



Conidial movement of nontoxigenic *Aspergillus flavus* and *A. parasiticus* in peanut fields following application to soil^{*,†}

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Abstract

The use of nontoxigenic strains of *Aspergillus flavus* and *A. parasiticus* in biological control effectively reduces aflatoxin in peanuts when conidium-producing inoculum is applied to the soil surface. In this study, the movement of conidia in soil was examined following natural rainfall and controlled precipitation from a sprinkler irrigation system. Conidia of nontoxigenic *A. flavus* and *A. parasiticus* remained near the soil surface despite repeated rainfall and varying amounts of applied water from irrigation. In addition, rainfall washed the conidia along the peanut furrows for up to 100 meters downstream from the experimental plot boundary. The dispersal gradient was otherwise very steep upstream along the furrows and in directions perpendicular to the peanut rows. The retention of biocontrol conidia in the upper soil layers is likely important in reducing aflatoxin contamination of peanuts and aerial crops such as corn and cottonseed.

Key words: aflatoxin, *Aspergillus flavus*, *Aspergillus parasiticus*, biological control, spore dispersal, peanuts

Introduction

Peanuts (*Arachis hypogaea* L.) are often invaded before harvest by *Aspergillus flavus* Link and *A. parasiticus* Speare, fungi that produce the carcinogenic aflatoxins. Soil serves as a reservoir for the two species [1, 2] and since peanuts fruit underground, the pods are in direct contact with soil fungal populations. Pods are most susceptible to invasion by aflatoxigenic fungi and subsequent aflatoxin contamination under conditions of late-season drought and elevated soil temperature [3–5]. *A. flavus* is the dominant species in peanuts as well as in aerial crops such as corn and cottonseed; however, *A. parasiticus* also may be an important contributor to aflatoxin contamination, particularly in peanuts [6–8]. *A. flavus* typically produces aflatoxins B₁ and B₂ and often another mycotoxin, cyclopiazonic acid, whereas *A. parasiticus* produces aflatoxins G₁ and G₂ in addition to the B aflatoxins

but not cyclopiazonic acid [9]. The genetic capacity of *A. flavus* to produce aflatoxins and cyclopiazonic acid is extremely variable, with many strains being nontoxigenic, and soil populations of *A. flavus* differ according to geographic region in their toxigenicity [10]. In contrast, *A. parasiticus* typically produces high levels of aflatoxins and nontoxigenic strains are rare [9].

Various management strategies necessary to reduce aflatoxin levels to within regulatory limits for animal feed and human food products result in economic losses to peanut growers, shellers, and processors [11]. Biological control through the application to soil of nontoxigenic strains of *A. flavus* and/or *A. parasiticus* has been used effectively to reduce pre-harvest aflatoxin contamination in peanuts [12, 13] and in corn and cottonseed [14, 15]. With biological control, the applied nontoxigenic strain presumably occupies the same ecological niche as native aflatoxigenic strains. When present at high densities in soil relative to native strains under conditions of drought stress in peanuts, the biocontrol strain colonizes most of the available infection sites in peanut seeds and

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thereby reduces overall aflatoxin contamination, although the precise mechanism of aflatoxin reduction is incompletely understood [16, 17].

Biocontrol strains of nontoxigenic *A. flavus* and *A. parasiticus* are applied to the soil surface as rice or wheat colonized by mycelia [13, 14] or as encapsulated mycelia in alginate pellets [18]. In peanuts, inoculum is applied to soil under the leaf canopy where soil moisture and high relative humidity result in extensive sporulation of the fungus on the substrate surface. Because of the subterranean fruiting of peanuts, it is necessary for the biocontrol strain to reach the pod zone and to remain there when pods become susceptible to invasion by *Aspergillus* species. This study documents the downward movement of conidia of nontoxigenic *A. flavus* and *A. parasiticus* in soil following natural rainfall. The downward movement of conidia observed with rainfall was further verified using controlled precipitation from a sprinkler irrigation system.

Materials and methods

Preparation of inoculum

Nontoxigenic *A. flavus* NRRL 21368 and *A. parasiticus* NRRL 21369, both of which are mutated to produce orange-brown conidia, were used in all experiments. The *A. flavus* color mutant was derived from a wild-type strain that does not produce aflatoxins or cyclopiazonic acid [13]. *A. parasiticus* NRRL 21369 was obtained from the color mutant NRRL 6111, which accumulates norsolorinic acid (a biosynthetic precursor to aflatoxin B₁) as well as B and G aflatoxins [19], through additional mutagenesis to produce a nontoxigenic strain [13]. Both nontoxigenic strains have been used in the biological control of aflatoxin in peanuts and corn [13, 15].

Fungal color mutants were grown on Czapek agar slants for 14 days at 30 °C. Conidia were scraped from the agar surface in 10 ml of sterile water with Tween 20 (50 µl/liter). For producing the inoculum, 2800-ml Fernbach flasks containing 500 g of long-grain rice and 150 ml distilled water were autoclaved and inoculated with 1 ml of conidial suspension (approximately 10⁷ conidia) of a single mutant strain. The cotton-plugged flasks were attached to a circular platform positioned at 75° from horizontal and rotated continuously at 2 rpm to keep the colonized grains separate and to restrict sporulation. The rice was incubated in

darkness at 30 °C for 4 days (*A. parasiticus*) and 5 days (*A. flavus*), oven-dried in shallow pans for 8 h at 50 °C followed by 12 h at 30 °C, and stored at 5 °C in plastic bags.

Rainfall experiment

Field plots. Two nonirrigated peanut fields of approximately 50 ha each in Randolph County, Georgia, were chosen to study the effect of rainfall on the movement of *A. flavus* and *A. parasiticus* conidia in soil. Field A was located 1.4 km south of Shellman and field B was 8.5 km southeast of Shellman. Soil in field A was classified as Greenville sandy loam (clayey, kaolinitic, thermic, Rhodic Paleudults) with a 0 to 2% slope [20]. Soil in field B was classified as Faceville sandy loam (clayey, kaolinitic, thermic, Typic Paleudults) with a 0 to 2% slope. Both soil types consist of sandy loam that is approximately 28 to 32 cm thick and overlays a Bt1 horizon of sandy clay. Fields A and B were planted with the peanut cultivar Georgia Green in rows 0.9 m apart on 29 April and 11 May 1998, respectively. Peanuts were cultivated using standard applications of fertilizer, herbicides, fungicides (for leaf spot), and insecticides [21]. Rainfall was measured with a standard rain gauge at each field.

A 9.2 × 9.2-m plot containing 10 rows of peanuts was delimited near the center of each field. Rice colonized by *A. flavus* and *A. parasiticus* color mutants was mixed in equal proportions by weight and was manually dispersed with a shaker on 10 July over the peanut canopy (45 to 50 cm wide) within the plot. The application rate for each species was 16.3 g/m of peanut row (177 kg/ha).

Soil collection. Two rectangular pits (L 60 cm × W 30 cm × D 40 cm) were excavated within each field plot on June 25 before inoculum application to quantify initial populations of wild-type species from *Aspergillus* section *Flavi* and populations of total filamentous fungi. For monitoring the downward movement of applied *A. flavus* and *A. parasiticus* color mutants, two additional pits were excavated on both 14 August (35 days after inoculum application) and 7 September (59 days). The length of the pits was parallel to the peanut row, with the sampling edge extending down through the pegging zone (approximately 10 cm from the peanut taproots). Soil from the vertical face of the pit was carefully scraped away to remove surface contamination. Using a sterile hollow pipe, a 30-cm horizontal

strip of soil (2-cm wide) was dug 6 cm into the face of the pit at sequential depths of 30, 20, 10, 5, and 0 cm.

The lateral movement of conidia away from the inoculated plots was examined by establishing four transect lines that were perpendicular to each side of the plots. The transect lines extended from the midpoint of the plot sides. The slope of the soil surface along the transect lines was measured with a transit instrument. Since the plots were delimited according to the east-to-west orientation of the peanut rows, east and west transect lines were parallel to peanut rows and north and south transect lines were perpendicular to the rows. Samples consisting of the upper 6 cm of soil (150 cm³ each) were collected from the east and west transects in the furrows between peanut rows. In the north and south transects, soil samples were collected either in the furrows or from under the peanut canopy, depending upon the predetermined distance from the plot. Immediately after inoculum application, soil samples were taken at 0.5 m outside of the plot boundaries. Soil along the transects was subsequently sampled 59 days following plot inoculation, starting at 0.1 m inside of the plot boundary and at intervals of 0.5, 1, 2, 3, 4, 6, 10, 16, 24, 34, 48, 66, 96 and 126 m outside of the plot. All soil samples collected during the experiment were dried in sealed paper bags at ambient temperature, thoroughly mixed, and stored at 5 °C.

Soil plating. For soil samples collected from different depths, 8.3 g of soil was added to test tubes with 25 ml of 0.2% water agar and vortexed for 30 sec. Soil suspensions were diluted with water agar when necessary. Densities of *Aspergillus* species were determined by spreading 0.2 ml aliquots of soil suspension on each of five plates of modified dichloran-rose bengal medium [1] and incubating for 2 to 3 days at 37 °C. Wild-type species belonging to *Aspergillus* section *Flavi* were identified as described by Horn and Dörner [1]. Colonies of *A. flavus* NRRL 21368 were distinguished from those of *A. parasiticus* NRRL 21369 under the stereomicroscope by the paler orange-brown conidia, larger conidial heads, and longer stipes.

Soil samples from along the transect lines were treated in a similar manner, except that 100 g of soil was blended with 300 ml of 0.2% water agar (pre-cooled to 5 °C) at low speed for 1 min in a Waring blender. Soil densities of total filamentous fungi were determined by diluting the above soil suspensions in 100 ml of water agar and spreading 0.2 ml on each of five plates of unmodified dichloran-rose bengal me-

dium [7]. Plates for filamentous fungi were incubated for 4 days at 25 °C.

Sprinkler irrigation experiment

Field plot. A 4.4 × 18.6-m plot was established in a peanut field located 2.1 km north of Sasser, Terrell County, Georgia. Soil was classified as Tifton sandy loam (fine-loamy, siliceous, thermic, Plinthic Paleudults) with a 2 to 5% slope [20]. This soil series consists of sandy loam that is approximately 24 to 30 cm thick and overlays a Bt1 horizon of sandy clay loam. The available water-holding capacity of the sandy loam is 10 to 12 cm/m of soil (3.0 to 3.6 cm of water in the upper 30 cm of soil). The cultivar Georgia Green was planted on 10 May 1999, with five rows running the length of the plot. Following the establishment of young peanut plants, the plot length was divided into six 3.1-m subplots. A barrier of 25-cm aluminum sheeting that was buried 5 cm surrounded each subplot. To protect the plot from rainfall after application of the inoculum, a 5.1 × 21.6-m shelter consisting of an anchored arched framework of PVC pipe covered by a sheet of translucent plastic was constructed over the plot. The edge of the plastic covering was situated 0.5 m above the soil surface to leave a gap for air circulation. The field received 17.2 cm of rainfall between planting and placement of the plastic over the shelter framework on 14 July. Rice inoculum of *A. flavus* and *A. parasiticus* color mutants was applied to peanut plants under the covered plot on 15 July as previously described for the rainfall experiment.

Sprinkler irrigation. Irrigation water was applied using the line source sprinkler technique described by Hanks et al. [22]. With this technique, water is applied in decreasing amounts with increasing distance from the sprinkler line source. One end of the plot (subplot 1) was adjacent to the line source and received the most water; the remaining subplots 2 to 6 extended away from the line source and received decreasing water. Impact sprinklers were spaced at 6.4-m intervals along the line source, and the sprinkler heads were 1.1 m above the soil surface. Four catchment containers (6.1-cm diameter opening) were placed in the center of each subplot parallel to the line source to determine the depth of water applied. Catchment containers were elevated on small stands to keep the openings above the peanut canopy.

The plot was irrigated during the night and early morning to reduce the effects of wind drift and evap-

oration. The plastic covering was removed from the shelter prior to irrigation and the first two sections of the PVC framework (built in 3.1-m sections) also were removed to eliminate obstruction to water exiting the sprinkler nozzles. Irrigation was performed at one-hour intervals that were separated by 0.5 h of no irrigation to minimize ponding. The first irrigation event (26 July) consisted of five one-hour irrigation intervals and the second irrigation event over the same plot (17 August) consisted of four one-hour intervals. Following each irrigation event, the shelter covering was reinstalled to exclude rainfall; therefore, rainfall was entirely excluded from the plot between the first and second irrigation events.

Soil core samples. Movement of color mutants to different soil depths was followed using a cylindrical 7.6 × 30.5-cm core soil sampler (Art's Manufacturing and Supply, Inc., American Falls, Idaho, USA). Three soil cores were removed from separate peanut rows in each of the six subplots 25 to 35 h following the first irrigation event, and the holes were refilled with uninoculated soil. A second set of soil cores similarly was obtained 50 to 60 h following the second irrigation event. Soil cores were removed from near the catchment containers under the peanut canopy (approximately 8 to 16 cm from the peanut taproots) where the colonized rice showed sporulation. Therefore, soil cores were taken approximately 1.5 m from the sprinkler line source in subplot 1 and at 3.1-m increments thereafter in other subplots, with cores from the most distant subplot 6 being 17.0 m from the line source.

A sterilized aluminum retaining cylinder (7.3-cm internal diameter) was inserted into the core soil sampler. The retaining cylinder, which contained the soil column, was divided into 2-cm sections from 0 to 8 cm for the upper portion of soil, followed by 4-cm sections from 8 to 28 cm. Soil from the bottom 2 cm was discarded. Data were reported for the midpoint values (1, 3, 5, 7, 10, 14, 18, 22, and 26 cm) for these sections. After removal of the retaining cylinder from the core soil sampler, the soil column was divided between the sections using monofilament fishing line. Each section of the cylinder plus soil was then transferred to a sterile surface. The center of the section was cut out with a sterile aluminum tube (4.7-cm internal diameter), air-dried in a sealed paper sack, and dilution plated (8.3 g) for colonies of *Aspergillus* species as previously described for the rainfall experiment. The remaining soil from each section was placed in a

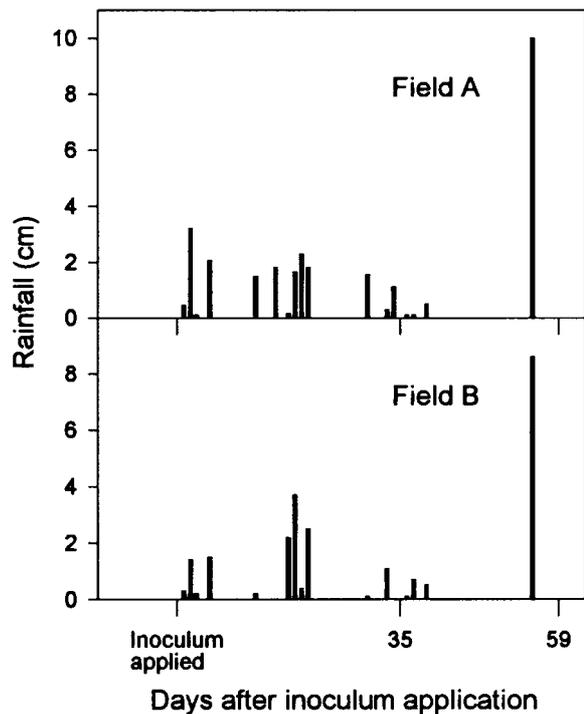


Figure 1. Precipitation in fields A and B during the rainfall experiment for up to 59 days after inoculum application.

plastic bag and used for determining volumetric water content according to the procedure described by Gardner [23].

Statistics. Soil densities of *A. flavus* and *A. parasiticus* from the sprinkler irrigation plot were analyzed with a three-factor ANOVA of log-transformed values followed by Tukey mean separation tests ($P = 0.05$). Data were examined using SAS statistical package, Version 7 (SAS Institute, Cary, North Carolina, USA).

Results

Rainfall experiment

Wild-type populations of species belonging to *Aspergillus* section *Flavi* were dominated by *A. flavus* and *A. parasiticus* before application of inoculum to plots in fields A and B (Table 1). *A. tamaritii* Kita and *A. caelatus* B. W. Horn were less abundant. Soil densities of *Aspergillus* species and total filamentous fungi dropped off dramatically at 30 cm in field A where soil samples were often from the compacted Bt1 horizon, but not in field B where the Bt1 horizon was generally below 30 cm.

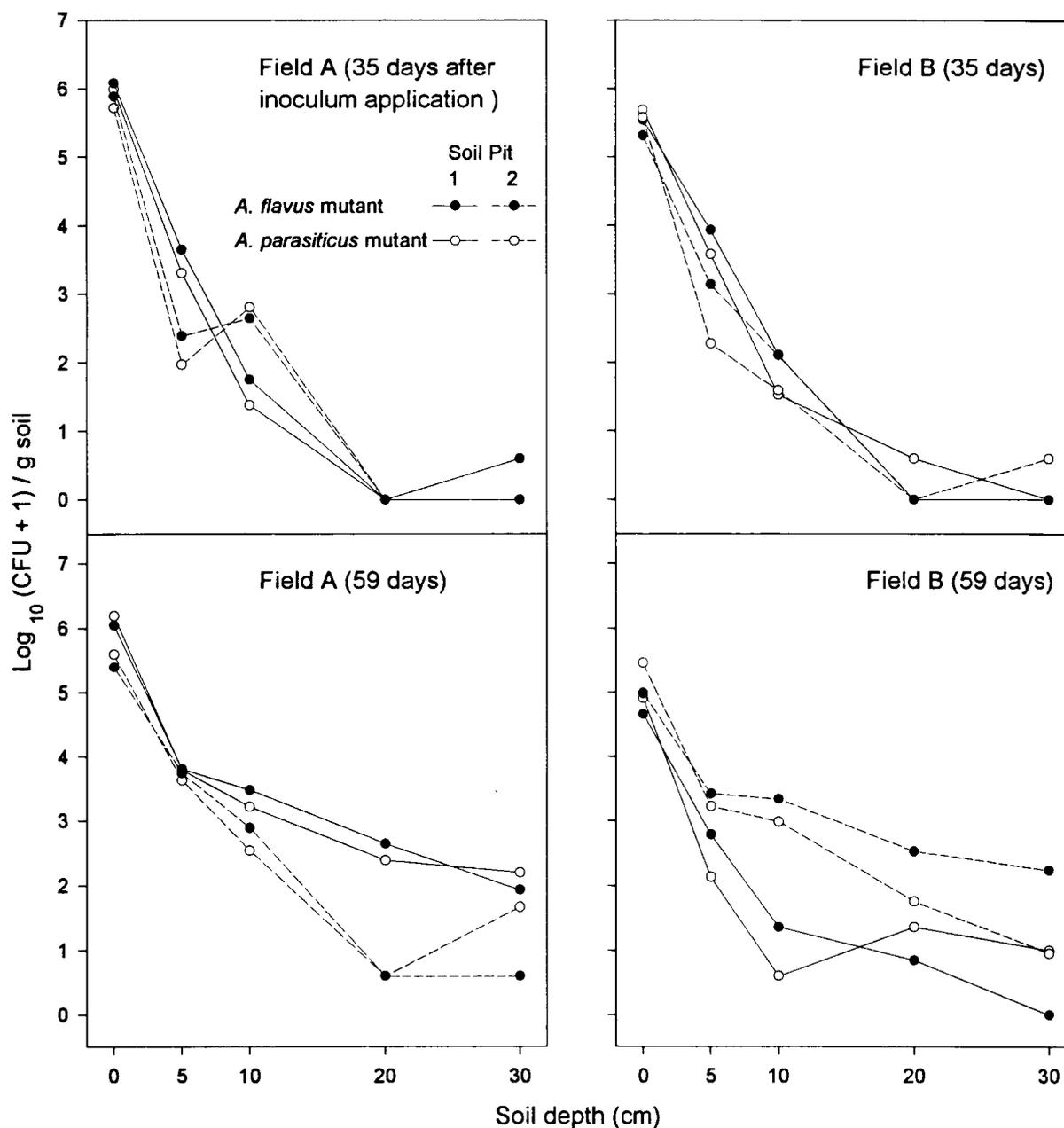


Figure 2. Movement of nontoxic conidial-color mutants of *A. flavus* and *A. parasiticus* to different soil depths in plots from fields A and B during the rainfall experiment 35 and 59 days after addition of inoculum. Shown are data from two excavated pits in each field.

Inoculum consisting of rice colonized by nontoxic *A. flavus* and *A. parasiticus* was applied to plots in fields A and B on the day after a rainfall of 3.6 cm. Following inoculum application, respective cumulative rainfall amounts for fields A and B were 18.0 and 13.6 cm (0 to 35 days after inoculum application) and 10.7 and 9.9 cm (36 to 59 days) (Figure 1).

During the latter period, most of the rainfall occurred on 3 September (55 days after inoculum application). Immature conidial heads were visible on rice grains under the peanut canopy after 24 h and were fully sporulating by 48 h. Rice grains that had fallen between the rows (no canopy) showed little sporulation. Inoculum was observed on the soil surface for up to 2 weeks after

Table 1. Soil populations of wild-type species from *Aspergillus* section *Flavi* before application of nontoxigenic conidial-color mutants

Soil depth (cm)	CFU/g ^a				Total filamentous fungi ($\times 10^3$)
	<i>A. flavus</i>	<i>A. parasiticus</i>	<i>A. tamarii</i>	<i>A. caelatus</i>	
Field A					
0	290 \pm 252.4	75 \pm 41.0	0 \pm 0.0	0 \pm 0.0	73 \pm 16.9
5	154 \pm 141.4	204 \pm 79.2	7 \pm 0.0	42 \pm 50.2	49 \pm 7.5
10	185 \pm 152.0	205 \pm 59.4	23 \pm 4.2	42 \pm 31.8	104 \pm 16.5
20	120 \pm 41.7	172 \pm 68.6	10 \pm 4.2	23 \pm 14.1	81 \pm 8.1
30	0 \pm 0.0	0 \pm 0.0	0 \pm 0.0	0 \pm 0.0	5 \pm 1.2
Field B					
0	65 \pm 46.0	439 \pm 299.1	0 \pm 0.0	24 \pm 12.0	57 \pm 19.9
5	104 \pm 73.5	263 \pm 105.4	0 \pm 0.0	23 \pm 22.6	56 \pm 3.8
10	114 \pm 77.8	309 \pm 202.2	2 \pm 2.1	12 \pm 6.4	65 \pm 3.2
20	73 \pm 25.4	265 \pm 21.2	0 \pm 0.0	15 \pm 16.3	45 \pm 17.2
30	52 \pm 73.5	75 \pm 106.1	0 \pm 0.0	7 \pm 9.2	35 \pm 42.3

^aMean \pm SD based on soil samples from two excavated pits.

application; thereafter, the rice grains had deteriorated to the point where they could no longer be detected.

Thirty-five days following inoculum application to fields A and B, densities of mutant *A. flavus* and *A. parasiticus* were highest in the upper 2 cm of soil, dropped sharply to <700 CFU/g at a depth of 10 cm, and were very low (<5 CFU/g) at 20 and 30 cm in depth (Figure 2). Mutant densities showed a similar pattern 59 days after application of inoculum, except that higher densities (<500 CFU/g) at 20 and 30 cm were sometimes observed. An underlying Bt1 horizon consisting of sandy clay was present in both fields starting at a depth of 26 to 32 cm; peanut feeder roots were often observed to a least 30 cm in depth.

Peanut rows in fields A and B were planted in an east-to-west direction and the soil surface sloped downward (<1.5%) toward the east and west, respectively (Figure 3). Mutants of *A. flavus* and *A. parasiticus* were not detected in soil in any direction at 0.5 m outside of the plot boundary immediately following application of the rice inoculum. During the first two weeks following soil inoculation, a small amount of sporulating inoculum from under the peanut canopy washed into the furrows and subsequently 2–5 m downslope from the plot boundary. Fifty-nine days after applying the inoculum to the plot in field A, *A. flavus* and *A. parasiticus* mutants were present at approximately 3300 CFU/g in furrows between peanut rows 96 m east of the plot boundary (Figure 4). In field B, mutants were detected at approximately 100 CFU/g

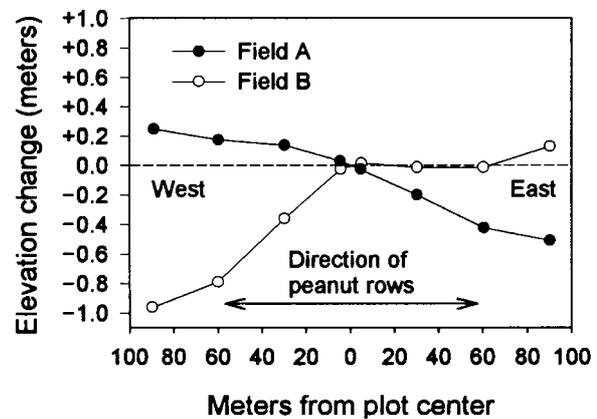


Figure 3. Elevation change in fields A and B relative to the center of each plot in the rainfall experiment; east-to-west direction of the peanut rows is indicated.

in furrows that were 96 m west of the plot boundary. In both fields, long-distance dispersal coincided with the downward slope of the soil surface in the direction of the peanut rows (Figure 3). Both fields leveled off at 126 m and color mutants were not detected at this distance. Mutant densities in soil generally decreased to <50 CFU/g at 1 to 10 m from the plot boundaries in other directions that were either upslope along the furrows or were perpendicular (north and south) to the direction of the peanut rows (Figure 4).

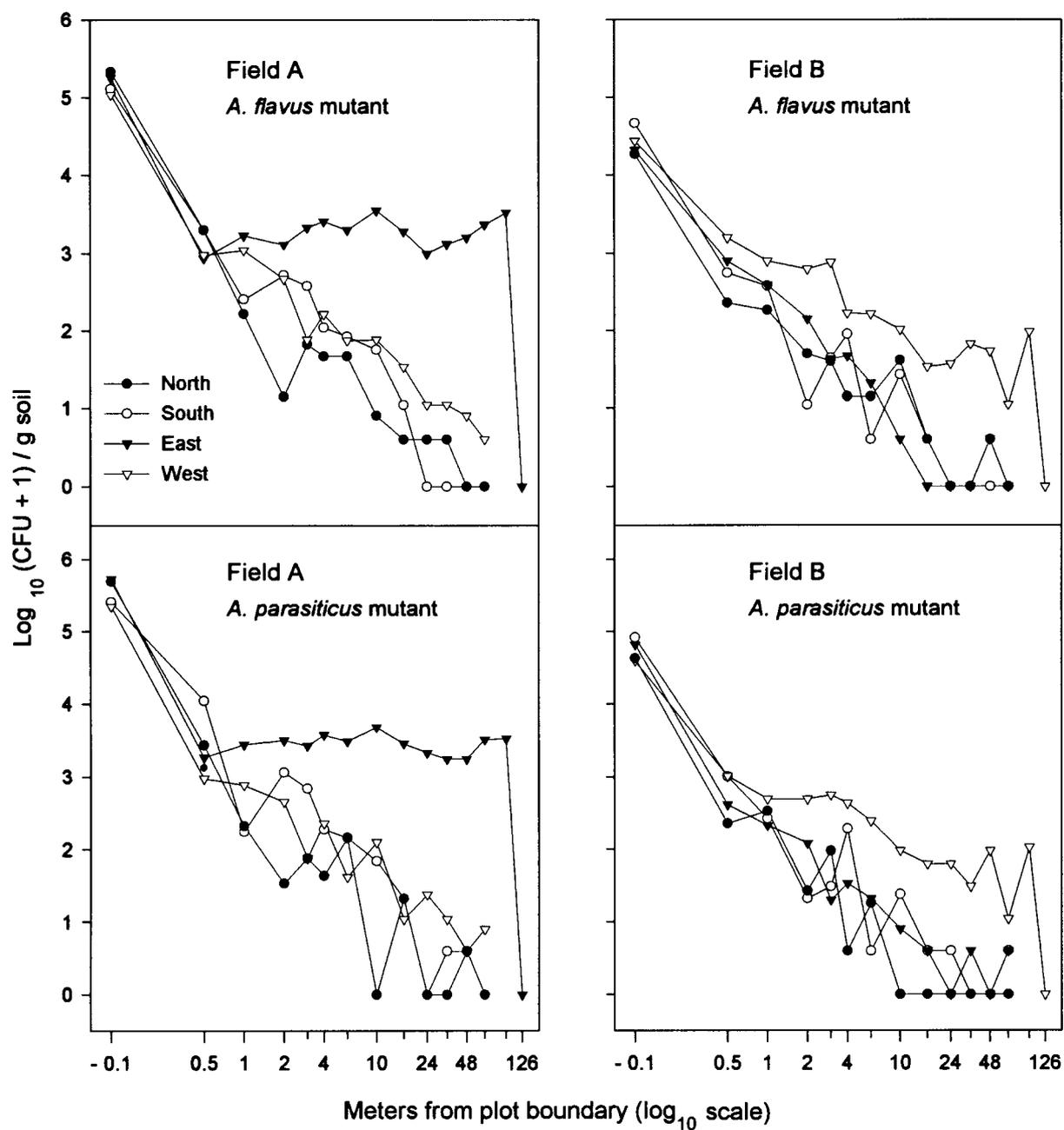


Figure 4. Lateral movement of nontoxicogenic conidial-color mutants of *A. flavus* and *A. parasiticus* in fields A and B during the rainfall experiment. The upper 6 cm of soil was sampled along transects that extended north, south, east, and west of the plot boundary 59 days following application of fungal inoculum to the surface of the plot.

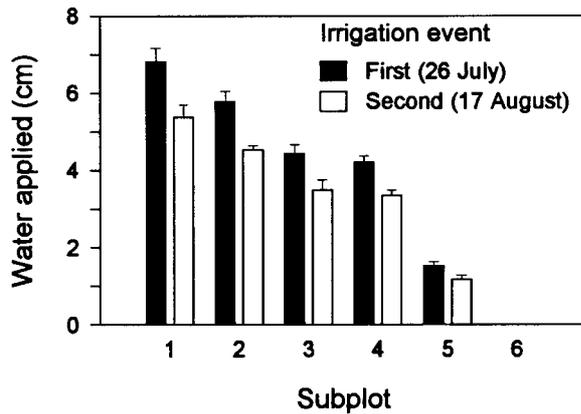


Figure 5. Amount of water applied from the sprinkler irrigation system during two separate irrigation events. Subplots 1 to 6 are located increasing distances from the irrigation source; the amount of applied water was measured in the center of each subplot. Bars represent the mean \pm SD of four measurements.

Sprinkler irrigation experiment

Rice grains colonized by mycelium of nontoxigenic conidial-color mutants of *A. flavus* and *A. parasiticus* were densely covered with sporulating heads after 48 h under the peanut canopy. During the first and second irrigation events, water applied from the line source sprinklers decreased in a linear manner with increasing distance from the sprinkler heads (Figure 5). Subplot 6 furthest from the line source served as a control and did not receive any water. The low standard deviations indicate that water obtained from catchment containers was fairly uniform within each subplot.

Water content by soil depth following the first and second irrigation events is shown in Figure 6. Both irrigation events resulted in a higher soil water content for subplots 1 to 4 compared to subplots 5 and 6 due to the gradient of decreasing water with increasing distance from the irrigation line source (see Figure 5). The overall water content values of the first irrigation event were slightly higher than those of the second irrigation event (Figure 6), which corresponds to the different periods of evapotranspiration between water application and soil sampling in the first irrigation event (25 to 35 h) and second irrigation event (50 to 60 h). The water content of soil in subplot 6 (control, no water applied) after the first irrigation event increased with depth due to soil moisture conditions established by rainfall before erection of the shelter.

Soil densities of nontoxigenic *A. flavus* and *A. parasiticus* were highest (6.0×10^5 to 1.7×10^6

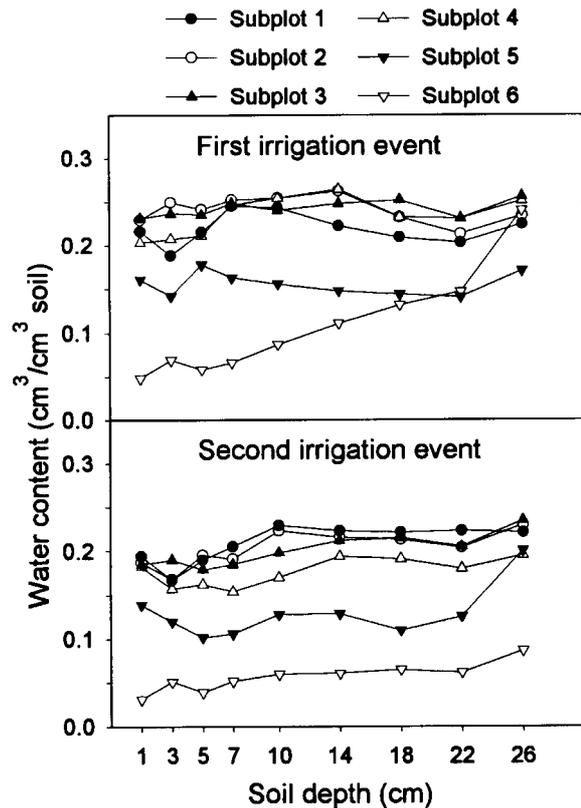


Figure 6. Soil water content at different depths following sprinkler irrigation on 26 July (first irrigation event) and 17 August (second irrigation event). Subplots 1 to 6 are located increasing distances from the irrigation source that correspond to decreasing amounts of water applied (see Figure 5). Each datum point is the mean of three soil samples.

CFU/g) in the upper 2 cm of soil following both irrigation events (Figure 7). The first application of water resulted in a steep density gradient according to soil depth, with densities generally decreasing to <1000 CFU/g at 3 to 5 cm and to <100 CFU/g at 5 to 10 cm (excluding subplot 6 control where no water was applied). With soil depths of 3 to 7 cm in the first irrigation, densities of nontoxigenic *A. flavus* and *A. parasiticus* appeared to increase slightly with increasing amounts of applied water in the subplots (Figure 7). However, at any given soil depth, densities were significantly different ($P \leq 0.05$) among subplots only between the nonirrigated subplot 6 and subplots 1 to 5 at depths of 3, 5 and 7 cm. There were no significant differences in fungal density between the first and second irrigation events in *A. flavus* [$F(1, 216) = 0.01$; $P > 0.05$] and *A. parasiticus* [$F(1, 216) = 3.23$; $P > 0.05$].

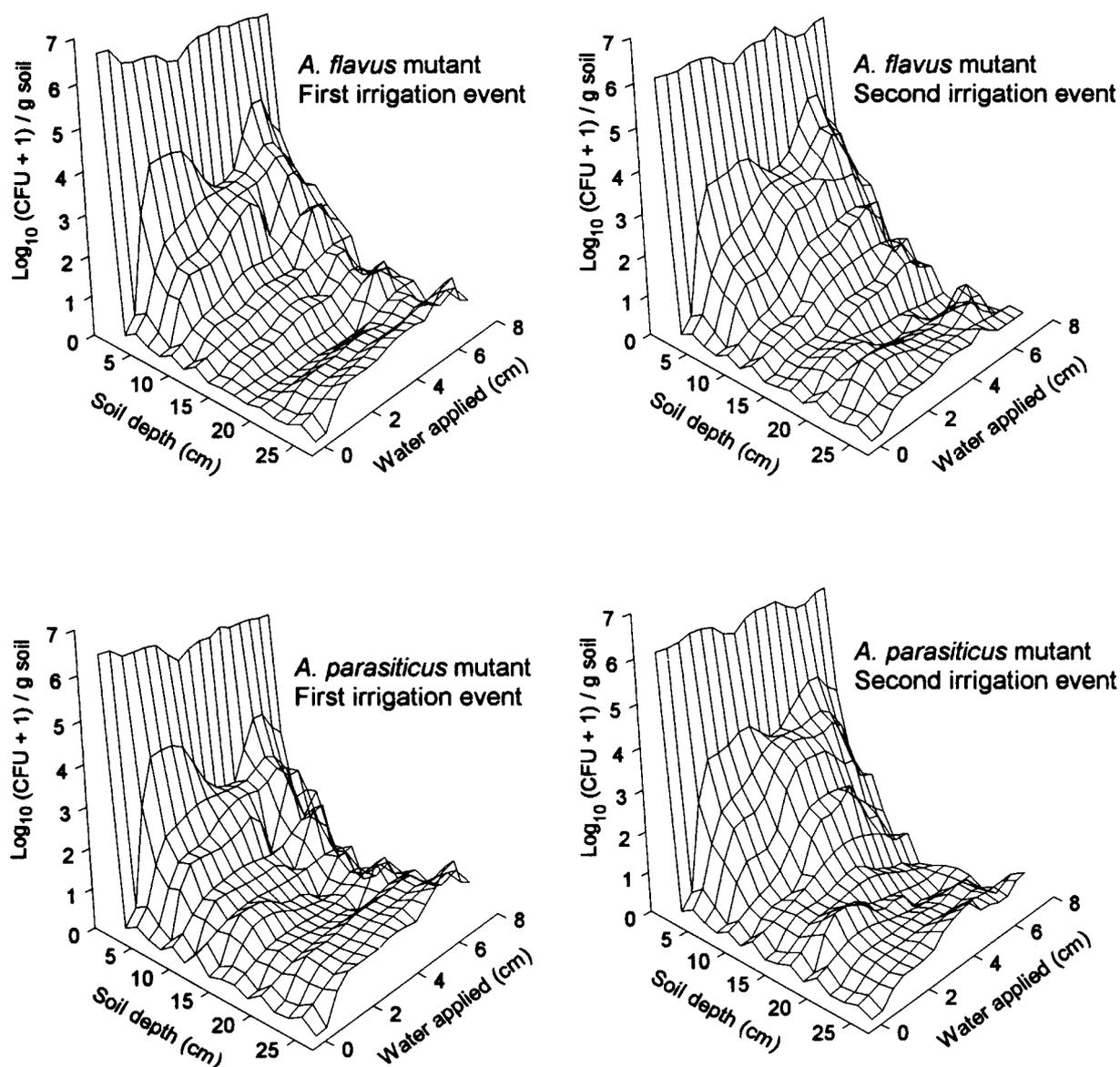


Figure 7. Movement of nontoxicogenic conidial-color mutants of *A. flavus* and *A. parasiticus* to different soil depths after separate sprinkler irrigations on 26 July (first irrigation event) and 17 August (second irrigation event). Densities of mutants are the mean of three soil samples, and water amounts are the mean of four measurements in each of six subplots (see Figure 5).

Discussion

Downward movement of conidia

In the rainfall experiment, moderately high wild-type populations of *A. flavus* and *A. parasiticus* were initially present in soil to the compacted Bt1 horizon, or well beyond the depth of 6 cm for peanut pod development [24]. Therefore, effective biological control may depend on the movement of conidia of non-

toxicogenic *A. flavus* and *A. parasiticus* from the soil surface to the pods. In soil samples with high densities of nontoxicogenic color mutants, conidia of *A. parasiticus* were recognizable and abundant under the microscope, which suggests that colonies obtained from dilution plating represented conidia originating from the rice inoculum. However, some of the colonies also could be the result of secondary invasion of soil organic matter.

Nontoxigenic *A. flavus* and *A. parasiticus* were applied to three different soil types, two of which were exposed to natural rainfall and the third to sprinkler irrigation. In all cases, conidial densities were highest in the top layer of soil and relatively few conidia were present below 10 cm. Soil characterization data from the sprinkler irrigation plot indicate that the upper 30 cm of soil had a water-holding capacity of approximately 3.0 to 3.6 cm. Water applied to subplots 1 to 4 during each of the two irrigation events exceeded the water-holding capacity of the soil; hence, drainage below 30 cm should have occurred. The flow of water through soil clearly had a minimal effect on the downward movement of conidia from the soil surface.

The dry, powdery conidia of *Aspergillus* and *Penicillium* species are strongly hydrophobic and thus do not readily suspend in water without the addition of a surfactant. In a laboratory study in which water was applied to a soil column, greater than 98% of *A. flavus* conidia remained in the surface layer and very few reached a depth of 10 to 15 cm [25]. Similar results were obtained with *P. cyclopium* Westling following the addition of water to a sand column [26]. Furthermore, Dobbs and Hinson [27] showed that conidia of *P. nigricans* Bainier (Thom) retained their hydrophobic properties after 5 weeks of incubation in moist soil. The latter two studies involving *Penicillium* species also demonstrated that fungal spores with a mucilaginous coating are readily suspended in water and as a consequence, easily pass through a sand column. Therefore, the restriction of nontoxigenic *A. flavus* and *A. parasiticus* conidia to the uppermost soil layers despite repeated rainfall and applied irrigation water may be due to their hydrophobicity.

Soil properties also greatly influence the movement of fungal spores, and even wetttable mucilaginous spores often show limited movement through some types of soil following application of water [28, 29]. Adjacent particles of soil create pore spaces which are interconnected by narrow openings called necks. For spores to pass through soil, they must travel through a microscopic streamline created by a series of water-filled pores [30, 31]. Downward movement ceases if a spore encounters either an air-filled pore or a neck diameter that is too small for passage. Wilkinson et al. [31] demonstrated that the downward movement of zoospores and cysts of *Phytophthora megasperma* Drechs. decreased as the percentage of fine soil particles increased; hence, spores moved very little in silt loam but were recovered to a depth of 30 cm in sand. Similarly, Krauss and Deacon [29]

examined the movement of mucilaginous spores from the soil surface beneath peanut plants of a pimaricin-resistant mutant of *Mucor hiemalis* Wehmer. The fungus was not detected below 22 cm in nonplanted soil despite high amounts of tropical rainfall (36 cm over the 51-day course of the experiment). They concluded that under conditions of high soil moisture, the small neck diameters associated with soil pores were the limiting factor in spore movement. In contrast, spores were transported up to 60 cm along the peanut taproot (assayed by root colonization), presumably in water films surrounding the root cortex. Spores also move to considerable depths through soil cracks; however, earthworm channels may remain air-filled even following heavy rain [30]. Further work is necessary to separate the effects of soil properties and conidium hydrophobicity in the retention of nontoxigenic *A. flavus* and *A. parasiticus* near the soil surface.

Lateral movement of conidia

Despite the minimal slope in the two peanut fields used in the rainfall experiment, surface runoff along peanut furrows following rainfall dispersed nontoxigenic *A. flavus* and *A. parasiticus* downstream for nearly 100 m from the treated plots. Conidia were most likely the unit of dispersal since the rice inoculum was washed only a short distance from the plots. Additional research is needed to determine whether the conidia are freely dispersed with water or are bound to soil particles during dispersal. Café-Filho and Duniway [32] and Neher and Duniway [33] also have shown that plant pathogenic *Phytophthora* species are dispersed from point sources for up to 70 m downstream along irrigation furrows. In this study, the hydrophobicity of conidia and their resultant retention near the soil surface may have contributed to the lateral dispersal due to surface water runoff.

Dispersal gradients in this study that were either upstream along the furrows or in directions perpendicular to the peanut rows were very steep. Steep gradients from an area source of inoculum can be the result of wind and/or rain dispersal and are influenced by characteristics of the inoculum source (size, shape and height), weather (wind direction/speed and rainfall intensity), crop structure and spore type [34]. The dry conidia of *Aspergillus* species are adapted for wind dispersal, although they also may be dispersed directly or indirectly by rainfall. Raindrops striking a conidium-bearing surface release spores by mechanical shaking of the substrate or by a puff mechanism

in which air turbulence from the spreading raindrop dislodges the spores [35]. Dry conidia of *A. flavus* also are splash dispersed but because they are nonwetable, conidia are carried on the surface of the splash droplets [25]. The plant canopy restricts both wind and rain dispersal from ground-level inoculum [36, 37]. In this study, some of the rice inoculum washed into the exposed furrows, thereby exposing conidia to the direct effects of wind and rainfall. Dispersal gradients in this study were measured solely by the presence of the fungi in soil, and sampling of air and splash droplets is necessary to better define the mode of dispersal.

Biological control

This study clearly demonstrates that applied nontoxigenic *A. flavus* and *A. parasiticus* remain near the pod zone of peanuts where they would be most effective in reducing aflatoxin through competition with native toxigenic populations of *Aspergillus* species. A high inoculum rate (16.3 g/m of row per species) was used in these experiments to monitor the movement of conidia in soil. However, this level of application would not be economically feasible under standard conditions of peanut cultivation. Dorner et al. [13] showed a 74% reduction of aflatoxin in peanuts with as little as 2 g/m of combined nontoxigenic *A. flavus* and *A. parasiticus*. The loss of inoculum through water runoff following a much lower application rate may be a factor in the effectiveness of biocontrol strains in reducing aflatoxin.

The retention of conidia near the soil surface also may have important implications for biological control in aerial crops. In infection of corn and cotton by *A. flavus*, conidia dispersed from soil by wind and insects comprise the primary inoculum [38–40]. Therefore, a high soil-surface population of applied nontoxigenic strains should facilitate the reduction of aflatoxin in these crops.

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