin (sum of B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub>) concentrations (ng/g) in peanuts from 1997<sup>a</sup>

Treatment	Commercial size category						
	Jumbo	Medium	Number 1	Splits	Oil stock	Damage	Total <sup>b</sup>
Control	54.4 a	136.6 a	207.0 a	1.5 a	1684.8 a	10340.8 a	405.5 a
Rice	1.4 a	6.4 a	20.9 a	15.6 a	174.4 b	1274.4 a	43.9 b
Pesta	0.0 a	5.7 a	10.8 a	12.8 a	74.0 b	643.8 a	20.4 b
Corn flour	0.0 a	3.3 a	15.2 a	5.1 a	62.6 b	1698.5 a	29.9 b

<sup>a</sup> Means in a column followed by the same letter are not significantly different (Student–Newman–Keuls method,  $P > 0.05$ ).

<sup>b</sup> The weighted aflatoxin concentration for all size categories combined.

### 3.4. Aflatoxin contamination

Mean aflatoxin concentrations for individual size categories and total kernels are presented in Tables 2 and 3. Although treatments generally appeared to result in less aflatoxin in 1996 compared with untreated controls, differences were not statistically significant because of wide variation in the individual values. The highest value for total kernels from a control subplot was 231.0 ng/g compared with high values of 10.4, 45.6, and 45.1 ng/g from subplots treated with rice, Pesta, and corn flour formulations, respectively. In 1997 (Table 3) aflatoxin concentrations were significantly reduced by all formulations in the oil stock category and in total kernels. The overall average reduction in total kernels for all formulations was 92%, ranging from 89.2% for peanuts treated with the rice formulation to 95.0% for peanuts treated with Pesta.

In 1996 there was not a statistically significant correlation between aflatoxin contamination and percent kernel infection by wild-type ( $P = 0.13$ ,  $n = 48$ ) or color mutant ( $P = 0.53$ ,  $n = 48$ ) strains of *A. flavus* and *A. parasiticus*. In 1997 reduced infection of kernels by wild-type strains was significantly correlated with reduced aflatoxin contamination ( $r = 0.50$ ,  $P < 0.001$ ,  $n = 48$ ), but the correlation between aflatoxin and infection by color mutants was not significant ( $P = 0.35$ ,  $n = 48$ ).

## 4. Discussion

Although the three formulations tested in this study did not contain equivalent loads of the color mutant

strains, they all delivered competitive quantities of nontoxigenic color mutants of *A. flavus* and *A. parasiticus* to peanut soils. There was a surprisingly large percentage of color mutants in soil from untreated subplots throughout the study. This probably resulted from the close proximity of treated subplots to untreated subplots. Each environmental control plot contained an untreated subplot along with subplots treated with each of the three formulations. In these types of studies where a large inoculum of *A. flavus* and *A. parasiticus* is added to soil, it is difficult to completely prevent movement of fungi into untreated subplots when they are in close proximity to each other.

The highest overall quantity of *A. flavus* and *A. parasiticus*, which was predominately the applied color mutants, was achieved with the cultured rice formulation. The color mutants were always greater than 99% of the overall population of *A. flavus* and *A. parasiticus* in soil to which the rice formulation was applied. At planting and harvest in 1997, populations in Pesta and corn flour-treated subplots were about half those in rice-treated subplots, and the percentage represented by color mutants was not as great, particularly at harvest. However, these differences apparently had no effect on subsequent aflatoxin contamination since all three treatments resulted in similar, significant aflatoxin reductions.

The application rate used in this study (10 g/m of row) was the same as one used in a prior study of the effect of inoculum rates of the cultured rice formulation on aflatoxin contamination of peanuts Dorner et al., 1998). Reductions in aflatoxin achieved by all formulations in this study were similar to those achieved by that same rate of inoculum used in this earlier study. Therefore, the delivery of an adequate population of nontoxigenic strains was achieved by all formulations even though the population and percentage of nontoxigenic strains was highest for the cultured rice formulation.

In 1997 a reduced percentage of seeds infected by wild-type strains of *A. flavus* and *A. parasiticus* was correlated with reduced aflatoxin concentrations. No such correlation was found in 1996, and infection percentages of wild-type *A. flavus* and *A. parasiticus* were significantly lower in peanuts from both the rice and corn flour-treated subplots, but not in peanuts from subplots treated with Pesta. In 1997, only treatment with the rice formulation resulted in a significant reduction in infection by wild-type strains. There were no statistically significant differences in infection percentages by color mutants in 1996, and in 1997 only peanuts from the rice-treated plots had a significantly higher infection rate by the applied color mutant strains. Nevertheless, aflatoxin concentrations were similarly reduced by all formulation treatments. Poor correlations between infection percentages and aflatoxin contamination have been found in other studies as well (Cole et al., 1985; Hill et al.,

1983; Horn et al., 1994; Sanders et al., 1985). A possible explanation for these poor correlations is that *A. flavus* infection and growth are separate events. Surface disinfection followed by plating of seeds usually shows whether or not *A. flavus* is present inside the seed, but it gives no indication of the degree of *A. flavus* growth that may have occurred.

Infection without subsequent growth by the fungus is possible because of the capacity of peanuts to produce stilbene phytoalexins in response to fungal infection, which inhibit fungal growth (Wotton and Strange, 1985, 1987). This production of phytoalexins occurs when the water activity of peanuts is sufficiently high (>0.95). As the water activity decreases as a result of prolonged drought stress, the capacity for phytoalexin production is lost while sufficient water is still available for *A. flavus* growth and aflatoxin production (Dorner et al., 1989). Therefore, mere infection of peanuts by *A. flavus* and *A. parasiticus* is a poor predictor of aflatoxin contamination because there is no indication of the amount of fungal growth that has or has not occurred. Another problem associated with using infection percentages concerns the method used for surface disinfection of the seeds. When a group of seeds is surface disinfected together, as was done in this study, there is the possibility that all fungal propagules are not killed when an individual seed contains a high level of fungal growth and sporulation. A large number of seeds will, therefore, appear to have been infected when that was not the case. This problem has been reviewed and studied by Sauer and Burroughs (1986), and they concluded that it was not always possible to achieve contact between spores and sodium hypochlorite because of air bubbles, cracks, surface hairs, debris, etc., on seed surfaces.

The primary concern of the present work was to determine the efficacy of different formulations of nontoxigenic strains of *A. flavus* and *A. parasiticus* in reducing aflatoxin contamination of peanuts. Although none of the numerical reductions seen in 1996 was statistically significant, the average reduction by all formulations of 86% coupled with the statistically significant average reduction of 92% in 1997 indicates that all formulations were effective in reducing aflatoxin contamination. The technology of incorporating conidia into granules such as Pesta and pregelatinized corn flour are viable options to consider for commercial production of aflatoxin biocontrol formulations.

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