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Effect of Inoculum Rate of Biological Control Agents on Preharvest Aflatoxin Contamination of Peanuts

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Studies were conducted during 1994 and 1995 in the environmental control plot facility at the National Peanut Research Laboratory to determine the effect of different inoculum rates of biological control agents on preharvest aflatoxin contamination of Florunner peanuts. Biocontrol agents were nontoxigenic color mutants of *Aspergillus flavus* and *Aspergillus parasiticus* that were grown on rice for use as soil inoculum. Three replicate plots (4.0 × 5.5 m) were treated with 0, 2, 10, and 50 g/m of row (0, 20, 100, and 500 lb/acre, respectively) of an equal mixture of the color mutant-infested rice in 1994, and the same plots were retreated in 1995. Aflatoxin concentrations were determined by high performance liquid chromatographic analysis of all peanuts. Treatment means for total kernels in 1994 were 337.6, 73.7, 34.8, and 33.3 ppb for the 0, 2, 10, and 50 g/m treatments, respectively. Regression analysis indicated a trend toward lower aflatoxin concentrations with increasing rates of inoculum ($R^2 = 0.40$; $P < 0.05$). For the same repeated treatments in 1995 aflatoxin concentrations in total kernels averaged 718.3, 184.4, 35.9, and 0.4 ppb. Regression analysis revealed a stronger relationship between inoculum rate and aflatoxin concentrations ($R^2 = 0.66$; $P < 0.05$) in the second year of treatment. Compared with untreated controls, the 2, 10, and 50 g/m treatments produced respective reductions in aflatoxin of 74.3, 95.0, and 99.9% in the second year. The data indicated not only a treatment-related effect, but also that a higher degree of control might be achieved when plots or fields are retreated with biocontrol agents in subsequent years.

Key Words: **biological control; aflatoxin; *Aspergillus flavus*; *Aspergillus parasiticus*; *Arachis hypogaea*; peanut; groundnut; mycotoxin; formulation.**

INTRODUCTION

Aflatoxins are potent hepatotoxic, carcinogenic metabolites of the fungi, *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare (CAST, 1989). The four naturally produced aflatoxins are designated B_1, B_2, G_1, and G_2 and will hereafter be collectively referred to as aflatoxin. Aflatoxin-producing fungi can invade and grow in various agricultural commodities, including peanut (*Arachis hypogaea* L.), resulting in their contamination with aflatoxin. Preharvest aflatoxin contamination of peanuts occurs under conditions of heat and drought stress during the latter stages of the growing season, and when such climatic conditions exist over a wide area of peanut-producing regions, significant contamination of the crop occurs (Blankenship *et al.*, 1984; Cole *et al.*, 1986; Dorner *et al.*, 1989; Hill *et al.*, 1983; Petit *et al.*, 1971; Wilson and Stansell, 1983). Because peanuts are primarily used for food, strict regulatory limits for the amount of aflatoxin in finished peanut products have been established. The U.S. Food and Drug Administration (FDA) has an action level of 20 ppb of total aflatoxins in food products; thus, peanuts contaminated above that level cannot be used for food, which can result in great economic losses to the peanut industry in certain years.

In an effort to reduce or eliminate aflatoxin contamination in peanuts and other crops, several research strategies are being explored, including development of organisms for biological control. Dorner *et al.* (1992) showed that introduction of a nonaflatoxin-producing strain of *A. parasiticus* to peanut soils resulted in aflatoxin reductions in edible-grade peanuts ranging from 83 to 98%. In studies with cotton, Cotty (1994) reported reduced aflatoxin concentrations in cottonseed from plots treated with a nontoxigenic strain of *A. flavus*. In these studies, the introduced strains of *A. flavus* and *A. parasiticus* dominated the soil microflora and preferentially invaded the developing crop at the expense of the native, toxigenic strains. This use of a biocompetitive agent for the purpose of biological control has, thus, showed great promise as a strategy for reducing preharvest aflatoxin contamination.

In previous studies, inoculum of the biocompetitive agent has been delivered by sprinkling an homogenate of fungal cultures over peanut plants (Dorner *et al.*, 1992), by spraying conidial suspensions over cotton plants (Cotty, 1994), and by spreading colonized wheat seed on soil beneath cotton plants (Cotty, 1994). We

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have also tested an inoculum produced by culturing the biocompetitive agent on rice, which was spread over peanut plants. In preliminary studies, the rice formulation proved to be an effective inoculum. The purpose of this two-year study was to determine the efficacy of different rates of a mixture of rice inocula containing color mutants of *A. flavus* and *A. parasiticus* in reducing preharvest aflatoxin contamination.

MATERIALS AND METHODS

Cultures and inoculum preparation. Nontoxigenic color mutants of *A. flavus* and *A. parasiticus* were prepared for use as biocompetitive agents. A strain of *A. flavus* (NPL 45) that was isolated from a peanut in 1991 and found to be incapable of producing aflatoxin or known biosynthetic precursors of aflatoxin was irradiated under ultraviolet light to generate color mutants. A color mutant (NRRL 21368) was chosen that possessed most of the morphological characteristics of the parent strain. Likewise, a toxigenic color mutant of *A. parasiticus* (NRRL 6111), which had proven to be highly competitive in peanut soils (Cole *et al.*, 1986), was irradiated to produce other mutants that were nontoxigenic. Surviving colonies were isolated, grown in pure culture in yeast extract-sucrose (YES) liquid medium, and analyzed by thin layer chromatography (TLC) to identify nontoxigenic strains (Horn *et al.*, 1996). One was chosen (NRRL 21369) for these studies based on the criterion that it possessed most of the morphological characteristics of the parent strain.

Strains were maintained on Czapek agar slants at 5°C. 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) 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. Rice inoculum was then dried in a shallow pan in a forced-air draft oven at 50°C for 6 h or until the moisture content was $\leqq$ 7%. Rice inoculum was stored at 5°C until used.

Test plots. In both 1994 and 1995 the environmental control plot facility at the National Peanut Research Laboratory (Dawson, GA), was used to test different inoculum levels of color mutant-infested rice. 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 main plots of 12.0 × 5.5 m were each subdivided into three 4.0 × 5.5 m subplots by placing two partitions across the plots. Florunner peanuts were planted on April 19, 1994 and April 26, 1995 with six rows per plot. Each treatment was replicated three times, and replicates were completely randomized. Treatments included untreated control and equal mixtures of the *A. flavus* and *A. parasiticus* color mutant-infested rice inoculum applied at 2 g/m of row (20 lb/acre), 10 g/m (100 lb/acre), and 50 g/m (500 lb/acre). Rice inoculum was applied by sprinkling the preweighed amount over each row at 23 days after planting (DAP) in 1994. In 1995 the same subplots were reinoculated at the same rate at 47 DAP.

Peanuts were grown according to practices recommended by the Georgia Cooperative Extension Service until the time drought stress was imposed. In 1994, peanuts received a final irrigation at 86 DAP, soil temperature modification began 104 DAP, and plants were dug by hand 146 DAP. In 1995, final irrigation occurred 82 DAP, soil temperature modification began 90 DAP, and plants were dug 145 DAP. 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 and combined as a single category for each subplot.

Microflora analyses. In 1994 only, soil and peanuts were examined, respectively, for populations and colonization percentages of wild-type *A. flavus* and *A. parasiticus* as well as the color mutants. These determinations were not made in 1995. Samples of soil from each subplot were taken immediately after planting, 101 DAP, and immediately prior to harvest to determine fungal populations in the geocarposphere. 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). Dilution platings were made on plates containing 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, the jumbo, medium, and number 1 size categories were separately riffle divided four times to yield a subsample of approximately 6.25% for plating. The number of kernels plated varied and ranged from about 80 to 350 kernels per subsample, depending on the quantity of peanuts in each category (see Table 1 for weight of kernels in each category). All kernels for plating were surface sterilized 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 7 days at 30°C.

Aflatoxin analyses. After shelling, all peanuts from each subplot (except those used for microflora analysis)

were analyzed for aflatoxin by the high performance liquid chromatography (HPLC) method of Dorner and Cole (1988) with certain modifications. Peanuts were not subsampled for 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_{18} 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_1 and G_1, 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).

Statistics. To determine aflatoxin concentrations for combined categories (i.e., edible = jumbo + medium + number 1 + splits; inedible = oil stock + damage; 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) in the relevant categories was divided by the total kernel weight (g) of those categories. Aflatoxin concentrations were transformed to log (aflatoxin + 1) and data were subjected to regression analysis using the general linear models procedure of SAS. Soil population data for 1994 were log transformed and peanut colonization percentages for 1994 were transformed to the arc sine of the square root for regression analyses. Pearson product-moment correlation coefficients were determined between soil population and kernel colonization and between kernel colonization and aflatoxin contamination.

RESULTS AND DISCUSSION

Mean kernel weights and mean aflatoxin concentrations for each seed-size category for 1994 are presented in Table 1. Mean weights were similar for each category across the four treatments, except for an unusually high number of splits in the 50 g/m treatment. Splits are not a true size category, but they are generally larger-size kernels that split during shelling. The large number of splits in the 50 g/m treatment seems to correspond with a slight reduction in the percentage of total peanuts from that treatment in the jumbo- and medium-sized categories, which indicates that more of those peanuts split during shelling than did the larger-sized peanuts from the other treatments. The medium-size category had the greatest weight followed by the jumbo, number 1, oil stock, splits, and damage. Aflatoxin concentrations were quite variable, but they generally followed the pattern of less toxin in the larger seed size categories and the greatest concentration of toxin in the damaged kernels.

A summary of the analysis of the aflatoxin results for edible, inedible, and total kernels categories for 1994 appears in Table 2. With the exception of the 24.9 ppb value for edible peanuts from the 50 g/m rate, aflatoxin concentrations tended to decrease with increasing rates of biocontrol inoculum. Results of regression analysis for the edible category were not significant; however, regression analyses within the inedible and total kernels categories revealed a significant trend ($P < 0.05$) of lower aflatoxin concentrations with increasing inoculum rates. The R-squares of 0.51 and 0.40 for the inedible and total kernels categories were not strong, but given the extreme variabilities historically associated with aflatoxin contamination (Whitaker *et al.*, 1992, 1994; Dorner and Cole, 1997), any statistically significant trend or difference is noteworthy. The trend was stronger in the inedible category than in the total kernels category, which would be expected because the

TABLE 1

Mean Kernel Weights (g) and Mean Aflatoxin Concentrations (ppb) in Different Seed Size Categories for 1994

	Treatment[a]							
	Control		2 g/m		10 g/m		50 g/m	
Seed size	Weight	Toxin	Weight	Toxin	Weight	Toxin	Weight	Toxin
Jumbo	1032.1	36.9	1278.4	0.3	1275.2	0.2	1121.9	0.2
Medium	2187.1	62.2	2514.7	3.4	2425.6	1.0	2174.0	39.1
Number 1	646.0	0.6	662.3	25.3	669.3	3.2	605.0	37.7
Splits	47.9	312.8	82.6	0.5	96.4	1.8	248.0	0.0
Oil Stock	280.1	165.5	264.3	71.8	290.0	8.4	303.8	113.7
Damage	46.1	32,836.8	68.9	4203.8	66.8	1760.7	49.0	137.7

[a] Values are the means of three plots per treatment.

TABLE 2

Aflatoxin Concentrations (ppb) in Edible, Inedible, and Total Kernels Categories of Peanuts Grown in Plots Treated with Different Rates of Inoculum of the Biocontrol Agents in 1994[a]

Inoculum rate	Edible[b]	Inedible[c]	Total[d]
50 g/m	24.9	118.9	33.3
10 g/m	1.1	448.9	34.8
2 g/m	6.7	1042.8	73.7
Control	44.9	4202.3	337.6
R-square	0.05	0.51[e]	0.40[e]

[a] Values are the mean of three plots.
[b] Edible, the weighted average of the combined jumbo, medium, number 1, and sound split seed size categories.
[c] Inedible, the weighted average of the combined oil stock and damaged categories.
[d] Total, the weighted average of all seed size categories, including damage.
[e] Results of regression analysis significant at $P < 0.05$.

total category includes the 24.9 ppb concentration in the edible peanuts from the 50 g/m rate. Overall, these data indicated that treating soil with the biocompetitive fungi had the effect of reducing aflatoxin in peanuts and that there was a mild effect of higher inoculum rates resulting in lower aflatoxin concentrations.

Soil populations of wild-type *A. flavus/parasiticus* and introduced color mutants are presented in Table 3. Regression analysis showed no significant trend in the population of wild-type *A. flavus/parasiticus* as related to treatments with different inoculum rates. Although a moderate increase in the population of wild-type *A. flavus/parasiticus* in the control treatments appeared to occur between planting and harvest, it was not significant and not as dramatic as has been observed in the past (Dorner *et al.*, 1992). High populations of the color mutants were found at the midseason and harvest samplings, particularly in the 10 and 50 g/m treatments. However, this apparently did not affect the final populations of wild-type *A. flavus/parasiticus*, as there was no significant difference in those populations among treatments at harvest.

Regression analysis of the kernel colonization percentages, presented in Table 4, indicated that inoculum rate had no significant effect on the percentage of kernels colonized by either wild-type *A. flavus/parasiticus* or the color mutants. The average colonization by wild-type *A. flavus/parasiticus* in control plots of 9.4% appears higher than those from the treatments, but that value was skewed by an unusually high colonization in one plot of 21%. Importantly, total colonization of peanuts by both wild-type *A. flavus/parasiticus* and the color mutants was not increased by addition of the large population of color mutants in treated plots.

The fact that there was no significant effect of inoculum rate on the soil populations of wild-type *A. flavus/parasiticus* at harvest or on the colonization of peanuts by wild-type *A. flavus/parasiticus* is interesting, given the effect of inoculum rate on aflatoxin contamination in the total and inedible categories (Table 2). However, there were significant positive correlations between soil populations of wild-type *A. flavus/parasiticus* and percentage of kernels colonized by wild-type *A. flavus/parasiticus* ($r = 0.77$; $P < 0.01$) and between percentage of kernels colonized and aflatoxin in the total kernels category ($r = 0.71$; $P = 0.01$). Taken together, these data indicate that introduction of the competitive color mutants is having an impact on aflatoxin contamination of peanuts in the first year of inoculation, but that the effect may not be totally explained simply by the effect of the color mutants on the populations of wild-type *A. flavus/parasiticus* in the soil or on the initial colonization of peanuts by wild-type *A. flavus/parasiticus*.

Mean kernel weights and aflatoxin concentrations for 1995 are presented in Table 5. Weights were similar to those in 1994, and aflatoxin concentrations also showed a similar pattern as a function of seed size, with concentrations generally increasing with decreasing seed size in the control and 2 g/m treatments. Aflatoxin concentrations were uniformly low in the 10 and 50 g/m treatments, with the exception of the damage category in the 10 g/m treatment. The concentration of only 45.5

TABLE 3

Populations (CFU/g) of Wild-Type *A. flavus/parasiticus* and Color Mutants of *A. flavus/parasiticus* Used as Biocontrol Agents in Soils Sampled Immediately after Planting, 101 Days After Planting (DAP), and Immediately Prior to Harvest in 1994

	Planting		101 DAP		Harvest	
Inoculum rate	AF[a]	CM[b]	AF[a]	CM[b]	AF[a]	CM[b]
50 g/m	138	0	80	12,333	358	14,667
10 g/m	147	0	93	17,083	331	4915
2 g/m	106	0	89	613	343	2101
Control	149	0	228	16	1682	530

[a] The mean CFU/g of wild-type *A. flavus* and *A. parasiticus* from three plots.
[b] The mean CFU/g of color mutants of *A. flavus* and *A. parasiticus* from three plots.

TABLE 4

Percentage of Kernels Colonized by Wild-Type *A. flavus/parasiticus* (AF) and Color Mutants of *A. flavus/parasiticus* (CM) Used as Biocontrol Agents in 1994

Inoculum rate	AF	CM	Total
50 g/m	0.9	8.4	9.3
10 g/m	1.0	4.9	5.9
2 g/m	3.1	9.6	12.7
Control	9.4	3.9	13.3

TABLE 5

Mean Kernel Weights (g) and Mean Aflatoxin Concentrations (ppb) in Different Seed Size Categories for 1995

	Treatment[a]							
	Control		2 g/m		10 g/m		50 g/m	
Seed size	Weight	Toxin	Weight	Toxin	Weight	Toxin	Weight	Toxin
Jumbo	1102.2	8.5	1134.2	2.0	1177.2	0.3	1395.0	0.0
Medium	2155.0	100.3	2173.0	24.3	2344.4	8.5	2175.0	0.1
Number 1	686.5	257.5	702.6	53.0	734.9	0.2	669.0	0.0
Splits	129.2	84.0	140.9	1.1	141.0	0.0	139.2	0.0
Oil Stock	263.9	1096.7	256.9	144.8	276.5	11.0	244.8	0.2
Damage	99.4	18,923.9	99.7	6794.8	111.0	2207.7	66.9	45.5

[a] Values are the means of three plots per treatment.

ppb in the damaged and visibly moldy kernels from the 50 g/m treatment was an impressively low concentration for such peanuts, particularly when compared with damaged peanuts from control plots in both 1994 and 1995. Of the 66.9 g of peanuts in the damaged category from the 50 g/m treatment, approximately 30–40% were visibly molded with *Aspergillus* species. The concentration of 45.5 ppb of aflatoxin in the category is a strong indication that the introduced color mutants preferentially proliferated in those peanuts.

Results of the regression analysis of the 1995 aflatoxin data for the combined edible, inedible, and total kernels categories (Table 6) showed a definite treatment-related effect in all categories. As indicated by the *R*-squares, the trend toward lower aflatoxin with increasing rates of biological control inoculum was stronger in every category compared with 1994. Aflatoxin in total kernels averaged 0.4 ppb in the high-rate treatments compared with 718.3 ppb in the controls. In the poorest quality, inedible category, the mean aflatoxin concentration from the high-rate treatments was only 6.0 ppb compared with 6417.4 ppb in the controls. In edible peanuts there was practically no aflatoxin in the 50 g/m treatments (0.1 ppb), and the 4.5 ppb average in the 10 g/m treatments was also well below the 20 ppb action level set by the FDA. Compared with untreated controls, the treatments with 2, 10, and 50 g/m produced reductions in aflatoxin of 74.3, 95.0, and 99.9%, respectively (total kernel basis).

TABLE 6

Aflatoxin Concentrations (ppb) in Edible, Inedible, and Total Kernels Categories of Peanuts Grown in Plots Retreated with Different Rates of Inoculum of the Biocontrol Agents in 1995[a]

Inoculum rate	Edible[b]	Inedible[c]	Total[d]
50 g/m	0.1	6.0	0.4
10 g/m	4.5	433.0	35.9
2 g/m	22.9	1854.2	184.4
Control	101.6	6417.4	718.3
R-square	0.61[e]	0.58[e]	0.66[e]

[a] Values are the mean of three plots.

[b] Edible, the weighted average of the combined jumbo, medium, number 1, and sound split seed size categories.

[c] Inedible, the weighted average of the combined oil stock and damaged categories.

[d] Total, the weighted average of all seed size categories, including damage.

[e] Results of regression analysis significant at $P < 0.05$.

The greater control of aflatoxin contamination seen in 1995 compared with 1994 supports earlier results (Dorner *et al.*, 1992), suggesting that this type of biological control is more effective in the second year than in the first year of application. This is presumably because the introduced biocompetitive agents have had time to establish themselves to a greater extent. In this study, the same rate of inoculum was used on the same plots each year. Second-year results indicate the probable advantage of multiyear application of biocontrol agents for maximum control of preharvest aflatoxin contamination.

A. flavus and *A. parasiticus* color mutants were used in combination as biocompetitive agents in this study, whereas *A. parasiticus* (NRRL 13539) was used alone as the biocompetitive agent earlier (Dorner *et al.*, 1992). Because *A. flavus* and *A. parasiticus* are closely related species, we originally believed that an aggressive, competitive, nontoxigenic strain of *A. parasiticus* could dominate the soil microflora under hot, dry conditions, thus precluding the need to incorporate nontoxigenic *A. flavus* as well. However, in a study of the effect of *A. parasiticus* soil inoculum on invasion of peanut seeds, Horn *et al.* (1994) reported that even though higher soil populations of *A. parasiticus* compared with *A. flavus* were achieved after inoculation with *A. parasiticus*, *A. flavus* still appeared to dominate in peanut seeds. They suggested that using *A. flavus* or a combination of *A. flavus* and *A. parasiticus* might be more effective than using *A. parasiticus* alone. A comparison of the results of the present study with those of Dorner *et al.* (1992) lends support to that suggestion.

Although aflatoxin reductions were apparent using *A. parasiticus* alone (Dorner *et al*., 1992), they did not come nearly as close to elimination of aflatoxin as in the 50 g/m treatment in this study.

The results indicate that a high rate of inoculum applied over 2 years could have a significant impact on preharvest aflatoxin contamination. However, such a high rate is probably impractical from an economic standpoint. The 10 g/m rate yielded a significant reduction in aflatoxin compared to the control in 1995, and the mean aflatoxin concentration in edible category peanuts of 4.5 ppb was well below the FDA action level of 20 ppb. The practical implementation of this biological strategy will depend on the right combination of effectiveness and economic feasibility.

This study offers initial guidelines on the amount of this type of inoculum (color mutant- infested rice) that is needed for different degrees of control. However, much research remains in refining this strategy. More dominant and competitive strains of *A. flavus* and *A. parasiticus* might be found that would offer greater control at lower initial application rates. Formulations other than rice might be more effective in delivering biocompetitive agents to the field. The effect of long-term reapplication of these biocompetitive agents on the populations of wild types of *A. flavus* and *A. parasiticus* is not known. It may be that after an initial high-rate application, subsequent application rates could be reduced while still maintaining control. It is also possible that after several years, nearly absolute control of aflatoxin contamination could be achieved if the wild types are essentially replaced by the introduced strains. Long-term studies in fields following a peanut, corn, and cotton rotation are underway to gain answers to these questions.

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REFERENCES

Blankenship, P. D., Cole, R. J., Sanders, T. H., and Hill, R. A. 1984. Effect of geocarposphere temperature on pre-harvest colonization of drought-stressed peanuts by *Aspergillus flavus* and subsequent aflatoxin contamination. *Mycopathologia* **85,** 69–74.

Blankenship, P. D., Sanders, T. H., Dorner, J. W., Cole, R. J., and Mitchell, B. W. 1989. Engineering aspects of aflatoxin research in groundnuts: Evolution of an environmental control plot facility. *In* "Aflatoxin Contamination of Groundnut: Proceedings of the International Workshop" (S. D. Hall, Ed.), pp. 269–278. ICRISAT Center, Patancheru, A.P. 502 324, India.

CAST. 1989. "Mycotoxins: Economic and Health Risks." Council for Agricultural Science and Technology, 137 Lynn Avenue, Ames, IA 50010. 91 pp.

Cole, R. J., Hill, R. A., Blankenship, P. D., and Sanders, T. H. 1986. Color mutants of *Aspergillus flavus* and *Aspergillus parasiticus* in a study of preharvest invasion of peanuts. *Appl. Environ. Microbiol.* **52,** 1128–1131.

Cole, R. J., Sanders, T. H., Dorner, J. W., and Blankenship, P. D. 1989. Environmental conditions required to induce preharvest aflatoxin contamination of groundnuts: summary of six years' research. *In* "Aflatoxin Contamination of Groundnut: Proceedings of the International Workshop" (S. D. Hall, Ed.), pp. 279–287. ICRISAT Center, Patancheru, A.P. 502 324, India.

Cole, R. J., Sanders, T. H., Hill, R. A., and Blankenship, P. D. 1985. Mean geocarposphere temperatures that induce preharvest aflatoxin contamination of peanuts under drought stress. *Mycopathologia* **91,** 41–46.

Cotty, P. J. 1994. Influence of field application of an atoxigenic strain of *Aspergillus flavus* on populations of *A. flavus* infecting cotton bolls and on the aflatoxin content of cottonseed. *Phytopathology* **84,** 1270–1277.

Dorner, J. W., and Cole, R. J. 1988. Rapid determination of aflatoxins in raw peanuts by liquid chromatography with postcolumn iodination and modified minicolumn cleanup. *J. Assoc. Off. Anal. Chem.* **71,** 43–47.

Dorner, J. W., and Cole, R. J., 1997. Distribution of aflatoxin in grade sample components of farmers stock peanuts. *Peanut Sci.* **24,** 47–51.

Dorner, J. W., Cole, R. J., and Blankenship, P. D. 1992. Use of a biocompetitive agent to control preharvest aflatoxin in drought stressed peanuts. *J. Food Prot.* **55,** 888–892.

Dorner, J. W., Cole, R. J., Sanders, T. H., and Blankenship, P. D. 1989. Interrelationship of kernel water activity, soil temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought-stressed peanuts. *Mycopathologia* **105,** 117–128.

Hill, R. A., Blankenship, P. D., Cole, R. J., and Sanders, T. H. 1983. Effects of soil moisture and temperature on preharvest invasion of peanuts by the *Aspergillus flavus* group and subsequent aflatoxin development. *Appl. Environ. Microbiol.* **45,** 628–633.

Horn, B. W., Dorner, J. W., Greene, R. L., Blankenship, P. D., and Cole, R. J. 1994. Effect of *Aspergillus parasiticus* soil inoculum on invasion of peanut seeds. *Mycopathologia* **125,** 179–191.

Horn, B. W., Greene, R. L., Sobolev, V. S., Dorner, J. W, Powell, J. H., and Layton, R. C. 1996. Association of morphology and mycotoxin production with vegetative compatibility groups in *Aspergillus flavus*, *A. parasiticus*, and *A. tamarii*. *Mycologia* **88,** 574–587.

Joshua, H. 1993. Determination of aflatoxins by reversed-phase high-performance liquid chromatography with post-column in-line photochemical derivatization and fluorescence detection. *J. Chromatogr. A* **654,** 247–254.

King, A. D., Jr., Hocking, A. D., and Pitt, J. I. 1979. Dichloran-rose bengal medium for enumeration and isolation of molds from foods. *Appl. Environ. Microbiol.* **37,** 959–964.

Pettit, R. E., Taber, R. A., Schroeder, H. W., and Harrison, A. L. 1971. Influence of fungicides and irrigation practice on aflatoxin in peanuts before digging. *Appl. Microbiol.* **22,** 629–634.

Scott, P. M. 1995. Natural toxins. *In* "Official Methods of Analysis of AOAC International" (P. A. Cunniff, Ed.), 16th ed., Sec. 971.22, AOAC International, Arlington, VA.

Whitaker, T. B., Dorner, J. W., Dowell, F. E., and Giesbrecht, F. G. 1992. Variability associated with chemically testing screened farmers stock peanuts for aflatoxin. *Peanut Sci.* **19,** 88–91.

Whitaker, T. B., Dowell, F. E., Hagler, W. M., Jr., Giesbrecht, F. G., and Wu, J. 1994. Variability associated with sampling, sample preparation, and chemical testing for aflatoxin in farmers' stock peanuts. *J. AOAC Int.* **77,** 107–116.

Wilson, D. M., and Stansell, J. R. 1983. Effect of irrigation regimes on aflatoxin contamination of peanut pods. *Peanut Sci.* **10,** 54–56.