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Evaluation of selected geocarposphere bacteria for biological control of *Aspergillus flavus* in peanut

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Abstract

Selected bacterial strains isolated from the region of peanut pod development (geocarposphere) and two additional bacterial strains were screened as potential biological control agents against *Aspergillus flavus* invasion and subsequent aflatoxin contamination of peanut in laboratory, greenhouse, and field trials. All 17 geocarposphere strains tested delayed invasion of young roots and reduced colonization by the fungus in a root-radicle assay used as a rapid laboratory prescreen. In a greenhouse study, seven bacterial strains significantly reduced pod colonization by *A. flavus* compared to the control. In a field trial, conducted similarly to the greenhouse assay, pods sampled at mid-peg from plants seed-treated with suspensions of either 91A-539 or 91A-550 were not colonized by *A. flavus*, and the incidence of pods invaded from plants treated with either 91A-539 or 91A-599 was consistently lower than nonbacterized plants at each of five sampling dates. At harvest, 8 geocarposphere bacterial strains significantly lowered the percentage of pods colonized (> 51%) compared to the control. Levels of seed colonization ranged from 1.3% to 45% and did not appear related to aflatoxin concentrations in the kernels.

Introduction

Preharvest invasion of peanut (*Arachis hypogaea* L.) by the aflatoxigenic fungi *Aspergillus flavus* Link:Fr and *A. parasiticus* Speare is a serious problem in peanut production when hot and dry environmental conditions prevail. Although these fungi can invade nearly any developing peanut tissue, colonization of seed is most significant since aflatoxins may be synthesized. Aflatoxins are among the most potent carcinogenic compounds known to humans, therefore, amounts allowed in seed are federally regulated.

Currently, the only measure for reducing *A. flavus* colonization of peanut and aflatoxin production is irrigation, especially in the latter 4–6 weeks of the growing season (Wilson and Stansell, 1983). However, this is not a feasible control method for many growers. Resistance, rotation, addition of soil amendments and pesticide application have been inconsistent in minimizing aflatoxin contamination (Blankenship et al., 1985; Brenneman et al., 1993; Cole et al., 1985a; Pettit and Taber, 1968; Pettit et al., 1971). However, use of these practices may reduce plant stress and limit the incidence of pods and seed invaded by *A. flavus*.

The potential for biological control of preharvest aflatoxin contamination of peanut has recently been demonstrated using a competitive, atoxigenic strain of *A. parasiticus* (Dorner et al., 1992). Atoxigenic strains of *A. flavus* inoculated simultaneously or 24 hours after inoculation with a toxigenic strain reduced aflatoxin levels in both maize (Brown et al., 1991) and cottonseed (Cotty, 1990). These studies were based on the hypothesis that the introduced atoxigenic isolates would exclude or outcompete the toxigenic strain.

Another biological control strategy is the introduction of a biological control agent to the infection court to reduce or eliminate colonization by the target organism, disregarding the effect of that agent on the population of the target organism (Cook and Baker, 1983). The geocarposphere (Garren, 1966) microbial community is the last barrier for *A. flavus* prior to pod colonization. This soil community contains a more tax-

onomically diverse (Kloepper et al., 1992) and numerous (Kloepper and Bowen, 1991) bacterial population than rhizosphere soils or peanut soils devoid of peanut plants. In contrast, the rhizosphere has a greater population and variety of fungal organisms than the geocarposphere or other peanut soil (Kloepper and Bowen, 1991; Kloepper et al., 1992). The presence of aflatoxigenic fungi in both the root and pod zones make both habitats logical sources for selection of biological control agents. However, because the infection court lies at the pod surface and bacteria are more numerous than fungi in this region, geocarposphere bacteria may provide greater protection to pods against invasion by *A. flavus* and subsequent aflatoxin production than native fungi.

Over 150 strains of bacteria isolated from the geocarposphere of peanuts grown in Alabama (Kloepper et al., 1992) have been evaluated in preliminary studies for in vivo biological control against *A. flavus* using a seed and root-radicle assay (see Materials and methods). These assays were developed for peanut by modifying in vivo prescreens designed for detecting antagonists of *Rhizoctonia solani* on cotton (Kloepper, 1991). Eighteen isolates which demonstrated superior performance in the repeated screens were selected for further testing.

The objectives of this study were to evaluate the biological control potential of these 18 bacteria isolated from the geocarposphere and two additional strains not associated with developing pods in laboratory, greenhouse, and field tests for minimizing *A. flavus* colonization of peanut and subsequent aflatoxin contamination.

Materials and methods

Culture, storage, and inoculum preparation of bacteria and fungi

Bacteria used in all experiments were collected in 1991 from the geocarposphere of peanuts grown at the Alabama Agriculture Experiment Station located in Headland, Alabama. Two additional nongeocarposphere strains (91A-634 and GB0-3) were also used. Strain 91A-634 is a *Bacillus thuringiensis* isolate and GB0-3 (Gustafson Inc., Dallas, TX) is a *Bacillus subtilis* strain, both selected for enhanced *A. flavus* control using in vitro techniques. Bacterial isolates were identified by analysis of fatty acid methyl-esters using the Microbial Identification System 'Aerobe Library' software (Newark, Delaware) (Table 1). For long-term storage, bacteria were grown in tryptic soy broth (TSB) (Difco) for 48 h, centrifuged 5 min at 6,000 rpm, resuspended in TSB containing 20% glycerol (TSBG) and frozen in vials at -80°C.

Spontaneous rifampicin-resistant mutants of each strain were obtained by streaking the wild-type strains on tryptic soy agar (TSA) (Difco) amended with rifampicin at a concentration of 100 μg mL^{-1}. Isolates which did not develop resistance at this concentration were subsequently streaked on TSA amended with 50 μg mL^{-1} rifampicin. Colonies phenotypically similar to the parent at either 100 μg mL^{-1} or 50 μg mL^{-1} were selected and stored in TSBG at -80°C.

For the root-radicle assay, single colonies of bacteria were inoculated into centrifuge tubes containing 25.0 mL TSB and incubated for 48 h at room temperature with shaking. Each culture was washed twice with 0.2 *M* phosphate buffer (PB) (pH=7.0) and populations were adjusted to approximately 1×10^8 (log 8) cfu mL^{-1} with PB prior to root-radicle inoculation.

For the greenhouse experiment, cultures of rifampicin-resistant bacteria were prepared as above in TSB. After agitation for 48 h, 0.10 mL^{-1} was spread on TSA plates. Bacteria were suspended in 20.0 mL^{-1}, PB then adjusted to log 8 cfu mL^{-1} with PB.

Inoculum for seed and mid-peg bacterial applications in the 1993 field study was prepared as described above except wild-type isolates were used. Suspensions prepared for the mid-peg application were not adjusted to log 8 cfu mL^{-1} but were added to 7.23 L PB, resulting in final suspensions ranging between log 8 and log 9 cfu mL^{-1}.

Conidia from a toxigenic stock culture of *A. flavus*, isolated from peanut in 1990 and grown on potato dextrose agar (PDA) (Difco), were transferred to fresh PDA. Upon spore development, a single spore was transferred to another PDA plate and grown for 10 days. This single-spore isolate then was transferred to silica gel (Onkau and Sinclair, 1985) for long-term storage at -10°C.

Cultures of *A. flavus* were prepared by spreading infested silica gel particles on petri plates containing PDA. Cultures were grown for 7–10 d at room temperature. Conidial suspensions were made by pipetting 10.0 mL of a sterile 0.01% Triton-X solution onto the culture plate and scraping the culture with a glass rod. The number of conidia mL^{-1} was determined using a Petroff-Hausser counting chamber (Hausser Scientific Partnership, Horsham, PA). Serial ten-fold dilutions were made to achieve either a log 7 conidia mL^{-1}

Table 1. Effect of root-radicle bacterization on growth and colonization by *Aspergillus flavus*[a]

Treatment	Bacterial identification[b]	Hours to appearance of *A. flavus*	Hours to conidiation by *A. flavus*	Visual rating[c]
Water only		144.0**[d]	144.0**	1.00**
91A-503	No match	76.0**	110.4**	2.65**
91A-505	*Pseudomonas chlororaphis*	93.6**	109.6**	2.42**
91A-508	*Alcaligenes eutrophus*	86.4**	119.2**	2.93**
91A-512	*Bacillus megaterium*	94.4*	118.4**	2.40**
91A-520	*Clavibacter michiganense* subsp. *insidiosum*	86.4**	115.2**	2.70**
91A-529	*Pseudomonas aeruginosa*	120.0**	123.2**	2.67*
91A-531	*Enterobacter cloacae*	112.0**	126.4**	2.20**
91A-535	*Curtobacterium flaccumfaciens* subsp. *poinsettiae*	NT[e]	NT	NT
91A-539	*Bacillus laterosporus*	78.4**	120.0*	2.93*
91A-542	*Salmonella enteritidis*	91.2**	129.6**	2.33*
91A-543	*Pseudomonas aeruginosa*	100.8**	118.4**	2.40**
91A-550	*Burkholderia cepacia*	80.0**	115.2*	2.80**
91A-554	*Cellulomonas cartae*	89.6**	110.4*	2.93
91A-558	*Xanthomonas maltophila*	81.6	104.0	3.13
91A-581	*Burkholderia cepacia*	110.8**	133.6**	1.90**
91A-596	*Pseudomonas putida*	110.4**	129.6**	2.33**
91A-599	*Pseudomonas putida*	107.2**	126.4**	2.07**
91A-625	*Pseudomonas saccharophila*	107.2**	120.0**	2.27**
91A-634[f]	*Bacillus thuringiensis*	73.6	96.0	2.73**
GB0-3[f]	*Bacillus subtilis*	86.4*	108.8*	3.20
A. flavus only		38.4	75.2	4.30

[a]Experiments were terminated 144 h after bacteria-treated root-radicles were inoculated with *A. flavus*. Values are the means of three experiments.
[b]Bacteria were identified using the GC-FAME system (Netwark, DE).
[c]Percentage of root-radicle colonized was assessed using a visual rating based on a scale of 1–6 where 1=no coverage and 6=complete coverage.
[d]*, ** indicate a significant difference from the *A. flavus*-only control at p=0.10 and p=0.05, respectively.
[e]NT = not tested.
[f]Nongeocarposphere bacterial strain.

suspension for the root-radicle assay or a log 4 conidia mL^{-1} inoculum density for use in the greenhouse study.

Root-radicle assay

Bacterial strains isolated from the geocarposphere were evaluated for biological control potential against *A. flavus* in a rapid, in vivo prescreen using ungerminated (seed assay) or germinated peanut seed with root-radicles (root-radicle assay). Because of the similarity between the two assays, only methods for the root-radicle assay are described. Peanut seed cv. 'Florunner' were surface-disinfested by soaking in 0.5% sodium hypochlorite for one min followed by a two min soak in tap water. Seed were then germinated in petri plates (60×15 mm) containing water agar in a controlled environment growth chamber (30°C, 95% RH). Seed with root-radicles greater than 1.0 cm long and free of contamination were selected for use. Root-radicles were soaked 5 min in suspensions of bacteria (log 8 cfu mL^{-1}), transferred to water agar plates and returned to the growth chamber. Root-radicles used as positive and negative controls were treated the same way except they were soaked in sterile water. All root radicles except the negative controls were challenged

24 h later with 20 μL of a log 7 conidia mL^{-1} suspension of *A. flavus* and incubated for 144 h. The negative control was inoculated with 20 μL sterile water. This assay in conjunction with the seed assay was used in screening the initial 159 geocarposphere bacterial isolates (Bowen et al., 1992). Upon selection of the best 18 bacterial isolates, only the root-radicle assay was used for continued evaluation. In initial screenings (159 and 70 bacterial isolates), root-radicles and seed were examined once every 24 h after *A. flavus* challenge, through 144 h, to determine time intervals for mycelial appearance, conidiation, and complete coverage of the tissue by *A. flavus*. In the root-radicle assay, data were also collected on the number of root branches and final root length. Final evaluation of the best bacterial isolates (17 isolates) in the root-radicle assay did not include measurements of root branches or final root length, but a visual rating of the amount of root-radicle colonized by the fungus was made at the end of the study (144 hours after *A. flavus* challenge). The percentage of the root radicle colonized by *A. flavus* was assessed using a six point scale: 1=no coverage; 2=0 <25% coverage; 3=25% <50%; 4=50% <75%; 5=75% <100%; and 6=complete coverage. Seventeen of 18 bacterial isolates were screened in three experiments arranged in a completely randomized design with five replications per treatment. Isolate 91A-535 was not tested in these trials.

Greenhouse study

To assess the ability of bacteria to reduce *A. flavus* colonization of peanut under conditions more closely resembling those which may occur in the field, a whole-plant assay was developed for screening bacteria, selected from the in vivo assay, in the greenhouse. Nontreated peanut seed, cv. 'Florunner', were soaked 5 min in either phosphate buffer (control) or suspensions of rifampicin-resistant bacteria (log 8 cfu mL^{-1}) prior to sowing. Seed were sown in 11.4-litre pots containing a sandy loam field soil taken from the site of the 1993 field study. All bacterial strains except 91A-634 and 91A-581 were tested. A randomized complete block design was employed with 21 treatments and four replications. At peak flowering, each pot received a 25.0 mL soil drench of *A. flavus* inoculum (log 4 conidia mL^{-1}). Drought was achieved by allowing plants to wilt then bottom watering with approximately 300 mL water per pot from 70 days after planting (DAP) until 120 DAP (harvest). A mid-peg application of bacteria was made by drenching the soil surface with 25.0 mL of bacterial inoculum (log 8 cfu mL^{-1}). Control treatments received a soil drench of 25.0 mL phosphate buffer. At 120 DAP, whole plants were harvested and soil from the geocarposphere was collected.

Ten grams of geocarposphere soil per pot were added to autoclaved 250 mL Erlenmeyer flasks containing 90 mL of phosphate buffer, shaken for 1 h at 150 rpm and serially diluted. Appropriate dilutions were spiral-plated (Spiral Systems, Inc., Bethesda, MD) on TSA containing 100 mg L^{-1} cycloheximide for total bacterial populations, TSA amended with either 50 or 100 μg mL^{-1} rifampicin and 100 mg L^{-1} cycloheximide for populations of introduced bacteria, and on PDA for total fungal populations. TSA plates were incubated 48 h; TSA plates containing rifampicin were incubated 96 h in the dark; and PDA plates were incubated 7 d at room temperature. To quantify geocarposphere populations of aflatoxigenic fungi, dilutions were spiral-plated on M3S1B (Griffin and Garren, 1974), a selective medium for *Aspergillus* spp., and incubated 7 d at room temperature. Bacteria were enumerated using a laser colony counter and bacterial enumeration software (Spiral Systems, Inc., Bethesda, MD). Populations of total fungi and *A. flavus* were counted visually.

Pegs and pods were removed from harvested plants for assessing the incidence of *A. flavus* on these tissues. Pegs and pods were surface disinfested 1 min in a 0.5% sodium hypochlorite solution, rinsed in tap water for 30 seconds, placed on trays lined with cotton, and misted with a 5.0% saline solution to inhibit bacterial growth. The percentage of pegs and pods with visible *A. flavus* growth was determined after incubation in a controlled environment growth chamber (30°C, 95% RH) for 5 d.

Field biological control experiment

Nontreated 'Florunner' peanut seed were soaked 30 min in either bacterial suspensions or phosphate buffer (control) prior to planting. Nineteen bacterial strains were tested against a control. Isolate GB0-3 was not evaluated. Plots were located in a nonirrigated field with a previous history of *A. flavus* infestation at the Wiregrass Substation of the Alabama Agricultural Experiment Station near Headland, Alabama. Seed were sown on April 29, 1993 in two 7.6-m row plots using a randomized complete block design with 20 treatments and three replications. At mid-peg (7/27), one plant per plot was sampled to determine the effect of seed treatment with bacterial isolates on the inci-

dence of peg and pod invasion by *A. flavus*. After sampling, rows were treated with bacteria (1250 mL row^{-1} of a log 8–9 cfu mL^{-1} suspension) or phosphate buffer (control) at the same rate, using a commercial, hand-held sprayer. Between treatments, the spray containers were disinfested with a 0.5% sodium hypochlorite solution followed by two rinses with tap water. At bimonthly intervals, two plants from each plot were sampled and returned to the lab. Ten pegs and 10 pods were removed from each of the two plants for assessing the incidence of *A. flavus* on these tissues using methods described in the previous section.

Immediately prior to digging on September 21, 1993 (146 DAP), geocarposphere soil samples were collected from three sites within each plot and combined for quantifying the microflora populations. A portion of the pods from each plant was removed by hand for determining aflatoxin content and percentage of seed invaded by *A. flavus*. Analysis of geocarposphere soil and incidence of *A. flavus* on seed was made using methods described above. Pods harvested for determining aflatoxin levels in seed were dried 4 d in a 45°C oven. Total aflatoxin levels were quantitated using the Aflatest™ (Vicam, Somerville, MA) fluorescence column technique.

Data analysis

Treatment means were compared to control means using the Student's t-test procedure for each experiment (SAS Institute, 1985). Percentage of pegs and pods and seed colonized by *A. flavus* were arcsine-transformed prior to statistical analysis. Means resulting from these transformations were retransformed to percentages for presentation.

Results

Root-radicle assay

Seventeen of 19 bacterial strains screened significantly delayed the time to appearance and conidiation by *A. flavus* compared to the positive control (*A. flavus* only) (Table 1). Bacteria delayed fungal appearance from 76.0 h to at least 120 h after *A. flavus* challenge. Root-radicles treated with the fungus only were colonized more rapidly (38.4 h after inoculation). Conidiation of the fungus was delayed at least 20.8 h in the presence of bacteria, compared to the positive control. Sixteen isolates reduced the amount of root-radicle covered by *A. flavus* at the end of the assay (144 h) (Table 1). There was no apparent relationship between time to appearance or time to conidiation by *A. flavus* and the percentage of root-radicle covered.

Greenhouse and field study

The severity of drought imposed in the greenhouse study resulted in poor plant health and low yields of both pegs and pods. The incidence of *A. flavus* on pegs was inconsistent with the percentage of pods colonized among bacterial treatments from mature, greenhouse-grown and harvested field-grown peanuts (Table 2 and 9/21 of Table 3). The incidence of pegs colonized by *A. flavus* ranged from 1.5 to 29.7 percent in the greenhouse trial. In general, a greater percentage of pegs from field peanuts was invaded by the fungus (9/21 of Table 3). Significant reductions in the percentage of pegs colonized were observed among seven bacterial treatments applied in the field. No marked decreases in peg invasion were associated with bacterial treatments applied to peanuts grown in the greenhouse.

The proportion of harvested pods invaded by *A. flavus* was reduced by treatment with certain geocarposphere bacterial isolates in both whole-plant studies (Table 2 and 9/21 of Table 3). However, only 4 strains (91A-512, 91A-535, 91A-542, 91A-558) performed consistently well among the experiments. In the greenhouse trial, 7 bacterial treatments led to a reduction (>57%) in the percentage of pods colonized by *A. flavus* compared to the control. Five strains provided complete protection from *A. flavus* invasion. A comparable reduction (>51%) was observed in mature pods (9/21 of Table 3) from 8 treated plots although none of the treatments resulted in pods free from *A. flavus* invasion.

Mid-peg sampling (7/27) of the field study was conducted just prior to the mid-peg application of bacteria to determine the effect of seed application on peg and pod colonization by *A. flavus*. The incidence of pegs invaded by *A. flavus* was extremely high for all treatments and only significantly reduced by a single isolate (91A-599) (Table 3). Conversely, the incidence of pods colonized by the fungus was much lower. No pods were colonized from plots treated with isolates 91A-539 or 91A-550. Four other strains reduced the incidence of *A. flavus* on pods by greater than 67% relative to the control.

The proportion of pegs and pods invaded two weeks after the mid-peg bacterial treatment (8/11) was insignificant (data not shown). However, by the subse-

Table 2. Incidence of pegs and pods colonized by *Aspergillus flavus* in greenhouse-grown peanuts treated with bacteria at-plant and at mid-peg

Treatment	Percent colonization[a]	
	Pegs[b]	Pods[b]
Control	14.8	61.8
91A-503	15.8	20.2
91A-505	11.0	0.0**[c]
91A-508	18.7	11.7
91A-512	12.9	0.0**
91A-520	3.7	12.7
91A-529	13.8	0.0**
91A-531	4.8	0.0**
91A-535	8.0	9.3*
91A-539	8.4	11.7
91A-542	9.6	14.2*
91A-543	29.7	35.4
91A-550	21.2	11.7
91A-554	4.2	20.6
91A-558	4.8	0.0**
91A-596	2.4	19.1
91A-599	28.2	19.0
91A-625	1.5	16.7
GB0-3	1.8	35.4

[a] Values are the means of the arcsine transformation and retransformed to percent colonization by *A. flavus*.
[b] Incidence was assessed on pegs and pods of 120-day old peanut plants.
[c] * and ** indicate a significant difference from the control at $p=0.10$ and $p=0.05$, respectively.

quent sampling date (8/26) three strains were effective in minimizing both peg and pod invasion by the fungus, 6 other isolates reduced pod colonization only, and another strain lowered peg colonziation only.

Incidence of *A. flavus* on seed taken from harvested pods was reduced only by bacterial strain 91A-596 (data not shown). Levels of aflatoxins from seed did not differ significantly due to any treatment; however, in general, bacterized plants had lower aflatoxins in seed than control plants (data not shown).

Effect of geocarposphere bacterial treatments on indigenous bacterial and fungal populations in the peanut geocarposphere

Populations of bacteria, fungi and *A. flavus* were enumerated to quantify the effect of bacterial application on the peanut pod microflora of greenhouse grown plants. The proportion of rifampicin-resistant bacteria to total bacteria was not determined as a result of repeated contamination of the isolation media with fungi resistant to the incorporated fungicide, cycloheximide. Total bacterial populations in peanut geocarposphere were of similar density regardless of treatment; however, bacterial populations were much lower in the geocarposphere of plants treated with isolate 91A-558 than the control (data not shown). Population densities of bacteria ranged from 4.37 log cfu g^{-1} to 5.01 log cfu g^{-1} of sample. Total fungal populations ranged from 2.23 log cfu g^{-1} to 3.88 log cfu g^{-1} sample. Total populations of fungi were significantly higher for three strains (91A-505, 91A-520, 91A-625) than the control (3.20 log cfu g^{-1} sample) (data not shown). The proportion of the total fungal population represented by *A. flavus* (*A. flavus* population / total fungal population × 100) varied greatly depending upon the bacterial treatment. For the control, 41% of the total fungal population was composed of *A. flavus*, while for some isolates (91A-508, 91A-529, 91A-554), *A. flavus* populations were not detected in the geocarposphere at 120 DAP, and other isolates (91A-503, 91A-596) supported an *A. flavus* population approximately one log unit higher than the control (data not shown).

Populations of microorganisms in the geocarposphere of field-grown peanut at harvest generally were not affected by treatment with biological control candidates (data not shown). Total bacterial populations ranged from 4.81 log cfu g^{-1} to 5.99 log cfu g^{-1} sample. Total fungal populations ranged from log 3.39 log cfu g^{-1} to 4.22 log cfu g^{-1} sample. Of this total, populations of *A. flavus* often accounted for one half of the total. Only two isolates (91A-512 and 91A-531) significantly reduced populations of *A. flavus* in the geocarposphere (data not shown).

Discussion

Selected geocarposphere and nongeocarposphere bacteria tested in laboratory, greenhouse, and field biological control experiments demonstrated control activity against *A. flavus* invasion of peanut tissues. An in vivo root-radicle assay was selected for use as a prescreen prior to greenhouse and field trials for two reasons. The nature of the root-radicle assay allows for the interaction among the host, *A. flavus*, and the biological control agent. Historically, biological control agents have been selected based on in vitro antibiosis (Weller, 1988) even though this characteristic often has not correlated well with biological control performance in the

Table 3. Effect of bacterial treatment on the percentage of *Aspergillus flavus*-invaded pegs and pods from field-grown peanut at 3 samplings dates

Treatment[a]	Percentage of pegs and pods invaded by *A. flavus*[b]					
	7/27		8/26		9/21	
	Peg	Pod	Peg	Pod	Peg	Pod
Control	72.1	43.2	18.3	33.6	31.2	49.4
91A-503	81.1	no pods	13.4	6.2*	34.0	26.8
91A-505	72.1	36.0	28.1	27.3	30.1	24.9
91A-508	61.9	1.1**[c]	3.1*	10.0*	13.7**	30.1
91A-512	75.0	29.0**	14.4	23.8	19.7	23.7*
91A-520	73.6	14.6	21.1	16.2	38.1	22.5
91A-529	58.7	14.6	19.1	7.0*	42.2	38.6
91A-531	59.2	23.1	22.9	12.7	27.5**	51.4
91A-535	53.3	6.7	15.5	8.5*	10.0**	11.5**
91A-539	42.5	0.0**	14.1	10.1**	9.9**	27.5*
91A-542	60.2	5.3	3.5*	5.9*	12.4	16.2**
91A-543	66.9	10.0**	23.8	10.8*	8.7**	38.8
91A-550	43.8	0.0**	8.3	12.9	29.7	28.2
91A-554	64.0	8.2	12.3	11.5*	18.8**	38.6
91A-558	60.2	16.6	10.8	15.7	26.3	25.6**
91A-581	55.1	8.2	8.3*	12.3	13.9	11.2*
91A-596	47.7	28.0	10.0	15.8	22.9	15.3*
91A-599	32.1*	13.9**	4.8*	7.7*	10.8**	17.4**
91A-625	60.1	44.9	11.2	20.7	39.6	30.0
91A-634	88.8	24.1	15.2	20.7	31.9	43.7

[a]Treatments were applied at-plant and at mid-peg.
[b]Values are the retransformed means of arcsine-transformed data of the percentage of pegs and pods colonized by *A. flavus* from 3 replications within a single experiment.
[c]* and ** indicate a significant difference from the control at p=0.10 and p=0.05, respectively.

field (Fravel, 1988). By including the host in the test system, various biocontrol mechanisms could be manifested simultaneously, resulting in a more accurate evaluation of the candidate organism. Secondly, the prescreen is rapid and does not require considerable space. In contrast, an assay designed to examine the biological control effect of candidate organisms on *A. flavus* directly in the geocarposphere would require 2–4 months of plant growth, as well as considerable greenhouse or field facilities.

The root-radicle assay was a good prescreen for detecting bacterial biological control candidates against *A. flavus*. This was indicated in the greenhouse and field by significant reductions in the incidence of pods colonized as a result of treatment with bacteria selected from in vivo studies. Four strains consistently performed well in the two whole-plant studies. A reduction in the number of strains having a significant biological control effect in these whole-plant studies, compared to the root-radicle assay, reinforces the importance of plant testing in screening potential biological control agents.

The percentage of pods colonized by *A. flavus* at harvest was significantly lower with 7 bacterial strains in the greenhouse study (Table 2) and 8 bacterial strains in the field study (Table 3). However, only three strains were able to minimize *A. flavus* invasion in both whole-plant studies. The observed differences among isolate performance between the two whole-plant studies is most likely a result of contrasting environmental conditions. Prolonged drought stress in the geocarposphere of greenhouse-grown peanuts may have lowered survivability of certain introduced isolates as well as some components of the indigenous flora. Drought-tolerant geocarposphere strains, having a selective advantage, could persist and sustain biological control of *A. flavus*. In the field study, rainfall occurred weekly during the last 10 weeks of the growing season. This abundance of

water to the geocarposphere might favor those isolates which were unable to reduce *A. flavus* colonization of pods in the greenhouse when soil water was limiting. Furthermore, excessive moisture could eliminate superiority of drought-tolerant isolates by increasing the numbers of competitive, indigenous microorganisms.

Populations of total bacteria and *A. flavus* in the geocarposphere of greenhouse-grown peanut were approximately a log unit or more lower than the respective populations enumerated from field geocarpospheres. The quantitative differences between the greenhouse and field studies are most likely a result of contrasting water availability as discussed above. Within each study, the presence of *A. flavus* was not detected in pod-zone soils from some treatments; however, this absence of *A. flavus* was related only to significant reductions in pod colonization for one treatment in the greenhouse study and another treatment in the field study. This suggests that the observed control of *A. flavus* on harvested pods by treatment with certain geocarposphere bacteria was not due to direct antagonism.

Aflatoxin content of seed harvested from the field was below the 20 ppb federal limit even though seed colonization was extensive for some treatments. Cole et al. (1985b) reported high incidences of seed colonization by aflatoxigenic fungi without aflatoxin contamination when moisture and temperatures were unfavorable for aflatoxin production. Maintenance of geocarposphere temperatures above 25.7°C but below 31.3°C, with drought stress in the latter third of the growing season, resulted in aflatoxin production even though *A. flavus*-type fungi colonized seed at temperatures above and below this range (Cole et al., 1985b). Thus, for a given level of colonization by *A. flavus*, the level of aflatoxin is affected by environmental conditions (Cole et al., 1985b). Based on the average weekly soil temperatures and total weekly rainfall in the 1993 growing season, conditions conducive to aflatoxin formation were not met for this study. When environmental conditions do not favor disease development, a biological control effect is not observed (Weller, 1988). Therefore, conclusions relative to the benefit of application of these bacterial isolates for aflatoxin reduction could not be made in the year of our study. Nevertheless, the ability of certain isolates to withstand drought and minimize invasion of developing peanut tissues in the greenhouse warrant further study under field conditions conducive to aflatoxigenic fungi and aflatoxin production.

The use of bacterial isolates in combination or individually at different developmental stages, may provide a greater potential for control of *A. flavus*-type fungi and subsequent aflatoxin production. As pointed out by Cook and Baker (1983), "complexes of adapted microorganisms are more stable and have better biological balance than single microorganisms". Furthermore, changes in climatic factors or microbial community structure during the growing season unfavorable to one organism may enhance the biological control activity of the other.

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